United States
Environmental Protection
Agency

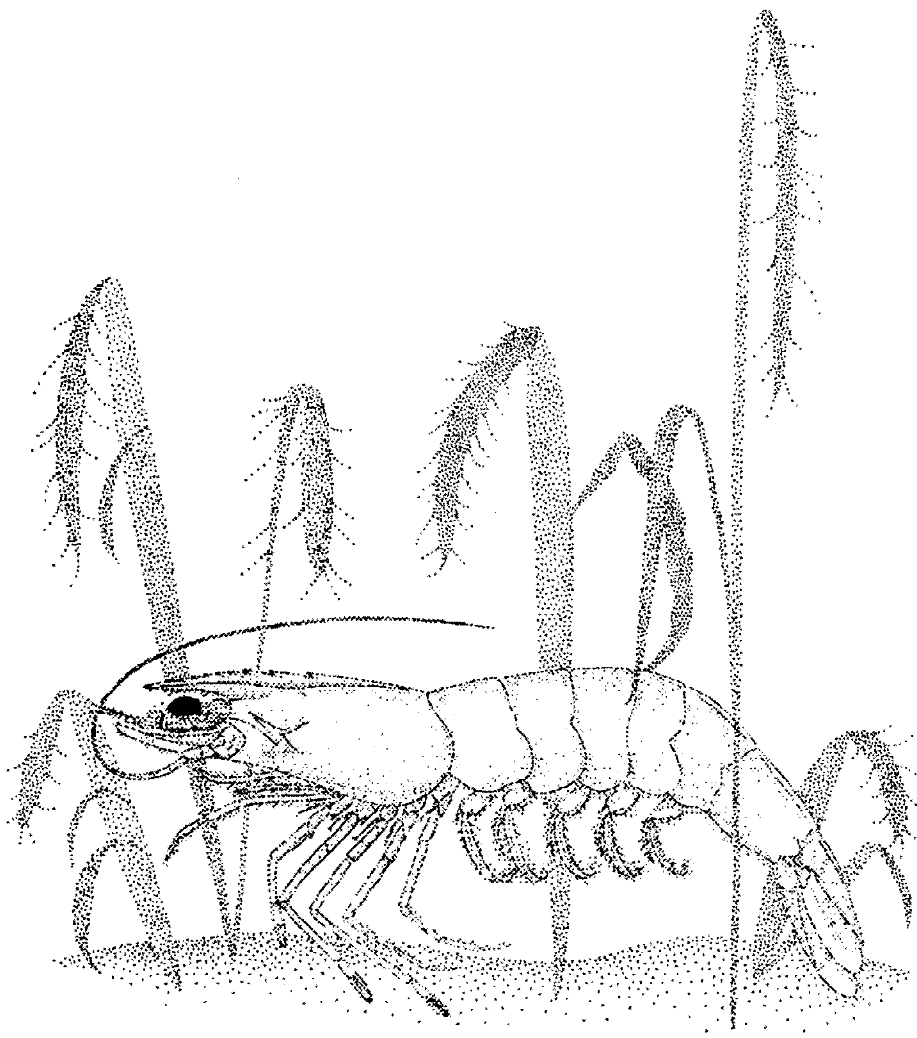
Office of Research and
Development
Washington, DC 20460

EPA/600/R-99/027
March 1999

EPA

Report on the Shrimp Virus Peer Review and Risk Assessment Workshop

Developing a Qualitative Ecological Risk Assessment



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REPORT ON THE SHRIMP VIRUS PEER REVIEW AND RISK ASSESSMENT WORKSHOP

Developing a Qualitative Ecological Risk Assessment

National Center for Environmental Assessment
Office of Research and Development
U.S. Environmental Protection Agency
Washington, DC

Printed on Recycled Paper

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DEDICATION

Ned Alcathe's valuable experience contributed to the development of the Joint Subcommittee on Aquaculture (JSA) Shrimp Virus Report. Early in the JSA Shrimp Virus Work Group's deliberations, Ned assisted in identifying issues and concerns regarding the potential role of shrimp processing in the shrimp virus problem. Despite being seriously ill, Ned joined the group of experts who participated in the January 1998 Shrimp Virus Peer Review and Risk Assessment Workshop; only a few weeks later, he died unexpectedly. In tribute to his technical contribution, his commitment to resolving this complex issue, and his warm, gentle spirit, we dedicate this document to Ned.

CONTENTS

CONTENTS (continued)

LIST OF TABLES

LIST OF FIGURES

FOREWORD

Environmental protection in the 1990s is pervaded by the language of risk, and environmental policies are set by the concepts and methods of risk assessment. Risk assessment and risk management provide the primary framework for decision-making at the U.S. Environmental Protection Agency (EPA), and EPA's primary mission is to reduce risks to environmental stressors. EPA's first Agencywide guidelines for ecological risk assessment, published in May 1998, provided a broad framework applicable to a wide range of environmental problems associated with chemical, physical, and biological stressors. However, although EPA has considerable experience in applying the ecological risk assessment paradigm to chemical contaminants, Agency experience for physical and especially biological stressors is limited. This report illustrates the applicability of the new ecological risk assessment guidelines to biological stressors such as nonindigenous pathogenic shrimp viruses.

Conducting the shrimp virus assessment illustrates several important points about the ecological risk assessment process. First is the importance of stakeholder involvement. Given that the shrimp virus issue involves sensitive socioeconomic and political issues, it was essential to hold meetings with stakeholders prior to completing the risk assessment and to conduct the risk assessment process openly. Second, although there are critical data gaps and uncertainties surrounding the shrimp virus issue, the ecological risk assessment process facilitates clear communication of available scientific information in a way that facilitates environmental decision-making. Finally, a primary objective of conducting a risk assessment is to support risk management activities. The use of this shrimp virus risk assessment as input to a subsequent risk management workshop provides this critical linkage. Overall, this assessment provides an excellent prototype for evaluating the risks associated with biological stressors.

William H. Farland
Director
National Center for Environmental Assessment

PREFACE

Public concerns over the potential introduction and spread of nonindigenous pathogenic shrimp viruses to the wild shrimp fishery and shrimp aquaculture industry in U.S. coastal waters have been increasing. Although these viruses pose no threat to human health, outbreaks on U.S. shrimp farms, the appearance of diseased shrimp in U.S. commerce, and new information on the susceptibility of shrimp and other crustaceans to these viruses prompted calls for action. In response, the Joint Subcommittee on Aquaculture (JSA) tasked a Federal interagency Shrimp Virus Workgroup with assessing the shrimp virus problem. Four Federal agencies are represented on the JSA Shrimp Virus Workgroup: the National Marine Fisheries Service (NMFS), the Animal and Plant Health Inspection Service (APHIS), the Environmental Protection Agency (EPA), and the Fish and Wildlife Service (FWS).

In June 1997, the Shrimp Virus Workgroup summarized the available information on shrimp viruses in a report to the JSA entitled "An Evaluation of Potential Shrimp Virus Impacts on Cultured Shrimp and on Wild Shrimp Populations in the Gulf of Mexico and Southeastern U.S. Atlantic Coastal Waters" (JSA Shrimp Virus Report [JSVR]). During July 1997, in cooperation with the JSA, EPA's National Center for Environmental Assessment (NCEA) sponsored a series of four stakeholder meetings to gather stakeholder input on the JSVR and the shrimp virus issue. The JSVR and the stakeholder (public) comments formed the basis for the shrimp virus peer review and risk assessment workshop, held during January 1998. Workshop participants considered several potential pathways of nonindigenous pathogenic shrimp viruses to wild shrimp populations, including shrimp aquaculture, shrimp processing, and "other" sources and pathways, and independently assessed risks using a qualitative risk assessment approach developed by the Aquatic Nuisance Species Task Force. The workshop report was revised based on comments provided by an external scientific review in July 1998.

This workshop report, together with the results of the independent scientific review, was used as the basis for a risk management workshop on shrimp viruses held on July 28-29, 1998, in New Orleans. The risk management workshop, jointly sponsored by the EPA Gulf of Mexico Program, NMFS, and the USDA Agricultural Research Service, developed options and strategies for managing the threat of shrimp viruses to cultured and wild stocks of shrimp in the Gulf of Mexico and southeastern U.S. Atlantic coastal waters.

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The National Center for Environmental Assessment (NCEA), Office of Research and Development, was responsible for the preparation of this report. The original document was prepared by Eastern Research Group under EPA Contract No. 68-C6-0041, Work Assignment No. 1-06. Bill van der Schalie of NCEA served as the EPA Work Assignment Manager and Kay Austin of NCEA was the technical lead for the project.

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1. EXECUTIVE SUMMARY

This report highlights issues and conclusions from the Shrimp Virus Peer Review and Risk Assessment Workshop, sponsored by the U.S. Environmental Protection Agency (EPA) in cooperation with the Joint Subcommittee on Aquaculture (JSA; National Science and Technology Council), held January 7–8, 1998, in Arlington, VA. The goals of the workshop were to:

- Complete a qualitative assessment of the risks associated with shrimp viruses, following the general risk assessment process developed by the Aquatic Nuisance Species Task Force.
- Evaluate the need for a future, more comprehensive risk assessment.
- Identify critical risk-relevant research needs.

The workshop focused on the scientific and technical aspects of the likelihood that nonindigenous viruses will become established in wild shrimp populations in the Gulf of Mexico and southeastern Atlantic coastal regions and on the potential ecological consequences of establishment. The workshop included 22 experts with varied backgrounds, including shrimp biology, toxicology, virology, marine ecology, ecological risk assessment, and shrimp aquaculture and processing. Before the workshop, participants received several background documents (ERG, 1997; JSA, 1997; ANSTF, 1996 [Appendix G]) and they were asked to prepare written premeeting comments for review by all participants. (These comments appear in Appendix C.) At the workshop, participants were divided into three groups, each of which was charged with evaluating the risks associated with one of the following categories of viral pathways:

- Aquaculture
- Shrimp processing
- Other potential sources

The risk that shrimp viruses pose to shrimp aquaculture operations was not considered as part of the scope of the workshop due to the limited time available; however, workshop participants believed that the risks to shrimp aquaculture should be given special attention as part of a subsequent technical or management workshop.

The qualitative risk assessment was conducted using the modified Aquatic Nuisance Species Task Force risk assessment approach (ANSTF, 1996; Appendix G). In developing the qualitative risk assessment, participants considered the following:

- Likelihood of viruses being present in the pathway
- Ability of the viruses to survive transit in the pathway
- Colonization potential of the viruses in native shrimp
- Spread potential of the virus within native shrimp populations
- Consequences of establishment

In general, workshop participants agreed that viruses could be associated with pathways leading to coastal environments and that they could survive in these pathways. Participants concluded that there is potential for viruses to colonize native shrimp in localized areas, such as an estuary or embayment, near the point of entry into the marine system. Some participants also noted that repeated viral introductions to an area will increase the risk of colonization.

Participants had widely divergent views on the potential for viruses to spread beyond the initial local area of colonization. This divergence largely reflects the high uncertainty associated with this aspect of exposure. Participants considered the potential for localized colonization and subsequent spread to be a critical aspect of evaluating the potential establishment of viruses in native shrimp.

Workshop participants discussed the impact that virus establishment could have on local shrimp populations (e.g., within an individual estuary). The participants determined that initial kill rates might be high but that the population would be likely to recover rapidly due to reintroduction of shrimp from other locales or compensatory increases in reproduction. Workshop participants concluded that the risk from viral introductions to the entire population of native shrimp along the southeastern Atlantic coast and within the Gulf of Mexico is relatively low, although there is a high degree of uncertainty associated with this evaluation.

The ability of workshop participants to address broader ecological risks in a comprehensive manner was limited by the time and information available. However, some participants thought that the issue of broader ecological risks is important and merits further consideration.

Workshop participants identified areas where further research and information would improve the assessment of risks and could help evaluate current conditions. They also identified actions for reducing uncertainty that should be given the highest priority, including:

- Improved diagnostic methods
- Surveys of wild shrimp populations for presence of the four nonindigenous viruses and for genetic composition
- Experiments to reduce uncertainties surrounding virus transmission and virulence
- Field epidemiologic studies

Participants identified other areas where additional research is needed to improve the ability to estimate risks to wild shrimp populations, including:

- Viral persistence
- Compensatory mechanisms
- Monitoring of imported shrimp
- Development of suitable population models
- Targeted surveys of nonpenaeid species to determine if they are susceptible to or carriers of nonindigenous viruses

Workshop participants believed that, given the existing knowledge base, it is currently not feasible to conduct a more comprehensive, quantitative assessment of the risks associated with nonindigenous shrimp viruses. Participants generally agreed that, at present, qualitative evaluations could be made, but they noted there is a great deal of uncertainty surrounding many key areas of the shrimp virus problem. Participants determined that there is a need to continue efforts to gather available data on shrimp virus effects and a need to conduct a systematic research effort that could be used to reduce the uncertainty of any subsequent risk assessments.

Workshop participants identified the following areas of concern where additional efforts should be focused:

- Management implications of shrimp viruses
- Risks of shrimp viruses to aquaculture operations
- Risks of shrimp viruses to nonpenaeid species

2. INTRODUCTION

This report highlights issues and conclusions from the Shrimp Virus Peer Review and Assessment workshop sponsored by the U.S. Environmental Protection Agency (EPA) in cooperation with the Joint Subcommittee on Aquaculture (JSA), held January 7–8, 1998, in Arlington, VA. The goals of the workshop were to:

- Complete a qualitative assessment of the risks associated with shrimp viruses, following the general risk assessment process developed by the Aquatic Nuisance Species Task Force (ANSTF)
- Evaluate the need for a future, more comprehensive risk assessment
- Identify critical risk-relevant research needs

The workshop focused on the scientific and technical aspects of the likelihood that nonindigenous viruses will become established in wild shrimp populations in the Gulf of Mexico and southeastern Atlantic coastal regions and on the potential consequences of such establishment.

This section provides an overview of the recently published JSA report (JSA, 1997) that formed the basis for the workshop, a description of the workshop process, and a discussion of the qualitative risk assessment approach used at the workshop. Section 2 of this document summarizes discussions held during the workshop on several aspects of the qualitative risk assessment process, and it contains a risk characterization developed by the workshop chair and breakout group chairs following the workshop's conclusion. Section 3 discusses actions for reducing uncertainty that were identified by participants during the workshop. The reports of each breakout group are contained in Appendix A.

2.1. JSA REPORT OVERVIEW

Dr. Kay Austin of EPA's National Center for Environmental Assessment, and a member of the JSA Shrimp Virus Work Group, discussed the work group's efforts to date and described events leading to the workshop. She provided an overview of the purpose, scope, and findings of the work group's report, entitled "An Evaluation of Potential Shrimp Virus Impacts on Cultured Shrimp and on Wild Shrimp Populations in the Gulf of Mexico and Southeastern U.S. Atlantic Coastal Waters" (hereinafter called JSA report) (JSA, 1997). Highlights of her presentation follow.

New, highly virulent viruses have been documented in foreign shrimp aquaculture. Consumer demand for shrimp continues to grow, and to meet this demand, the United States has greatly increased shrimp importation from areas of the world where pathogenic shrimp viruses are known to be endemic. Recent events have prompted calls for investigation into the actual risks to U.S. domestic resources. These events have included catastrophic viral outbreaks in shrimp aquaculture both in the United States and abroad, recent appearances of these organisms in shrimp in commercial retail stocks, and new information on the susceptibility of shrimp and other crustaceans to these organisms. While some of these viruses have severe and lethal effects in crowded aquaculture conditions, they are not known to pose threats to human health.

The U.S. shrimp industry (harvesting and processing alone) is valued at $3 billion per year. Imported shrimp account for more than 80% of the market. In 1995, imports exceeded domestic production by a ratio of four to one, amounting to 720 million pounds (in tails). The largest share of these imports comes from Latin America and Asia, areas of the world where shrimp viruses are endemic. Domestic aquaculture operations, in contrast, account for a much smaller portion of the U.S. market, ranging from 2 million pounds in 1991 to 4 million pounds in 1994.

The JSA, which is under the auspices of the President's National Science and Technology Council, formed the interagency Shrimp Virus Work Group in March 1996 to assess the risks associated with these emerging viral pathogens. Four Federal agencies are represented in the work group: the National Marine Fisheries Service (NMFS), EPA, the U.S. Fish and Wildlife Service (USFWS), and the U.S. Animal and Plant Health Inspection Service (APHIS). JSA charged the work group with developing a Federal interagency strategy to address the shrimp virus issue and to identify relevant research on viral stressors, their potential mode of transmission, and their potential for introduction to U.S. shrimp resources.

The work group recognized that the shrimp virus problem presents some unique issues in risk assessment. Members determined that the problem is a complex one that moves beyond the traditional single-chemical, single-species assessment process. The shrimp virus problem involves potentially nonindigenous viral stressors and has great potential to significantly impact the U.S. shrimp industry and other ecological components of coastal systems.

During its initial evaluation of the problem, the work group decided to base its approach on EPA's ecological risk assessment guidelines, which were published in draft form in 1996 (U.S. EPA, 1996). Because the work group determined that not enough information was available to complete an actual risk assessment, it followed a problem formulation approach that enabled the work group to summarize risk-relevant information available prior to January 1997 and to identify data gaps and critical research needs.

During its problem formulation activities, the work group developed a proposed management goal and identified potential viral sources, potential viral and other environmental stressors, and potential ecological effects. The work group also reached consensus on assessment endpoints and developed a conceptual model (Figure 1) that illustrates the linkages between human activities, viral stressors, and assessment endpoints of concern. The work group's report was completed in June 1997.

Significant findings of the JSA report include the following:

- Viral disease has been associated with severe declines in wild shrimp harvests in the Gulf of California. Populations of the blue shrimp, *Penaeus stylirostris*, and other less dominant species plummeted coincident with the observed occurrence of IHHNV disease in wild shrimp populations in the Gulf of California. The work group found that this is the best piece of epidemiologic information suggesting a link between introduced viruses and declines in wild shrimp populations. There remains considerable debate, however, regarding the validity of this association of disease and effects.

- Nonindigenous shrimp viruses have not been documented in wild U.S. shrimp populations; until recently, detection efforts have been minimal. Sampling techniques may have been inadequate, and the correct technology may not have been available to adequately detect the viruses.

- Numerous nonindigenous viral disease outbreaks have occurred in U.S. shrimp aquaculture since 1994, and frozen shrimp in commerce have been found to be contaminated with these viruses. Laboratory studies show that all life stages of shrimp are potentially at risk from at least one of the four viruses covered in the JSA 1997 report.

- Harvesting practices in foreign aquaculture could put U.S. domestic shrimp populations at risk. The work group learned that when an outbreak occurs in some foreign aquaculture operations, the affected crop is often harvested immediately and exported to avoid severe crop and monetary losses.

- Shrimp may be contaminated from a number of possible sources. The work group identified aquaculture and shrimp processing as two potentially important sources that may affect wild shrimp populations. Potential pathways for nonindigenous viruses to reach shrimp populations via these sources are shown in Figures 2 and 3. The work group also considered a number of other possible sources, such as live and frozen bait shrimp, ballast water, and natural spread by mechanisms such as hurricanes, floods, or animals. Research and display facilities also may be a source of exposure to wild populations.

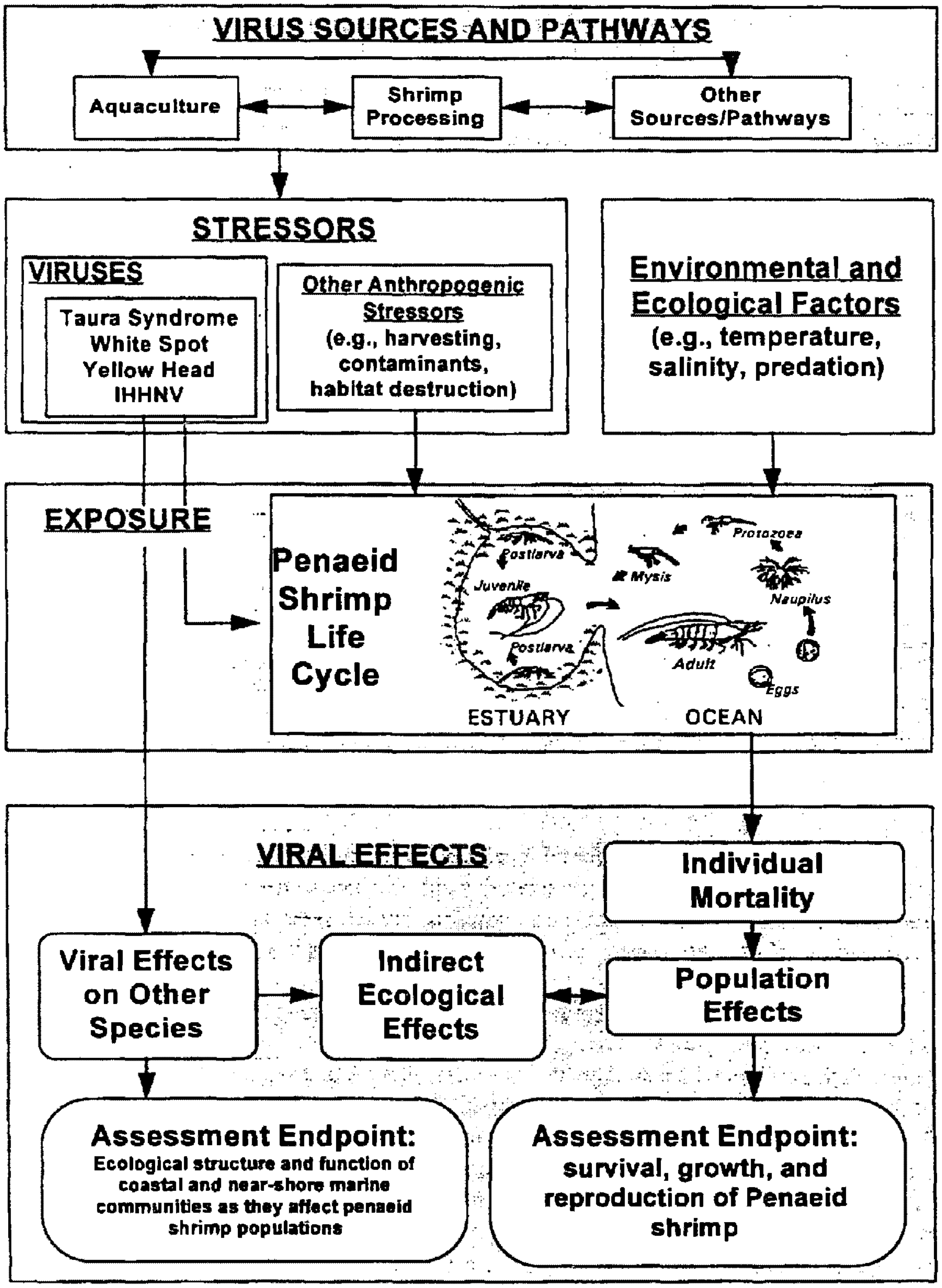

Figure 1. Shrimp virus conceptual model. This model was provided to workshop participants to assist with their discussions. Participants focused their discussions on viral stressors and direct effects on penaeid shrimp.

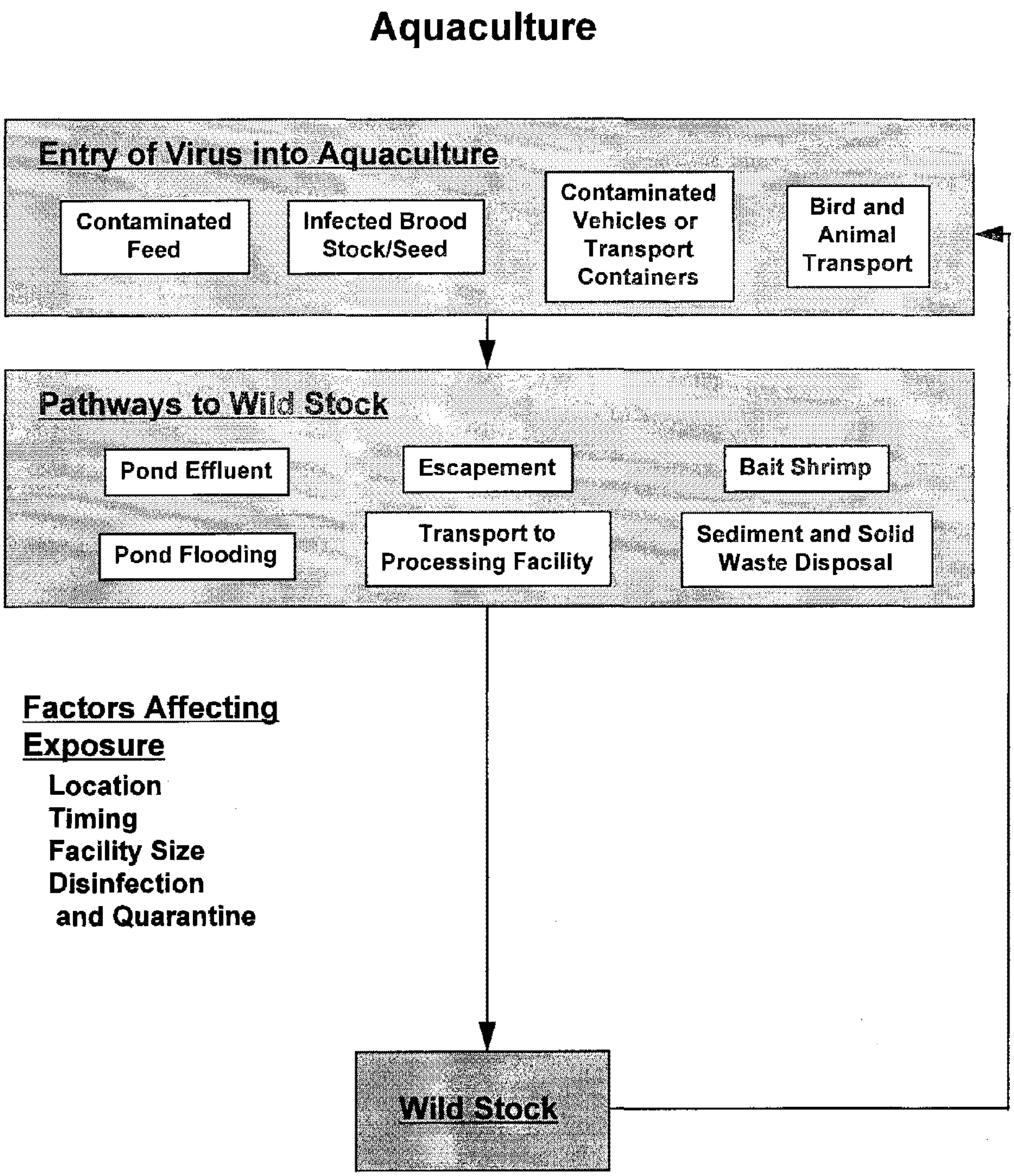

Figure 2. Conceptual model: virus sources and pathways for aquaculture.

Source: JSA, 1997.

Shrimp Processing

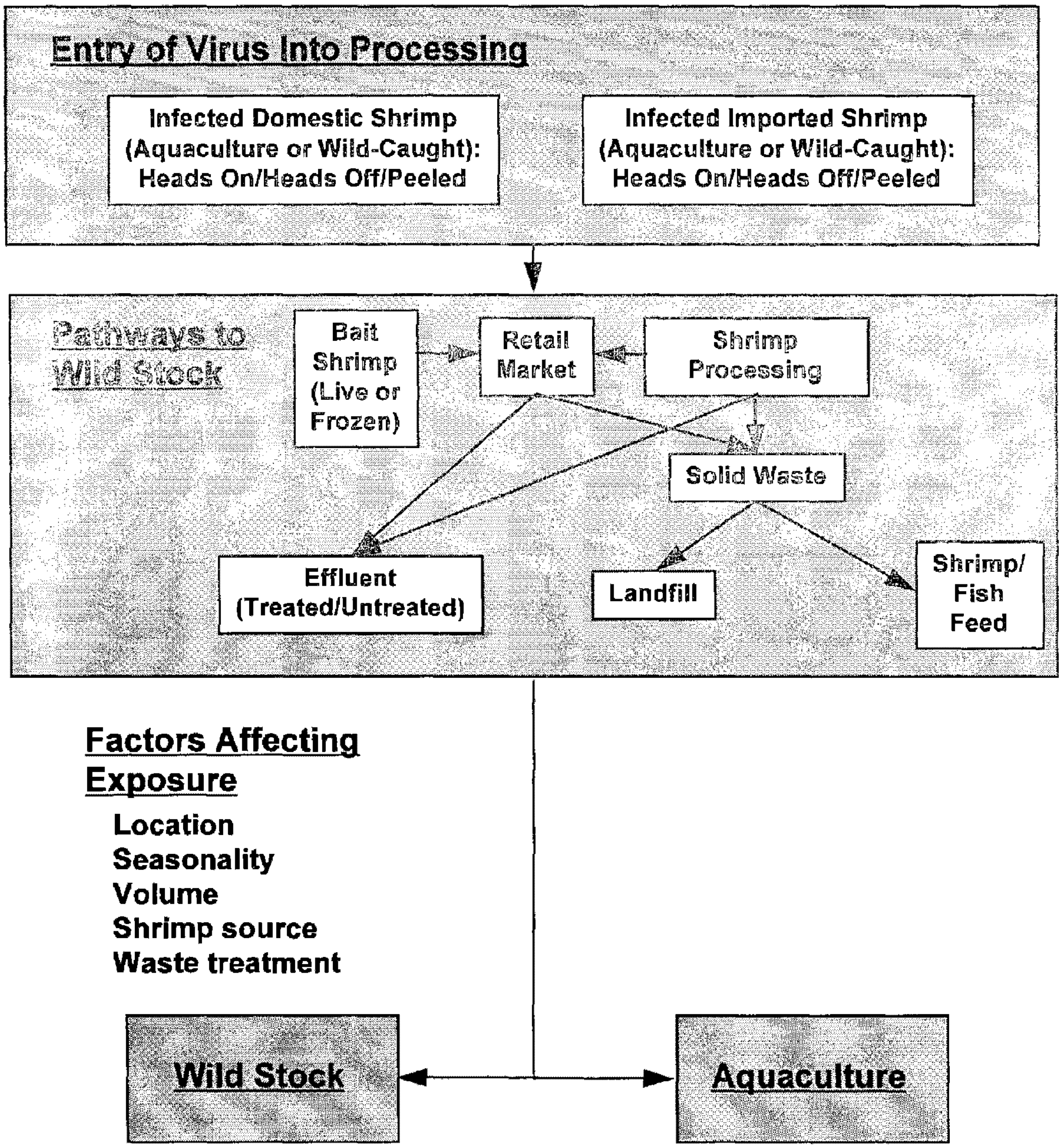

Figure 3. Conceptual model: virus sources and pathways for shrimp processing.
Source: JSA, 1997.

- Species other than shrimp may be at risk from these viruses. Viral disease could result in alterations to ecosystem structure or function, potentially affecting a wide range of endpoints, such as predator-prey relationships, competition, and nutrient cycling. Many other economically and ecologically important organisms that occupy coastal areas feed on juvenile shrimp, and impacts to these organisms could be serious if the wild shrimp populations on which they feed decline. Other organisms may be susceptible to disease themselves or serve as carriers of these viruses.

During July 1997, JSA and EPA sponsored public meetings in Charleston, SC; Mobile, AL; Brownsville, TX; and Thibodaux, LA, to gather stakeholder input on the shrimp virus issue and the JSA report. Stakeholders included individuals from the wild shrimp fishery industry, the shrimp aquaculture industry, the shrimp processing industry, environmental organizations, regulatory and resource management agencies, and the general public. The minutes of these stakeholder meetings were published in October 1997 (ERG, 1997).

2.2. PEER REVIEW OF JSA SHRIMP VIRUS REPORT

Prior to the workshop, Eastern Research Group (ERG) provided all experts with a number of documents and materials to help the experts prepare for the workshop and to assist them in developing a peer review of the JSA report. The materials provided included the JSA report (JSA, 1997), the minutes of the stakeholder meetings about the JSA report (ERG, 1997), and a copy of a qualitative risk assessment process for nonindigenous organisms (ANSTF, 1996). Panel members (Appendix B) were asked to review the material and prepare written comments to address questions on the following topics:

- Management goals, assessment endpoints, and the conceptual model
- Viral stressors and factors regulating shrimp populations
- Viral pathways and sources
- Stressor effects
- Comprehensive risk assessment and research needs

(Lists of the peer review experts and their breakout group discussion assignments are contained in Appendix B. The charge to experts and the experts' premeeting written comments are contained in Appendix C. Overheads prepared by the chairpersons that summarize the premeeting comments are contained in Appendix F.)

Below are summarized the peer review comments by expert panel members on aspects of the JSA report (excluding sections not directly relevant to the JSA report) important to developing a qualitative ecological risk assessment. The recommended modifications to information included in the JSA report were considered in discussions at the workshop and incorporated as appropriate into the qualitative risk assessment.

2.2.1. Management Goal, Assessment Endpoints, Conceptual Model, and Scope of the Assessment

The participants were charged with commenting on how well the JSA report's proposed management goal, assessment endpoints, and conceptual model reflected the dimensions of the shrimp virus problem. Those persons responding generally agreed that the proposed management goal adequately reflected the broader dimensions of the shrimp virus problem. However, a number of participants offered suggestions to further broaden the focus of the management goal to include the following: enlarging the report's geographic coverage to include the U.S. Pacific coast; focusing on risks to aquaculture; considering other potential pathogens (e.g., other viruses, bacteria, fungi) and other potentially susceptible organisms; addressing other environmental stressors potentially impacting wild shrimp populations (e.g., pollutants, coastal development); and evaluating the economic impacts related to developing (or not) alternative seafood production methods. One reviewer also indicated that the management goal may have been appropriate in 1996, as it assumed the four viruses were "new" and none had been established in U.S. coastal waters. However, the reviewer noted that there is growing evidence that at least one of the four viruses (WSSV) may have already become established. Other reviewers emphasized the need to keep the focus of the management goal and assessment endpoints narrow for the risk assessment to be manageable.

Participants generally agreed that the proposed assessment endpoints were adequate to address the scope of the problem. Many reviewers broadly interpreted or accepted the intent of the first assessment endpoint—"survival, reproduction of wild shrimp"—but they viewed the second proposed assessment endpoint—"ecological structure and function"—as too broad to be measurable or meaningful. Some believed that the second proposed endpoint should be deleted and replaced with one to address concerns for potential risks to a broad range of other marine organisms. A number of other recommendations were made to modify the proposed endpoints, including the following: emphasize risks of viruses to aquaculture, focus on ecological aspects not necessarily related to wild shrimp populations and harvest, and develop comprehensive data on the genetic structure and prevalence of viruses in natural populations. One expert expressed concern that the report did not focus enough on ecologically important, nonpenaeid species such as the grass shrimp.

Reviewers expressed a variety of opinions about the scope of the conceptual model and the risk assessment. While some experts indicated that the proposed conceptual model should be expanded to include the full range of probable risks and to develop a suite of related assessment endpoints, others emphasized that such an expansion would be overly ambitious for the initial phase of the risk assessment. It was recommended that all potentially important interactions should be identified in the conceptual model and connectivity between various endpoints or systems should be represented. One reviewer noted that the assessment might be expanded following the findings of the initial phase of the risk assessment.

2.2.2. Viral Stressors and Factors Regulating Shrimp Populations (Relevance of Laboratory Data, Human Health Concerns, Reliability of Available Identification Techniques)

Because of the lack of extensive field data on virus effects on wild shrimp populations, participants were asked to judge the relevancy of information on virus infectivity and effects from laboratory or intensive aquaculture to determine virus effects on wild shrimp populations. Opinions varied widely. Some participants viewed such information as totally irrelevant given conditions in an aquaculture setting, such as high densities that can potentially contribute to susceptibility, virus infectivity, and spread. Others noted that while this type of information could not be used to make reliable predictions about virus effects on wild populations, it is valuable in determining the potential for effects to occur in the wild. Reviewers noted that laboratory studies can be used to establish potential host range, and dose response data can be used to predict impacts to wild populations. In some cases, participants noted that such data can provide the best and most reliable information available.

The JSA report indicated that concerns for human health effects of shrimp viruses could be "ruled out" based on expert opinion and numerous observations, given the tremendous quantities of shrimp imports over the past 30 years and the lack of evidence of human health effects as a result of shrimp importation, processing, and consumption. Workshop participants were asked to comment on the report's conclusions. Opinions varied; some experts were more cautious and expressed the opinion that it was premature to completely rule out human health effects, while others with perhaps more extensive experience working with the viruses of interest offered a differing point of view. The latter group noted that while one can never be absolutely certain that a nonhuman host virus will not become infectious to humans, it is highly unlikely that shrimp viruses will affect human health. Participants noted that viruses co-evolve with their hosts and become highly adapted to particular hosts. Given the tremendous evolutionary distance between vertebrates and invertebrates, it is improbable that these viruses could infect humans or other vertebrates, even by mutating. However, factual evidence to clarify this uncertainty is lacking.

The JSA report expressed concern about the availability and reliability of methods for isolation and identification of shrimp viruses in wild populations and environmental media. Expert panel members were asked to comment on their understanding of this issue. Opinions were divided; some panel members responded that identification techniques are reliable and effective, while others pointed out that detection methods have yielded mixed results. It was noted that bioassays, histologic examination, and serologic methods have been applied, but their specificity and sensitivity have been difficult to assess. Panelists said that methods are available for only three or four of the viruses focused on by the JSA report, and the complex nature of this testing may not allow definitive conclusions to be made about occurrence. Most participants commented that additional research is needed to develop molecular, immunologic, and diagnostic techniques to screen for viruses in wild shrimp tissues, feed, and environmental media potentially contaminated by shrimp viruses. Given current technology, one expert noted that it is impossible to determine with certainty virus occurrence in large volumes of soil or water.

2.2.3. Viral Pathways and Sources

The JSA Shrimp Virus Work Group considered aquaculture and shrimp processing to be primary pathways of concern leading to exposure of pathogenic shrimp viruses to wild shrimp populations, and other sources were identified as secondary pathways for exposure. Workshop participants were asked to judge the acceptability of these sources as potential pathways for virus introduction to wild shrimp populations.

2.2.3.1. *Aquaculture*

One common theme expressed by participants was that there is little scientific evidence to either confirm or refute the occurrence of epizootics among wild shrimp associated with naturally occurring or introduced viruses. Participants noted that evidence indicates that one shrimp virus may be present in wild populations in U.S. coastal waters, but its source is unknown. One expert remarked that no convincing causal relationship has been established between outbreaks of virus in aquaculture facilities and virus transmission to wild stocks. The expert continued that although no direct link has been established, it does not rule out that it has occurred previously or will occur in the future. The expert added that, to date, wild populations have not been adequately monitored. He concluded that when monitoring is available, we may be able to track the movement of virus infection, eventually resolving this issue. One other reviewer also noted that without simultaneously isolating viruses from aquaculture and a geographically located shrimp population (possible with the development of gene probes), the role of aquaculture in infecting wild shrimp remains speculative. Participants emphasized that resolving this issue is highly problematic and a critical element to the risk assessment.

2.2.3.2. *Shrimp Processing*

The JSA report suggests that shrimp processing could be another primary source for introducing pathogenic shrimp viruses to U.S. coastal waters. Workshop participants were asked to consider how evidence from wild shrimp populations either supported or refuted the importance of shrimp processing as a potential source for shrimp virus. As with shrimp aquaculture, in general, panel members concluded that there was little scientific evidence to suggest a strong link between processed shrimp or shrimp process wastes and the occurrence of shrimp viruses in wild shrimp populations. However, several experts noted that disposal of wash water from shrimp processing facilities directly to receiving waters that support any phase of wild shrimp development should continue to be a concern. One theme reflected in reviewer comments was concern that the practice of some producers to harvest diseased shrimp for export makes this one of the more likely potential sources of virus contamination of wild shrimp populations (because the United States imports significant quantities of shrimp). One other reviewer concluded that even though shrimp processing could introduce virus, there is not enough known about viral persistence in nature to determine whether shrimp processing represents a realistic source of pathogenic shrimp virus introduction to wild shrimp.

2.2.3.3. *Other Potential Sources*

Workshop participants were also asked to consider the potential role of sources other than aquaculture and shrimp processing (secondary sources) in introducing pathogenic shrimp virus to wild shrimp. Those panel members responding considered bird feces and ballast water transfer as likely other potential sources. Natural spread, bait shrimp, and the introduction of secondary hosts were considered to be less important "other" potential sources. It was generally agreed that while those other sources suggested by participants and those listed in the JSA report could be plausible, their relative contribution was unknown. Participants expressed concern that management of these other potential sources could be problematic. However, one dissenting panel member expressed the opinion that an evaluation of the existing data with respect to the probabilities of transmission and establishment should be done for all potential sources.

The JSA report expressed concern that manufactured shrimp feed could be a potential other source of shrimp virus. Participants were asked to consider the importance of shrimp feed as a potential source of pathogenic shrimp viruses. Panel members were divided in their responses to this question. Many expressed the opinion that processing temperatures were adequate to eliminate shrimp feed as a source of shrimp virus, while others noted that temperatures may not be adequate and shrimp feed should continue to be of concern as a possible other source. However, one expert emphasized that the likelihood of shrimp feed as a source could be determined only by knowledge of virulence of the specific type of virus, its viability, and the length of time materials were held at temperatures, especially those processed at lower (70 °C) temperatures. To further eliminate shrimp feed as a potential other source, one participant noted that farms should be discouraged from using or supplementing manufactured food with natural feeds.

2.2.4. Stressor Effects

Participants were asked to consider how the available evidence regarding the effect of introduced shrimp virus on wild shrimp populations should be interpreted. In general, workshop participants indicated that there is little convincing information or scientific data on effects of introduced pathogenic shrimp viruses on wild shrimp populations. Some participants believed the available information, when considered carefully, could be useful in identifying underlying problems. One expert cautioned that available evidence should be considered individually for each virus and host system. Another expert noted that while there is clear evidence that viruses have been introduced to aquaculture, it is not known how these may relate to the observed declines such as those observed in the Gulf of California example considered by the JSA report. Reviewers also noted that such associations of virus occurrence cannot be made without considering the role of other important environmental factors in wild shrimp population declines, notably overfishing, El Nino, pollution, and environmental degradation. Reviewers indicated that there is a critical need for research to address this issue.

The JSA report discussed the importance of virus effects on nonshrimp species, and panel members were asked to comment on this issue. For the most part, participants agreed that potential virus effects on nonshrimp species are generally unknown but of significant concern. One respondent observed that it is well documented that some viruses can infect other crustacean species, noting that WSSV has been detected by polymerase chain reaction (PCR) in both cultured and wild shrimp, prawns, crabs, and other arthropods in different Asian countries. He concluded that the potential threat to U.S. shrimp, nonshrimp, and the ecosystem as a whole could not be ruled out. However, one member noted that while nonshrimp species are important ecologically, pathogenicity of viruses is usually species specific. In contrast, another participant commenting on the JSA report's discussion of effects on nonshrimp species thought that these effects should be considered not very great, and he pointed out that the report also failed to emphasize concern for effects on nonpenaeid shrimp species, such as the grass shrimp. This concern stemmed from the knowledge that other parasites of shrimp can be harmful to other marine organisms and that these pathogenic shrimp viruses could cause serious impacts to sport commercial fisheries by reducing available food sources such as the grass shrimp. Yet another expert noted that effects on nonshrimp species should be considered important, especially on susceptible species with low population levels. One participant felt this was an extremely important issue but noted it is probably difficult to evaluate on the short term.

2.3. SHRIMP VIRUS PEER REVIEW AND RISK ASSESSMENT WORKSHOP PROCESS

At the beginning of the workshop, the workshop chairperson, Dr. Charles Menzie (Menzie-Cura & Associates) reviewed the agenda (included in this report as Appendix D), explained the workshop's format, and reviewed the workshop's goals, which were to:

- Complete a qualitative assessment of the risks associated with nonindigenous shrimp viruses, following the general risk assessment process developed by the ANSTF
- Evaluate the need for a future, more comprehensive risk assessment
- Identify critical risk-relevant research needs

Dr. Menzie explained that the workshop report would be used to provide information for a proposed workshop to identify potential risk management options. The proposed workshop, sponsored by JSA and NMFS, was held in July 1998. Peer review experts were divided into three breakout groups, each of which was charged with evaluating the risks associated with one of three viral pathways (aquaculture, shrimp processing, and other potential sources).

Three experts in ecological risk assessment were selected as breakout group leaders: Dr. Wayne Munns (EPA Office of Research and Development), who facilitated discussions on aquaculture; Dr. John Gentile (University of Miami), who facilitated discussions on shrimp processing; and Dr. Anne Fairbrother (Ecological Planning and Toxicology, Inc.), who facilitated discussions on other potential sources. (See Appendix B for breakout group assignments.) After the workshop, Dr. Munns prepared the report of the aquaculture breakout group (Appendix A-1), Dr. Gentile prepared the report of the shrimp processing breakout group (Appendix A-2), Dr.

Fairbrother prepared the report of the other pathways breakout group (Appendix A-3), and Dr. Menzie prepared the qualitative risk assessment (Section 3). Workshop participants were asked to review and comment on the breakout group reports prior to preparation of this final document.

2.4. QUALITATIVE RISK ASSESSMENT METHODOLOGY

Mr. Richard Orr, of the U.S. Department of Agriculture, Animal, and Plant Health Inspection Services (USDA-APHIS), provided participants with an overview of the qualitative risk assessment methodology to be used at the workshop. The process was based on the ANSTF risk assessment approach (ANSTF, 1996), which provides a qualitative assessment of the probability and consequences of establishment of a nonindigenous species in a new environment. (A copy of the ANSTF report [1996] is contained in Appendix G.) Mr. Orr noted that the methodology may be used as a subjective evaluation, or it may be quantified to the extent possible depending on the needs of the analysis. He reviewed an assessment on black carp to illustrate the application of this process to a nonindigenous species. Both documents were provided to workshop experts as background information prior to the workshop.

Mr. Orr explained that the risk assessment model is divided into two major components: the "probability of establishment" and the "consequences of establishment" (see Figure 4, which contains the risk assessment model from the Report to the Aquatic Nuisance Species Task Force). These components of the model are further divided into basic elements that serve to focus scientific, technical, and other relevant information for the assessment. Mr. Orr discussed how the following elements could be used to estimate the probability of establishment of viral pathogens in wild shrimp populations:

- Probability of the nonindigenous organism being on, with, or in the pathway
- Probability of the organism surviving in transit
- Probability of the organism successfully colonizing and maintaining a population where introduced
- Probability of the organism spreading beyond the colonized area

The following elements are used in the ANSTF approach to evaluate the consequence of establishment of a nonindigenous species (see, Appendix G, p. 22):

- Economic impact
- Environmental impact

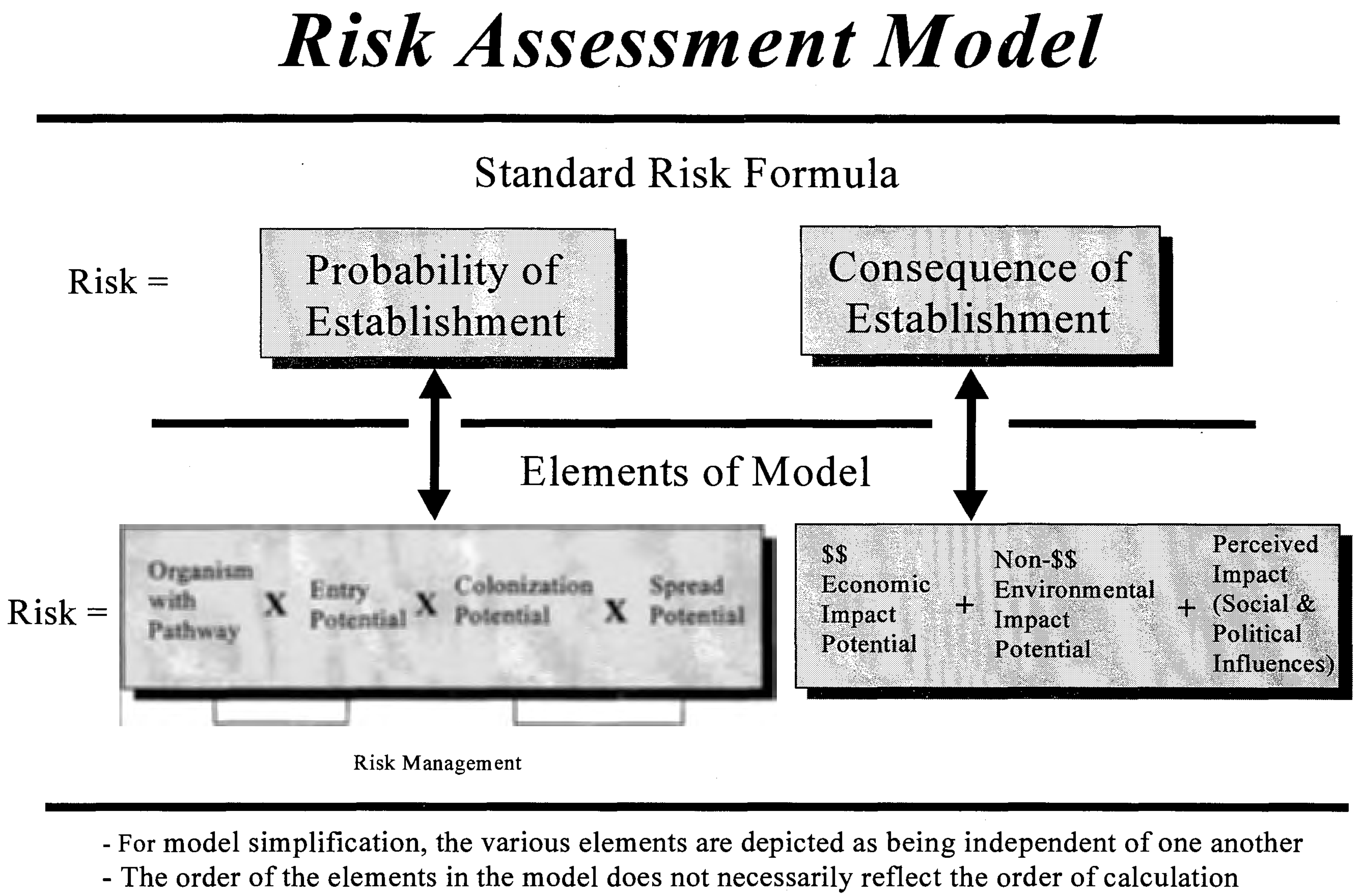

Figure 4. Risk Assessment Model from the Report to the Aquatic Nuisance Species Task Force

Source: Adapted from RAM, 1996.

- Impact from social and/or political influences

For the purposes of the Shrimp Virus Peer Review and Risk Assessment Workshop, only environmental impacts were evaluated. It was recommended that economic and perceived impacts of establishment be considered at a workshop on risk management options, which was held in July 1998.

Mr. Orr stressed that it is critical for the qualitative risk assessment to capture and communicate the uncertainty that surrounds the available information about shrimp viruses.

3. QUALITATIVE RISK ASSESSMENT

3.1. THE RISK ASSESSMENT PROCESS

Workshop participants began the risk assessment process by reviewing the management goal and assessment endpoints presented in the JSA report (JSA, 1997). Participants evaluated the risks associated with aquaculture, shrimp processing, or other potential sources. In the breakout groups, participants considered the ecological risks associated with each identified viral pathway. The evaluation of each pathway was conducted independently. It is important to note that participants did not attempt to rank the relative risk of the three identified sources.

Each breakout group evaluated both the potential for establishment of the viruses via the identified pathways and the potential ecological consequences of establishment. The breakout groups considered the four following elements of the potential for establishment of viruses via the identified pathways:

- Association of nonindigenous viruses with the pathway
- Entry of nonindigenous viruses into coastal waters via the pathway (including survival)
- Colonization/infection of shrimp at the local level
- Spread of nonindigenous viruses to the shrimp populations at large

To determine the probability of establishment of nonindigenous viruses, the breakout groups rated each of these elements as either low, medium, or high. The consequences of establishment were similarly rated. During their deliberations, the breakout groups were asked to identify the level of uncertainty (ranging from very uncertain to very certain) associated with each of the elements describing the potential for and consequences of establishment of nonindigenous pathogenic shrimp viruses.

Using the method set forth in the ANSTF (1996) report (Appendix G), workshop participants estimated the overall risk by compiling the risks associated with the individual elements of the process (i.e., [1] the four elements of the probability of establishment and [2] the consequence of establishment). The probability of establishment is determined by the lowest ranking of any of the four elements. For example, if elements under the probability of establishment had rankings of high, high, low, and medium, the overall probability of establishment would be considered low. This approach is reasonable because, for an organism to become established, each of the elements must occur. Assuming the elements are independent of each other, combining a series of probabilities will give a probability much lower than the individual element ratings. The conservatism of this approach is justified by the general high degree of biological uncertainty that is found throughout the process (ANSTF, 1996).

Rankings for the probability of establishment and the consequence of establishment are combined into an overall level of risk as shown in Table 1.

These rankings, which are based on expert judgment, should not be considered separately from the discussion and rationale provided by the workshop participants. As noted in the ANSTF (1996) report, "the strength of the Review Process is not in the element-rating but in the detailed biological and other relevant information statements that motivate them."

After evaluating the probability of establishment for their respective pathways and the consequences of establishment at the local and regional (e.g., Gulf of Mexico) population levels, the three breakout groups presented their findings in a plenary session. Breakout group findings are found in Appendices A-1, A-2, and A-3, while the main body of this document primarily reflects plenary discussions but incorporates breakout group findings when there was a lack of consensus. Following the conclusion of the expert workshop, the breakout group chairpersons and the workshop chairperson met to discuss the breakout group findings and their reports and to develop a risk characterization for the assessment using ANSTF methodologies.

Table 1. Combining the rankings for the probability of establishment and the consequences of establishment into an overall estimate of risk

If the overall probability of establishment is:	And the consequence of establishment is:	Then the overall risk ranking is:
High	High	High
Medium	High	High
Low	High	Medium
High	Medium	High
Medium	Medium	Medium
Low	Medium	Medium
High	Low	Medium
Medium	Low	Medium
Low	Low	Low

3.2. QUALITATIVE RISK ASSESSMENT RESULTS

This section summarizes discussions held during the workshop on several aspects of the risk assessment process:

- Management goal and assessment endpoints that frame the assessment (Section 3.2.1)
- The probability of establishment of shrimp viruses (Section 3.2.2)
- The consequences of establishment (Section 3.2.3)
- A characterization of the risks resulting from a combination of the probability and consequences of establishment (Section 3.2.4)

The reports of the three breakout groups are contained in Appendix A. Tables 2 through 5 provide the risk rankings assigned to various pathways by the breakout groups, which are summarized in Sections 3.2.2 through 3.2.4.

3.2.1. Management Goal and Assessment Endpoints

Workshop participants were asked to evaluate the completeness and adequacy of both the management goal and the assessment endpoints identified in the JSA report (JSA, 1997). In the ecological risk assessment process, the management goal is intended to reflect the management context of the assessment, while the assessment endpoints are explicit expressions of the environmental values to be protected, which serve as the focal points for an assessment.

The management goal identified in the JSA report is to:

- Prevent the establishment of new disease-causing viruses in wild populations of shrimp in the Gulf of Mexico and southeastern U.S. coastal waters while minimizing possible impacts on shrimp importation, processing, and aquaculture operations.

A number of participants thought that the management goal should be broadened to include risks to aquaculture operations. Participants concurred that these risks are important, but because of the limited time available for workshop discussions, they agreed that risks to aquaculture operations would not be considered during the workshop. Participants recommended instead that risks to shrimp in aquaculture operations and management of those risks be the subject of a separate workshop.

Table 2. Summary of aquaculture breakout group risk rankings

Refer to supporting discussion in the text to properly evaluate information presented in this table. The risk assessment process is described in Section 3.1 and Appendix G.

	Pathways to the environment				
Probability of establishment	**Escapement**	**Pond flooding**	**Pond effluent**	**Transport to processing facility**	**Sediment and solid waste disposal**
Association with pathway	High/very certain	High/very certain	High/very certain	High/very certain	High/very certain
Entry potential	High/very certain or low/reasonably certain[a]	Low/ very certain	Medium/very certain	Low/ reasonably certain	Low/ reasonably certain
Colonization potential	Low (or medium to high)[b]/very certain	Low (or medium to high)[b]/very certain	Low (or medium to high)[b]/very certain	Low (or medium to high)[b]/very certain	Low (or medium to high)[b]/very certain
Spread potential	Low/relatively uncertain to high/very uncertain[c]	Low/relatively uncertain to high/very uncertain[c]	Low/relatively uncertain to high/very uncertain[c]	Low/relatively uncertain to high/very uncertain[c]	Low/relatively uncertain to high/very uncertain[c]
Overall probability of establishment	Low to high	Low	Low to medium	Low	Low
Consequences of establishment	Low to medium/very uncertain	Low to medium/very uncertain	Low to medium/very uncertain	Low to medium/very uncertain	Low to medium/very uncertain
Overall risk estimate	Low to high	Low to medium	Low to medium	Low to medium	Low to medium

[a]High if pond is infected and shrimp escape from pond; low otherwise.

[b]Some breakout group members believed that the potential was medium and would be high if the aquaculture industry expands significantly along the Gulf Coast.

[c]The breakout group could not reach consensus; opinions on entry potential ranged from low to high.

Table 3. Summary of shrimp processing breakout group risk rankings

Refer to supporting discussion in the text to properly evaluate information presented in this table. The risk assessment process is described in Section 3.1 and Appendix G.

		Pathways to the environment			
Probability of establishment		**Treated effluent**	**Untreated effluent**	**Landfill**	**Shrimp/fish feeds**
Association with pathway		High/ very certain	High/ very certain	High/ very certain	High/very certain
Entry potential		Low/very certain	High/very certain	Medium/ reasonably certain	Low/ very certain
Colonization potential		Low/ very certain	Medium/ moderately certain	Low/ reasonably uncertain	Low/ very certain
Spread potential		Low/ very certain	Medium/ moderately certain	Low/ reasonably uncertain	Low/ very certain
Overall probability of establishment		**Low**	**Medium**	**Low**	**Low**
Consequences of establishment	**Local**	Low-medium/ reasonably uncertain	Low-medium/ reasonably uncertain	Low-medium/ reasonably uncertain	Low-medium/ reasonably uncertain
	Large scale	Low/highly uncertain	Low/highly uncertain	Low/highly uncertain	Low/highly uncertain
Overall risk estimate	**Local**	Low-medium	Medium	Low-medium	Low-medium
	Large scale	Low	Medium	Low	Low

Table 4. Summary of other pathways breakout group risk rankings for likely pathways to the environment

Refer to supporting discussion in the text to properly evaluate information presented in this table. The risk assessment process is described in Section 3.1 and Appendix G.

Probability of establishment	**Ballast water**	**Bait shrimp**		**Shrimp feed**		**Animal vectors**
		Foreign	**Domestic**	**No heat**	**Heat-treated**	
Association with pathway	High/moderately certain	High/ moderately certain	Low/very certain	Medium/ moderately certain	Medium/ moderately certain	High/very or reasonably certain[a]
Entry potential	High/very certain	High/very certain	High/very certain	High/very certain	Low/very certain	High/reasonably certain
Colonization potential	Low/moderately certain	High/very uncertain	High/very uncertain	Medium/very uncertain	Medium/very uncertain	Medium to high/ relatively uncertain
Spread potential	Medium/very uncertain	Medium/very uncertain	Medium/very uncertain	Medium/very uncertain	Medium/very uncertain	Medium/very uncertain
Overall probability of establishment	Low	Medium	Low	Medium	Low	Medium

[a]Very certain for gulls and freshwater and marine invertebrates; reasonably certain for other vertebrates.

Table 5. Summary of other pathways breakout group risk rankings for secondary or incidental pathways to the environment

Refer to supporting discussion in the text to properly evaluate information presented in this table. These pathways were rated individually by breakout group members, and there was no group discussion of these ratings. Consequences of establishment were not rated for these pathways. The risk assessment process is described in Section 3.1 and Appendix G.

Probability of establishment	**Natural spread**	**Research and display facilities**	**Human sewage**	**Fishing vessels**	**Hobby and ornamental displays**	**Live seafood distribution**	**Other crustacean aquaculture**	**Incidental introductions**
Association with pathway	Medium/very uncertain	Low/ moderately certain to high/very certain	Medium/ very uncertain	Low to medium/ moderately certain	Low/ moderately certain	Low/ reasonably uncertain	Low/very uncertain to medium/ moderately certain	Low/very uncertain
Entry potential	High/very uncertain	High/ moderately to very certain	Medium/ very uncertain	High/ reasonably certain	High/ moderately certain	High/ moderately certain	Low/very uncertain to medium/ reasonably certain	Low/very uncertain
Colonization potential	High/very uncertain	Low/very certain to high/very uncertain	Medium/ very uncertain	Medium/ reasonably uncertain	Low/ moderately certain	Low/ reasonably uncertain	Low/very uncertain to medium/very uncertain	Low/very uncertain
Spread potential	High/very uncertain	Low/ relatively certain to high/very uncertain	Medium/ very uncertain	Medium/ very uncertain	Medium/very uncertain	Medium/very uncertain	Low/very uncertain to medium/very uncertain	Low/very uncertain
Overall probability of establishment	Medium	Low to medium	Medium	Low to medium	Low	Low	Low to medium	Low

The JSA report (1997) identifies two assessment endpoints:

The JSA report (1997) identifies two assessment endpoints:

- Survival, growth, and reproduction of wild penaeid shrimp populations in the Gulf of Mexico and southeastern U.S. Atlantic coastal waters
- Ecological structure and function of coastal and near-shore marine communities as they affect wild penaeid shrimp populations

Workshop participants elected to focus their efforts on the first assessment endpoint (direct effects to wild shrimp populations) for the following reasons:

- Risks to wild shrimp populations are of primary concern
- Information on secondary effects is even more limited than information on direct effects on shrimp
- There was limited time available at the workshop for evaluating all possible direct and indirect effects.

Participants recognized the potential for direct effects on organisms other than penaeid shrimp and the potential for indirect effects; however, these effects were not discussed in detail during the workshop. They are, however, a potential concern for resource managers.

3.2.2. Probability of Establishment

This section summarizes breakout group discussions concerning the elements of the probability of establishment, which include association with pathway, entry potential, colonization (infection) potential, and spread potential.

Workshop participants recognized that differences among the four viruses could result in variations in the risk rankings associated with the elements comprising the probability of establishment for an individual virus. For example, if one virus were to survive longer than another virus in the marine environment, it could affect the entry potential ranking. However, the breakout groups decided to consider the potential for establishment of nonindigenous viruses as a single group but agreed to identify any unique differences that might alter risk rankings. A summary of the characteristics of the four viruses is contained in Table 6.

Table 6. Virus persistence, virulence, and infectivity

	IHHNV	TSV	YHV	WSSV
Persistence (1 = least, 4 = most)	**3.5**	**3.5**	**1.5**	**1.5**
Virulence to Gulf of Mexico species (1 = least, 4 = most)	1	2	3	4
	Relative infectivity			
Penaeus setiferus				
Larvae	—	—	ND	ND
Post-larvae	—	++	—	++
Juvenile	+	+	++	++
Adult	ND	+	ND	ND
Penaeus duorarum				
Larvae	—	—	ND	ND
Post-larvae	—	—	—	++
Juvenile	+	+	++	+
Adult	ND	ND	ND	ND
Penaeus aztecus				
Larvae	—	—	ND	ND
Post-larvae	—	+	—	++
Juvenile	+	+	++	+
Adult	ND	ND	ND	ND

Infectivity:

ND = No data
+ = Infectious
++ = Mortality
— = Tried but negative

3.2.2.1. *Association With the Pathway*

Breakout groups concluded with moderate to high certainty that there is a high likelihood that viruses are present in the aquaculture pathway, shrimp processing pathway, and some of the other potential pathways.

3.2.2.1.1. ***Aquaculture.*** The occurrence of nonindigenous viruses in U.S. aquaculture operations is well documented. As summarized in the JSA report, TSV has been identified in disease outbreaks in Hawaii, Texas, and South Carolina (Lightner, 1996a,b). IHHNV was first identified in Hawaii (Lightner et al., 1983a,b) and was subsequently observed in farms in South Carolina, Texas, and Florida (Fulks and Main, 1992). WSSV and YHV also have been documented at a shrimp farm in Texas (Lightner, 1996a,b). WSSV and YHV are considered to be of Asian origin; TSV and IHHNV are thought to have originated in Latin America. Workshop participants noted that the origins of these viruses are not always traceable to their ultimate sources, but it was suggested that their introduction to the United States may have resulted from the importation of infected shrimp from other regions of the world (e.g., Latin America and Asia).

3.2.2.1.2. ***Shrimp processing.*** Shrimp viruses can be brought into the United States with imported shrimp that are subsequently processed or used for other purposes (e.g., feed, bait shrimp, and retail sale). Of the shrimp processed in the United States, 80% of the total crop is foreign and 20% is domestic in origin. Pathogenic viruses have been identified in imported shrimp sold in this country. Breakout group members concluded with high certainty that the probability of association is high for all pathways considered (effluent, landfill, and shrimp and fish feed).

3.2.2.1.3. ***Other pathways.*** Other "primary" pathways described in the JSA report and considered by workshop participants include ballast water, bait shrimp, animal vectors, and shrimp feed. There appear to be no data on the occurrence of shrimp viruses in ballast water (or any of its components). Nonetheless, it is known that many organisms are discharged routinely with ballast water (including species of mysid shrimp, some of which have colonized bays and estuaries with devastating effects). There is, therefore, a high probability that ballast water could contain shrimp viruses, whether free living, attached to particulate matter, or in dead or infected shrimp.

Anglers use shrimp as bait when fishing in estuaries for fish that eat shrimp. They purchase bait from bait shops, or they use shrimp sold in grocery stores for human consumption. Bait shrimp generally are smaller than those sold for human consumption. They may originate from aquaculture facilities that have harvested their shrimp prior to full growout because of a viral outbreak. Some participants thought that Latin American and Asian producers may freeze these small shrimp and ship them to the United States for sale as bait, while the larger, uninfected shrimp will be sold at premium prices for human consumption. Therefore, there is a high probability that some imported bait shrimp may contain viruses.

Both live and frozen shrimp may be sold as bait. However, only native species of aquaculture shrimp may be harvested and sold as live bait. Some states (e.g., South Carolina) allow the use of nonnative farm shrimp as frozen bait. Native shrimp used in aquaculture are known to sometimes carry indigenous viruses (such as baculovirus, BP) but to date, there is no evidence that they carry nonindigenous viruses. Furthermore, any of these shrimp that are harvested early due to perceived disease problems are likely to be sold as frozen bait rather than as fresh bait. Therefore, there is a low probability that live shrimp used for bait will carry nonindigenous viruses.

Shrimp feed is made from soy protein, fish protein (including anchovies and menhaden), shrimp heads, and other types of shrimp and crustaceans (e.g., *Artemia*). Because the heads and other body parts of infected shrimp can carry a high concentration of viruses, workshop participants believed that there is a medium probability that the shrimp parts used as an ingredient in shrimp feed may be contaminated with the viruses. Although pathogenic nonindigenous viruses may be associated with this pathway, workshop participants concluded that the viruses are likely to be destroyed during processing of the shrimp feed (see Section 3.2.2.2).

Animal vectors such as gulls and freshwater and marine invertebrates were considered as another possible source for viral entry. For example, gulls and other scavengers, such as raccoons, are often seen feeding on dead shrimp and other organic matter associated with aquaculture facilities that have undergone viral outbreaks. Workshop participants believed there was a high probability of viral association with this pathway.

Workshop participants considered a number of other pathways to have a low to medium probability for viral association. Due to time limitations, these pathways, including natural spread of the viruses, research and display facilities, human sewage, fishing vessels, hobby and ornamental displays, live seafood distribution, other crustacean aquaculture, and incidental introductions, could not be discussed in detail at the workshop.

3.2.2.2. *Entry Potential*

Entry potential includes the probability of viruses surviving in transit and the probability of their transport to coastal waters. Each breakout group recognized that the entry potential of nonindigenous viruses depends on the pathway of arrival. For example, the survival and entry characteristics of viruses found in shrimp processing effluents may be quite different from those found in ship ballast waters. In addition, the breakout groups recognized that entry potential depends on location. For example, viruses associated with shrimp that are raised, processed, or disposed of in locations far inland are less likely to reach coastal waters than are viruses that are associated with shrimp that are raised, processed, or disposed of along the coast. Workshop participants evaluated subpathways within each of the major pathway categories (aquaculture, shrimp processing, and other source pathways) and described entry potentials for viruses as ranging from low to high. Participants found the level of certainty associated with these evaluations to be quite variable.

3.2.2.2.1. ***Aquaculture.*** The aquaculture breakout group considered the six subpathways from aquaculture to wild shrimp stocks identified in the conceptual model contained in the JSA report. Many breakout group members believed that the escapement subpathway (including both accidental and intentional releases, as well as "escape" via transport of shrimp tissue by the predatory activities of other animals) was the most likely route of release of viruses to the environment and that viruses were likely to survive when transported via this pathway. (As discussed in the following paragraphs, however, some breakout group members believed that the sediment and effluent pathways, which the group tabled because of a lack of crucial data, may also be important.)

The aquaculture breakout group noted that the entry potential via escapement (and other pathways) is likely to be related to the conditions in the pond (i.e., the presence and degree of infection by the viruses), the life stage of the shrimp (e.g., postlarvae may be more likely than adult shrimp to escape by passing through engineering controls), and the design of pond control systems. They concluded with relatively high certainty that the probability of surviving in transit would be high if conditions are favorable but assigned a low probability of survival if they are not.

The aquaculture breakout group had considerable discussion about the ability of viruses to survive in pond effluents and sediments. There is suggestive evidence about this potential pathway. TSV has been documented in water but not specifically in effluent waters. A workshop observer communicated results of an experiment that suggest that caged shrimp exposed in infected ponds developed disease (shrimp developed disease when exposed within 1 to 2 days to experimentally inoculated water, but they did not develop disease when exposed on days 3 to 5 following inoculation) (R. Laramore, personal communication, 1998). In 1995, HSF, Ltd., and the Arroyo Aquaculture Association conducted several trials in which cages were floated within a shrimp growout pond that had experienced a TSV epidemic and with pond water in tanks. The cages were suspended above the pond bottom and stocked with juvenile *P. vannamei*.

One participant noted that no TSV was detected in shrimp exposed for 30 days under these conditions. These results suggest that TSV may be transmitted during the acute but not the chronic stages of the disease. Other data suggest that IHHNV can survive in water in an infective state for at least 24 days (Glover et al., 1995). Another participant noted that viruses can spread quickly from pond to pond on aquaculture facilities, but it is not known how this transmission occurs. Based on this information, the aquaculture breakout group estimated that there is a medium potential that effluents released from infected farm ponds are a viable pathway for exposure to native populations; however, the breakout group was very uncertain about this estimate.

Pond flooding, sediment and solid waste disposal, and transport of shrimp to processing facilities were thought to have low likelihoods for entry potential, with uncertainties ranging from reasonably certain to very certain.

3.2.2.2.2. ***Shrimp processing.*** The shrimp processing breakout group identified two subpathways for which there is a medium to high potential for viruses to enter coastal areas: untreated effluents from shrimp processing facilities and solid wastes from disposal facilities near coastal areas that receive waste from shrimp processing facilities. The breakout group concluded with high certainty that there is a low potential for viable shrimp viruses to survive in effluents that are treated and disinfected at municipal facilities, and therefore, there is a low potential for entry of viable shrimp viruses to coastal areas from this pathway.

The shrimp processing breakout group estimated that approximately 50% of shrimp processing liquid effluent is untreated and that virus-contaminated discharges may therefore be released regularly into the environment. The breakout group was very certain that the probability of the organism surviving in transit—and therefore entering the environment through this pathway—is high.

Because of the uncertainties associated with the amounts of material reaching landfills, the types of vectors, and the threshold amount of virus required to infect the wild and aquaculture populations, the shrimp processing breakout group found it difficult to assess the probability of establishment of shrimp viruses from solid waste disposal facilities. Most breakout group members generally agreed that the shells, and particularly the heads, of foreign farmed and wild shrimp are highly likely to contain viruses. Considering these factors, breakout group participants concluded that these viruses are likely to persist for some time in landfill settings. Land crabs and seagulls are thought to be possible vectors for moving viruses from the landfills to estuarine waters. When these animals consume virus-contaminated materials, viruses might pass through their digestive systems in an infective state. The breakout group noted that TSV remains infective following gut passage in gulls, and the breakout group on other pathways suggested several other possible vectors for viral transmission. It is not known whether the concentrations and frequency of virus introduction from such vectors is sufficient to infect wild and aquaculture shrimp populations. The shrimp processing breakout group was reasonably certain that there is a medium probability of entry potential from coastal landfills to estuaries.

3.2.2.2.3. ***Other pathways.*** The other pathways breakout group found that the entry potential of viruses in ballast water, bait shrimp, and animal vectors is high. The group determined that while it is not likely that the freezing process used for bait shrimp will significantly reduce the virulence and infectivity of the virus, the effects of freezing may be virus specific. For shrimp feed, breakout group participants concluded that the probability of survival in transit depends on whether or not the feed meal is heat treated to temperatures sufficient to inactivate all viruses. It is thought that some of the viruses (e.g., TSV) may survive and maintain infectivity, even when heated to temperatures greater than 100 °C. While most of the fish meal produced in the United States is subjected to temperatures that appear to be sufficient to kill the viruses, breakout group members were unable to provide published data that would confirm this supposition. Moreover, several participants believed that other countries do not always heat-treat their meals, which would increase the potential for viable viruses to be present in the feed. The other pathways breakout group concluded that the transit survival probability is low for heat-treated feed and high for untreated feed. In contrast, the shrimp processing breakout group was very certain that feed was processed at temperatures sufficient to inactivate the viruses. Additional research will be necessary to resolve this issue.

3.2.2.3. *Colonization Potential*

Workshop participants agreed that the potential for viruses to colonize coastal areas is one of the most critical aspects of evaluating the potential for establishment. Workshop participants concluded that there is a high potential for viruses to be associated with many of the pathways identified in this report, but a low to high potential that these pathways could lead to introduction of viruses. The breakout groups were certain about association of viruses with these pathways and their entry potential through the pathway; however, they had a high degree of uncertainty about colonization potential.

3.2.2.3.1. ***Aquaculture.*** Many members of the aquaculture breakout group concluded that colonization potential was low (very uncertain). The rating of low was based on a lack of evidence that viruses had become established in wild shrimp populations in the United States as a result of aquaculture. Breakout group participants noted that colonization potential is likely virus specific and dependent on shrimp species and specific life stage. However, some breakout group members ranked colonization potential as medium, particularly for pond effluents, noting that these could provide continuous potential input of virus in coastal systems and as high if aquaculture expands further along the Gulf coast.

3.2.2.3.2. ***Shrimp processing.*** Colonization potential varied with pathway. Breakout group members considered colonization potential low for treated effluent, and they were very certain of this because they believed the disinfection processes would kill viruses. Colonization potential was also considered low for solid waste in landfills, but here there was reasonable uncertainty because of the absence of virus to shrimp dose-response data and the uncertainties associated with frequency and concentration of viruses associated with vectors at landfills. Shrimp and fish feeds were considered to have low colonization potential with high certainty because high temperatures used in processing feed were believed sufficient to kill pathogenic viruses. Colonization potential was judged to be medium for untreated effluent with medium uncertainty, because persistence, infectivity, and virulence of viruses in receiving waters will vary depending on numerous factors.

3.2.2.3.3. ***Other pathways.*** Colonization potential ranged from low to high depending on the pathway. For ballast water, colonization potential was considered low (moderately certain). On the basis of experience with other organisms, few organisms introduced into new environments survive to colonize. For penaeid shrimp and viruses, colonization depends on the point of discharge (e.g., nearshore vs. open ocean) and a number of other factors such as transmission and infectivity that are poorly understood outside of laboratory or aquaculture situations. For shrimp/fish food, colonization potential was thought to be medium (very uncertain). Introduction could occur via food used in aquaculture or chumming. The potential for viruses to colonize as a result of introduction from animal vectors was thought to be medium to high (relatively uncertain). Shrimp are likely to feed on bird feces that may contain viruses. Likelihood would increase in areas of high vector density (e.g., many seagulls where a shrimp die-off has occurred in an aquaculture pond). Finally, breakout group members thought that colonization potential from infected bait shrimp would be high (very uncertain) because bait shrimp are deposited in areas where native shrimp are known to occur.

In general, breakout groups believed that, for most subpathways, there is either a low or medium likelihood that, once introduced, viruses would be able to colonize native shrimp at a local level (i.e., within specific estuaries or embayments). The exceptions were high likelihoods of colonization noted for bait shrimp and, in the view of a few, aquaculture. In support of their conclusions, the breakout groups identified the following factors:

- Colonization potential is likely to be related to the magnitude of the source and the frequency of introductions. Therefore, large, frequent sources may have a greater likelihood of colonization than small, intermittent sources.

- Colonization potential is likely to be related to the medium in which the viruses are introduced. For example, viruses introduced within live or dead shrimp are thought to have a greater likelihood of colonization than are viruses introduced via water.

- There is no clear evidence to suggest that colonization has occurred in wild shrimp populations, despite a history of outbreaks in aquaculture operations, the presence of shrimp processing operations, discharges of ballast water, and the use of bait shrimp. (Although recent evidence suggests that WSSV-like viruses found in wild shrimp populations in South Carolina coastal waters may not differ from Asian isolates of the virus [Lo et al., in press], the significance of this observation is unclear.)

3.2.2.4. *Spread Potential*

The breakout groups viewed the potential spread of viruses beyond the initial locus of colonization as an area of great uncertainty. The aquaculture breakout group did not reach consensus on spread potential; estimates ranged from low (relatively uncertain) to high (very certain). Although viral diseases can spread rapidly between aquaculture ponds, participants recognized the difficulty in extrapolating from the spread of disease in aquaculture farms to that in wild populations. Factors such as population density and the time course of the disease may be important. For shrimp processing, spread potentials were judged to be low for both treated effluent (very certain, due to destruction of viruses by disinfection) and shrimp and fish feeds (very certain, because participants believed that high temperatures used in processing feed would kill pathogenic viruses). Spread potential for untreated effluent was considered medium (moderate uncertainty; persistence, infectivity, and virulence of viruses in receiving waters are sources of uncertainty and will vary depending on numerous factors). The spread potential for solid waste in landfills was considered high (reasonably certain). The other pathways breakout group believed that colonization potential for the four main pathways it considered was medium (very uncertain). During plenary discussion of the reports from the individual breakout groups, workshop participants generally believed that there is a medium probability that viruses could spread beyond the initial locations of colonization.

Breakout groups identified a number of factors significant to evaluating the potential spread of introduced viruses, such as the degree of interaction that would occur among individual wild shrimp and the spatial scale over which shrimp might "mix." Stocks of *P. setiferus* in the southeast Atlantic are thought to be fairly genetically homogeneous, as are the northern and southern populations in the Gulf of Mexico. Workshop participants believed that this suggests the potential for substantial interaction over broad geographic regions, which would promote the spread of viral infection. However, genetic homogeneity may not be the case for other penaeid species. The potential for spread also depends in large part on the time course of the disease, as well as the density of shrimp in wild populations. Breakout group members determined that low shrimp densities are likely to hinder disease spread, whereas high densities are likely to promote transmission. Spread potential is also host dependent and virus specific. It was noted that TSV and IHHNV have low spread potential, and the spread potential of YHV and WSSV is currently unknown. A WSSV-like virus has been found in a variety of crustaceans in southeastern Atlantic waters, but it is unknown at this time if it is the same as the Asian strain of WSSV. (Recent evidence suggests, however, that WSSV-like viruses found in wild shrimp populations in South Carolina coastal waters may not differ from Asian isolates of the virus [Lo et al., in press]). This evidence suggests a potential for colonization and spread, but it is unclear whether the WSSV-like viruses are indigenous, or if nonindigenous, when they may have been introduced. Finally, as noted in the JSA report, the presence of other stressors (e.g., low dissolved oxygen and extreme salinity) is also likely to influence the potential for spread of the disease.

3.2.3. Consequences of Establishment

In continuing to assess the risks to wild populations of shrimp viruses, the breakout groups evaluated the potential ecological effects associated with the establishment of pathogenic shrimp viruses. The breakout groups approached this step of the qualitative risk assessment process by considering the available information on the direct effects of viruses on shrimp. Breakout groups also examined possibly analogous situations based on experience with other diseases and invertebrates. Breakout groups discussed possible effects on ecological structure and function but, due to the limited time available, gave primary attention to direct effects on wild shrimp populations. In the absence of documented information or firsthand knowledge, experts relied primarily on professional judgment to evaluate the consequences of establishment. The breakout groups concluded that there is a high degree of uncertainty in assessing the consequences of establishment.

3.2.3.1. *Direct Consequences to Shrimp Populations*

In considering the possible consequences of shrimp viruses to shrimp populations at the local level and at the scale of the entire populations or stock, breakout groups evaluated three types of effects:

- Mortality of the infected animal
- Reduction in reproductive rates
- Alteration of the genetic structure of the population

3.2.3.1.1. ***Mortality effects.*** Breakout group experts concluded that the direct consequences of the establishment range from low to medium and that effects on the mortality of shrimp are more likely to occur at the local level than at the scale of the entire population or stock. The breakout groups determined that the probability of colonization at a local level is greater than the probability that viruses would spread beyond the local level to a regional population. It is thought that WSSV and YHV are more likely than IHHNV or TSV to cause acute mortality but that IHHNV and TSV are more likely to become endemic following introduction.

3.2.3.1.2. ***Reproductive effects.*** Breakout group experts focused primarily on factors that would affect reproductive output or recruitment. Experts were aware of no information describing adverse viral effects on the reproductive potential of infected individuals (indicating a potentially important data gap). One participant noted that reproductive output of infected *P. vannamei* brood stock appears to be unaffected by viral infection. However, in contrast to the previous statement, individual growth impairment in offspring of *P. vannamei* infected with IHHNV has been documented (Fulks and Main, 1992). Assuming that fecundity of female *Penaeus* is an increasing function of size (a phenomenon common in other invertebrate species), workshop participants considered that stunted growth of offspring could result in reduced reproductive output of the second generation. Individual growth impacts could therefore cause population-level effects, although an analysis of any changes in reproduction on shrimp population dynamics would be required to support this assertion. Workshop participants noted that epidemiologic models show that in "r-selected" species, effects on reproduction can have greater effects on population size than mortality effects. (Penaeid shrimp can be characterized as "r-selected" organisms because they display an annual life history pattern with high reproductive output and high mortality during early life stages.)

3.2.3.1.3. ***Effects on genetic structure and fitness.*** Breakout group participants discussed the potential effects of virus colonization on the genetic structure and fitness of wild shrimp populations. One breakout group thought that rapid reductions in population abundance resulting from viral disease could have unknown but potentially important effects on genetic structure by limiting genetic variability (the "founder effect"). One participant cited evidence from Thailand indicating that shrimp populations in the south of Thailand are much less genetically diverse than those from the northern part of the country. It has been hypothesized that this is due to the release of shrimp from aquaculture into the wild. One breakout group discussed the importance of understanding whether genetic resistance to viruses differs among populations. Further knowledge of genetic variability among Gulf Coast shrimp is necessary to make accurate predictions about which area has the highest potential for an epizootic.

3.2.3.1.4. ***Other information.*** Other information or lines of evidence that affected the experts' professional judgments about the potential consequences of establishment are summarized below:

- Penaeid shrimp can be characterized as "r-selected" organisms because they display an annual life history pattern with high reproductive output and high mortality during early life stages. Thus, penaeid shrimp populations that suffer population reductions in one year can exhibit rapid recovery, and this may reduce the long-term consequences of short-term impacts. In reviewing available information, the breakout group concluded that mass mortalities of adult shrimp may have relatively short-term impacts on standing shrimp stocks. For example, some natural stressors on shrimp (e.g., cold temperatures or freshwater flooding) are known to cause short-term reductions in populations at the local level. Because of high fecundity and migratory behavior, *P. setiferus* is capable of rebounding from a very low population size in one year to a large number in the next, if environmental conditions are favorable. This has been observed off the South Carolina coast several times in the past 50 years (Linder and Anderson, 1956; McKenzie, 1981). In another case, an increase in reproductive output of the Honduran population of *P. vannamei* was reported during a 1994 TSV outbreak. This provides anecdotal support for the concept that demographic compensatory responses may occur in disease-depleted populations, although it was noted that the population changes could have been caused by other factors (Laramore, 1997).

- Along with anecdotal information about the possible long-term effects of viral infections in Latin American and Asian shrimp populations, observations by some workshop participants indicated that direct mortality effects could be relatively transitory. Also, based on the observation that resistance to IHHNV appears to have increased in all populations tested since the identification of this virus in Hawaiian stocks, it was suggested that initial outbreaks could lead to enhanced resistance to future viral infection.

  It should be noted, however, that some workshop participants were concerned that the ability of viral pathogens to persist at low levels in a population could result in long-term adverse population effects. For example, participants noted the purported virus-induced declines in the population abundances of *P. stylirostris* in the Gulf of California began in 1987 and lasted 6 to 7 years, with stocks now reported to have returned to preoutbreak levels. (The role of IHHNV as the cause of the initial population decline has been the subject of much debate, however.)

- Based on observations from aquaculture situations, it appears that local colonization of shrimp viruses could result in local mortalities of shrimp. For example, TSV and others viruses are known to cause mass mortality on shrimp farms. Experiments with these viruses have documented mortality rates of up to 100%. One participant noted that in South Carolina, survival on commercial farms affected by TSV dropped from 63% in 1995 (the year prior to the TSV outbreak) to 19% in 1996 (the year of the TSV outbreak).

- Lines of evidence from other crustacean species indicate an association between an introduced biological agent and subsequent environmental impacts. For example, a crayfish species introduced from California to Europe may likely have served as a carrier to spread the freshwater crayfish plague throughout Scandinavia (Unestam and Weiss, 1970). Unlike short-term natural stressors (e.g., changes in temperature or salinity), an introduced disease organism (biological stressor) is likely to persist in the population.

- No empirical data exist to indicate that historical releases of shrimp virus to the Gulf of Mexico or to southeast Atlantic coastal waters have resulted in population-level impacts. However, no well-designed studies have been conducted to examine the epidemiologic conditions within these waters.

3.2.3.2. *Effects on Ecological Structure and Function*

Workshop participants observed that the introduction of nonindigenous shrimp viruses could affect ecological conditions apart from any direct effects on shrimp. Because these indirect effects were not a focus of this workshop, experts made only a limited attempt to characterize these consequences. Despite these limitations, some of the discussion related to this topic may be helpful to risk managers and is included in this report.

The aquaculture breakout group discussed instances in which other invertebrate species have experienced severe disease consequences. Participants viewed these examples as relevant to the effects of nonindigenous pathogenic viruses on shrimp:

- The near decimation of oysters (*Crassostrea virginica*) by the protozoan pathogens *Haplosporidium nelsoni* and *Perkinsus marinus*, called MSX and dermo disease, respectively (Haskin and Andrews, 1988; Andrews, 1996; Burreson and Ragone-Calvo, 1996), has resulted in significant changes in the oyster reef habitat throughout Chesapeake Bay and dramatically reduced the rate at which bay water was filtered by feeding bivalves (Kennedy, 1996).

- Insect/virus associations were described in which high abundances of the host species promote rapid outbreaks of viral disease, followed by dramatic declines in the host, near disappearance of the virus, and reestablishment of the host.

- The introduction into Scandinavia of North American crayfish that were carriers of the freshwater crayfish plague *Aphanomyces astaci* (Unestam and Weiss, 1970) had significant consequences.

Workshop participants believed that, in the absence of data on nonindigenous shrimp viruses in the wild, these and similar examples could serve as models for extrapolating potential consequences of viral establishment for shrimp populations. These examples may also serve as models for how ecological systems might be affected by viral outbreaks in shrimp. Either application would require careful analysis to identify similarities and differences relative to the shrimp virus situation.

The other pathways breakout group discussed the potential for viruses to affect estuarine ecology by infecting other species of shrimp, such as grass shrimp. Grass shrimp (*Paleomonetes sp.*) are an important part of the estuarine food web. Many species of fish (and penaeid shrimp) rely on this species as an important prey item. Data from Thailand suggest that grass shrimp may be carriers of one or more of these viruses, but data on infectivity rates and effects for Thai grass shrimp are lacking. On the other hand, it was noted that observations in South Carolina confirmed the presence of large populations of apparently healthy *Paleomonetes* in tidal areas near TSV-infected shrimp farms.

3.2.4. Risk Characterization

Using the ANSTF approach (ANSTF, 1996; Appendix G), workshop participants characterized the risk of viral introductions to wild penaeid shrimp populations by combining the probability of establishment of the virus with that of the presumed ecological consequences (see Section 3.1). Workshop participants assessed risks to local populations, which the experts generally defined as the population within a single estuary; they also considered the long-term effects on the entire population of native shrimp in the Gulf of Mexico and southeastern Atlantic coastal waters.

The risks estimated by the individual breakout groups are summarized in Tables 2 through 5. The discussion in this section is based on those risk estimates but emphasizes overall conclusions drawn during plenary workshop discussions among all breakout group participants.

3.2.4.1. *Risk to Local Populations*

Workshop participants concluded that the probability of establishment of shrimp viruses in a local estuary ranges from low to medium. The probability of establishment depends primarily on the colonization potential of the particular viruses. However, the probability of establishment could become much greater if virus is introduced repeatedly to the estuary over a long period. Workshop participants generally believed that the impact of such an establishment on the local shrimp population might involve high initial kill rates followed by rapid recovery due to reintroduction of shrimp from other locations. Therefore, workshop participants characterized the overall long-term risk of nonindigenous pathogenic virus introductions to the shrimp populations in a local estuary as generally low to medium. (The possibility of longer-term effects is suggested and discussed in Section 3.2.3.1).

Although workshop participants had very little time to consider the risks posed by nonindigenous shrimp viruses to other components of the estuarine ecosystem, many believed the level of risk to be medium, although uncertainty surrounding this risk estimate is very high. Of particular concern to participants was co-infection of important food web species, such as grass shrimp and crayfish. Because both penaeid shrimp and grass shrimp are important food sources for many other estuarine organisms, participants noted that the loss of this food base could have significant effects on other species. Following an initial viral kill of shrimp, fish or wildlife populations that depend on shrimp and other crustaceans as prey sources may take longer to recover than shrimp populations.

Participants raised concerns about the lack of information on the transmissibility of disease from one estuary to another through migration of diseased or infected shrimp. Participants thought that survivors of a local epizootic could move out to sea to reproduce, possibly infect other shrimp and offspring, and then move into adjacent or nearby estuaries. Such an event would expand what appears to be a localized risk into large-scale risk; however, each breakout group that evaluated the potential for spread by natural processes rated the probability of this occurrence as low. Therefore, the risk of a local infection having large-scale consequences is characterized as medium.

3.2.4.2. *Large-Scale Risk*

Workshop participants characterized the risk from viral introductions to the entire population of native shrimp along the southeastern Atlantic coast and within the Gulf of Mexico using the same analysis of the establishment pathways combined with that of the potential consequences of establishment on a large geographic scale. Workshop participants concluded that the consequences of virus introduction to the population as a whole would be relatively insignificant, and they characterized the risk as low.

Some participants expressed concern that the genetic structure of the population might be altered, and if viral resistance were linked with certain other important genes, overall fitness of the shrimp could be lowered. One participant noted that alterations to the genetic structure of the population could make the shrimp more susceptible to future infections and to simultaneous environmental stressors, such as weather changes or reduced estuarine salinity, thereby potentially increasing the risk potential. Furthermore, some participants stressed that uncertainty about the long-term ecological consequences of viral introduction will remain high until the effects of virus infection on reproduction can be determined.

3.2.4.3. *Summary*

Overall conclusions by workshop participants concerning the risks posed by nonindigenous pathogenic shrimp viruses may be summarized as follows:

- Based on information currently available, most workshop participants believed that the risk to native shrimp from introduction of nonindigenous viruses is low to medium, although uncertainty is high.

- Most participants agreed that local effects should be given a higher risk ranking than large-scale effects because local effects are more likely to occur.

- Participants suggested that the large amount of uncertainty associated with this risk characterization could be reduced through appropriate laboratory and field studies. The lack of evidence of conclusive viral impacts on worldwide shrimp populations does not derive from published systematic studies but rather is anecdotal.

Furthermore, by analogy, other marine invertebrates have experienced severe local impacts from exposure to pathogens (as has been noted in oyster populations in Chesapeake Bay). Also, viruses that have become established in terrestrial insect populations can cause cyclic epizootics and population crashes. Therefore, participants concluded that there is an urgent need to continue efforts to gather available data on shrimp virus effects and to conduct a systematic research effort that could be used to reduce the uncertainty of any subsequent risk assessments.

3.3. RISK MANAGEMENT RELEVANCE

Although this report does not recommend risk management actions, it contains information that may help risk managers with their decisions by:

- Providing insight into the pathways by which shrimp viruses could potentially enter and become established in the marine environment

- Identifying potential consequences to wild shrimp populations at local and stock levels

- Suggesting specific actions and studies that can reduce the uncertainties associated with evaluating the potential risks of shrimp viruses on wild shrimp populations

The ability to make quantitative estimates of the risks of viruses to wild populations of penaeid shrimp is constrained by the amount and type of information that is currently available. The majority of workshop participants believed that it is unlikely that the information required to complete a quantitative risk assessment will be available within the foreseeable future. At present, qualitative evaluations can be made.

The ability of workshop participants to address broader ecological risks in a comprehensive manner was limited by available information, but participants agreed that this important issue merits further consideration. Furthermore, while the topic of risks that nonindigenous pathogenic shrimp viruses pose to shrimp aquaculture operations was not part of the scope of the workshop, workshop participants agreed that these risks should be given special attention as part of another technical or management workshop.

4. ACTIONS FOR REDUCING UNCERTAINTY

The qualitative risk assessment conducted during the workshop revealed several critical sources of uncertainty. Further improvement in the ability to estimate risks to wild populations of shrimp will require reducing uncertainty in these key areas.

Workshop participants discussed the relative importance of actions for reducing uncertainty. Some participants stressed that, to reduce uncertainty, risk management actions need to occur in parallel with research, monitoring, and other actions. Most workshop participants generally believed that particular emphasis should be given to the following actions for reducing uncertainty:

- Improved diagnostic methods
- Surveys of wild shrimp populations for the presence of nonindigenous viruses and for genetic composition
- Experiments to reduce uncertainties surrounding virus transmission and virulence
- Field epidemiological studies

4.1. DIAGNOSTIC METHODS

Workshop participants determined that improvements to existing diagnostic methods and development of new diagnostic tools are very high priorities. Several participants noted that without adequate diagnostic methods, other risk assessment elements cannot be well studied or adequately evaluated. Other participants noted that many valuable diagnostic tools currently exist. Several key needs were identified during the workshop:

- There is a significant need to develop new diagnostic procedures. Some molecular probe applications and bioassay tests are available, although several workshop participants noted that the sensitivity of existing bioassay tests needs to be improved. One participant also cited the need to develop cell culture tests for crustacea, noting that new technologies are available to assist in developing cell cultures, but money and lack of equipment have been major obstacles.

- Tests for infectivity are needed to establish the threshold number of viruses that would be required for colonization potential. At least two tests should be employed, such as a PCR and ELISA or a PCR and a bioassay.

- Current diagnostic applications are focused on detecting viruses in the animal itself. Although some preliminary efforts have been made to detect viruses in environmental media (e.g., to identify the presence of WSSV using water concentration techniques and PCR), techniques to detect viruses in effluent streams, sediment, and other environmental media need to be improved.

- There appears to be considerable variability among laboratories in the procedures for using available diagnostic tools. Procedures for using diagnostic tools should be standardized so that both the credibility and limitations of diagnostic tools can be established.

4.2. SURVEYS OF WILD SHRIMP POPULATIONS

Participants identified the need to survey native shrimp populations to develop baseline information on viruses in wild stocks. It was noted that some monitoring activity has been conducted in the coastal waters of South Carolina and Texas. Participants generally believed that it was important to proceed with field surveys despite the current limitations of diagnostic methods. Participants suggested that because of these limitations, current survey efforts should include the archiving of samples to be evaluated pending development of improved diagnostics.

Workshop participants noted that monitoring surveys should include genetic characterization of wild populations. To date, only limited studies have been conducted. (In one study that is under way, molecular techniques are being used to determine the degree of genetic variability between populations of *P. setiferus* in the Gulf of Mexico and the U.S. southeastern Atlantic coastal region.) Participants suggested that surveys should be focused both in areas that may have experienced the release of nonindigenous viruses and areas where it is unlikely that prior release has occurred.

4.3. EPIDEMIOLOGY OF SHRIMP VIRUS TRANSMISSION

Workshop participants identified a need for well-designed experiments to improve understanding of the pathogenicity of viruses in native shrimp. In particular, studies are needed on virulence, distribution in various shrimp tissues, and rates of transmission, susceptibility, and recovery. Some suggested that laboratory experiments would be hindered by inadequacies in current techniques to identify pathogens and by the absence of diagnostic methods specific to identifying viruses in various environmental media. Given existing techniques for quantifying the amount of virus present, participants noted that currently it is most feasible to conduct qualitative transmission studies in which the amount of virus is estimated on a relative basis.

In other discussions, participants identified the need to understand not only mortality effects but also the consequences of infection on shrimp reproduction and growth. It is recognized that there are significant differences in viral pathogenesis among the four different viruses and the relative ability of the viruses to affect mortality, growth, and reproduction.

Participants also identified the need to develop a better understanding of the transmission of viruses from one species to another (i.e., between penaeid species and between penaeid and nonpenaeid species).

One participant stated that the most important reason to improve understanding of the epidemiology of shrimp viruses is to help identify mitigation measures (e.g., for aquaculture as a pathway).

4.4. FIELD EPIDEMIOLOGIC STUDIES

In addition to laboratory-based experiments, most participants believed that a parallel effort involving field epidemiology could yield information helpful for understanding the prevalence and potential effects of viruses in wild shrimp populations. Field epidemiologic studies may not provide the same level of understanding of detailed mechanisms as would laboratory experiments.

Participants suggested that field epidemiologic studies could make use of existing information from Latin America and Southeast Asia. Information would be sought on:

- The extent to which native shrimp populations in these areas may have been exposed to viruses
- The presence of viruses within these populations
- The observed effects (or lack thereof) of viruses on shrimp abundance and recruitment
- Possible ecological effects

Others suggested that the known locations of shrimp virus prevalence around the world should be documented and mapped so that potential sources can be identified.

4.5. LOWER PRIORITY RISK-RELEVANT RESEARCH AREAS

Workshop participants identified other areas, in addition to the four priority areas listed previously, where additional research is needed to improve the ability to estimate risks to wild shrimp populations.

4.5.1. Viral Persistence

Some participants noted the need to develop better techniques and to conduct experiments to evaluate the persistence of viruses in effluent streams, sediment, and other environmental media. It was noted that experiments should couple viral persistence with viral infectivity. For example, participants noted that IHHNV can be detected in sediments for 24 days; however, the duration of infectivity is unknown.

4.5.2. Compensatory Mechanisms

Participants believed that it is important to develop a better understanding of the compensatory mechanisms of native shrimp species in response to viral disease outbreaks. Research is needed to:

- Understand genetics and disease resistance (i.e., the need to improve understanding of the relationship between population genetics and the identification of disease-resistant phenotypes and how particular phenotypes develop resistance to a particular virus).
- Determine whether shrimp populations compensate for increased mortality with increased reproduction.
- Compile information on the shrimp immune-like response to viral infection. It was noted that coupling our understanding of target-organ sensitivity with information about resistance will improve the ability to predict which shrimp are likely to become carriers.

4.5.3. Monitoring of Imported Shrimp

Participants identified the need to monitor virus levels in imported shrimp using tests such as PCR and bioassay. Some experts suggested that, in terms of risk reduction, monitoring imported shrimp should be a higher priority than monitoring wild shrimp populations because of the high volume of imported shrimp.

4.5.4. Development of Suitable Population Models

Suitable population models are needed to evaluate the consequences of various virus-induced mortality or reproductive impairment scenarios. Because of the commercial importance of shrimp, workshop participants believed that it is highly likely that population models exist for these species. Additionally, a large body of catch statistics could be subject to time series analysis in concert with known periods of virus outbreaks or other environmental stressors, such as storm events. These types of data may be available for foreign fisheries as well. By using population models, constants for infection and transmission rates, and transport and fate, a modeling framework could be created to examine specific hypotheses. Sensitivity analyses could then be performed to determine which parameters are most important and contribute the most uncertainty. Research could then be directed to reduce uncertainty.

4.5.5. Other Risk-Related Research Needs

Other risk-related research needs identified by workshop participants include:

- Procedures for disinfection and eradication of large-scale outbreaks in aquaculture settings
- Genetic and biochemical characterizations of the viruses
- Research to improve understanding of factors that exacerbate expression of viral disease under conditions of high densities and high nutrients found in aquaculture settings
- Targeted surveys of nonpenaeid species (e.g., grass shrimp, crayfish, and micro-crustacea) to determine if they are susceptible to, or carriers of, nonindigenous viruses

5. SUMMARY

This section provides a brief summary of the results of the workshop. Topics include the qualitative risk assessment process; the need for a future, more comprehensive risk assessment; risk-relevant research needs; and areas of additional concern.

5.1. QUALITATIVE RISK ASSESSMENT PROCESS

Workshop participants conducted a qualitative assessment of risks by considering the:

- Likelihood of viruses being present in the pathway
- Ability of the viruses to survive transit in the pathway
- Colonization potential of the viruses (in native shrimp)
- Spread potential of the virus within native shrimp populations
- Consequences of establishment

In general, workshop participants believed that viruses could be in pathways leading to coastal environments and that they could survive in these pathways. Participants concluded that there is some potential for viruses to colonize native shrimp in a localized area, such as an estuary or an embayment, near the point of entry into the marine system. Participants had widely divergent views on the potential for viruses to spread beyond the initial local area of colonization, and this divergence reflected the large uncertainty associated with this aspect of exposure. Participants considered the potential for localized colonization and subsequent spread to be a critical aspect of evaluating the potential establishment of viruses in native shrimp.

Workshop participants considered the consequences of virus establishment at a local level (e.g., within an individual estuary) as well as within the offshore stocks. Participants discussed the impact of such an establishment on the local shrimp population. Initial kill rates might be high, but the population would be likely to recover rapidly due to reintroduction of shrimp from other locales. Workshop participants characterized the risk from viral introductions to the entire population of native shrimp along the southeastern Atlantic coast and within the Gulf of Mexico as low. Concern was expressed that certain effects (e.g., effects on genetic structure of shrimp and on the ecological system) may be difficult to assess.

5.2. COMPREHENSIVE RISK ASSESSMENT NEEDS

Most workshop participants concluded that, given the current knowledge base, it is infeasible to conduct a more comprehensive, quantitative estimate of risk. Most participants believed that, at present, qualitative evaluations can be made, but these are accompanied by large uncertainties. Participants agreed that there is a need to continue efforts to gather available data on shrimp virus effects and to conduct a systematic research effort that could be used to reduce the uncertainty in any subsequent risk assessments.

5.3. RESEARCH NEEDS

Workshop participants identified a number of areas in which further research and information would improve the assessment of risks and the evaluation of current conditions, with particular emphasis on the following areas:

- **The improvement of existing and the development of new diagnostic methods for viruses in shrimp and environmental media.** These methods are essential for all research studies and monitoring programs and for determining if viruses are present in imported shrimp, cultures used for aquaculture, and other possible pathways.

- **Surveys of wild shrimp populations.** Baseline information on the presence of viruses in native shrimp populations would provide insight into the extent to which populations already carry viruses. Baseline information would also be useful for supporting epidemiologic studies. Baseline studies could proceed even though there are limitations with current diagnostic methods. Well-designed studies would be enhanced by including an examination of the genetic structure of the populations.

- **Epidemiology of shrimp virus transmission.** Workshop participants identified a need for well-designed experiments to improve understanding of the pathogenicity of viruses in native shrimp.

- **Field epidemiologic studies.** In addition to laboratory-based experiments, participants believed that a parallel effort involving field epidemiology could yield information helpful for understanding the prevalence and potential effects of viruses in wild shrimp populations.

5.4. ADDITIONAL AREAS OF CONCERN

Workshop participants identified the following areas of concern, in which additional efforts should be focused:

- **Management implications of shrimp viruses.** It was recommended that a risk management workshop be held, focusing on impacts to natural resources and on possible impacts on shrimp importation, processing, and aquaculture operations.

- **Risks of shrimp viruses to aquaculture operations.** Workshop participants also recommended that a separate workshop be held on this topic.

- **Risks of shrimp viruses to nonpenaeid species.** Because this workshop was limited to evaluating the direct effects of viruses on wild shrimp populations, participants recommended that additional effort be directed toward evaluating nonpenaeid shrimp species (e.g., grass shrimp) and other species (e.g., crabs, amphipods, and copepods) that could be impacted by viruses.

6. REFERENCES

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APPENDIX A

BREAKOUT GROUP REPORTS

APPENDIX A. BREAKOUT GROUP REPORTS

Workshop participants were organized into three groups, each of which was charged with evaluating the risks associated with one of the following categories of viral pathways:

- Aquaculture
- Shrimp processing
- Other potential pathways

Dr. Wayne Munns (EPA Office of Research and Development) led the aquaculture group, Dr. John Gentile (University of Miami) led the shrimp processing group, and Dr. Anne Fairbrother (Ecological Planning and Toxicology, Inc.) led the "Other Pathways" group. Prior to the workshop, participants were given their breakout group assignment (Appendix B) and provided premeeting materials for their consideration in preparing for the workshop (Appendix C). At the discretion of each breakout group chair, observers were provided an opportunity to participate in discussions during breakout group sessions.

The breakout groups applied an adaptation of the risk assessment procedure described in the Aquatic Nuisance Species Task Force (ANSTF) report (RAM, 1996; Appendix G) to evaluate the ecological risks associated with each identified viral pathway (see also Section 2.1). Each breakout group evaluated and ranked elements of both the potential for establishment of the viruses via the identified pathways and the potential ecological consequences of establishment, should it occur. Breakout groups also identified the level of uncertainty (ranging from very uncertain to very certain) associated with these rankings.

After the workshop, Dr. Munns prepared the report of the Aquaculture Breakout Group (Appendix A-1), Dr. Gentile prepared the report of the Shrimp Processing Breakout Group (Appendix A-2), and Dr. Fairbrother prepared the report of the "Other Pathways" Breakout Group (Appendix A-3). Workshop participants had a chance to review and comment on the breakout group reports prior to preparation of the final document.

A-1. Report of the Aquaculture Breakout Group

A.1.1 INTRODUCTION

This breakout group was charged with assessing the risk associated with introduction of nonindigenous virus to wild shrimp populations from the shrimp aquaculture pathway (see Figure A-1).

Prior to implementing the ANSTF process, the Aquaculture Breakout Group addressed two questions. First:

1. Should the evaluation consider the four primary viruses (IHHNV, TSV, WSSV, and YHV) separately or as a group?

The breakout group recognized that consideration of differences among the viruses and in their relationships with host penaeids could lead to different ratings of the elements comprising probability of establishment; however, given the time constraints for completing the risk assessment, the breakout group decided that the viruses would be considered as a group whenever possible, but unique differences would be identified that might contribute to distinctly different conclusions about elements of the probability of establishment.

The second question addressed by the group was:

2. Should the evaluation consider risks of viruses directly to aquaculture operations in addition to the two assessment endpoints identified in the JSA report?

In its initial deliberations, the breakout group noted that aquaculture operations have already experienced outbreaks of viral infection, some of which have been catastrophic. This suggests that, because of the obvious risks to aquaculture, a further assessment to estimate these risks is not necessary at the present time. The breakout group decided instead to recommend to risk managers that action is needed to minimize risks to aquaculture from future outbreaks. Effective mitigation of this risk is likely to require evaluation of viral pathways to aquaculture operations; therefore, some future pathway analysis may be necessary. For this assessment, the breakout group decided to consider sources and pathways leading to aquaculture only if they provided information relevant to aquaculture as a source of viruses to wild populations of shrimp.

A summary of risk ratings discussed by the aquaculture breakout group is provided in Table A-1.

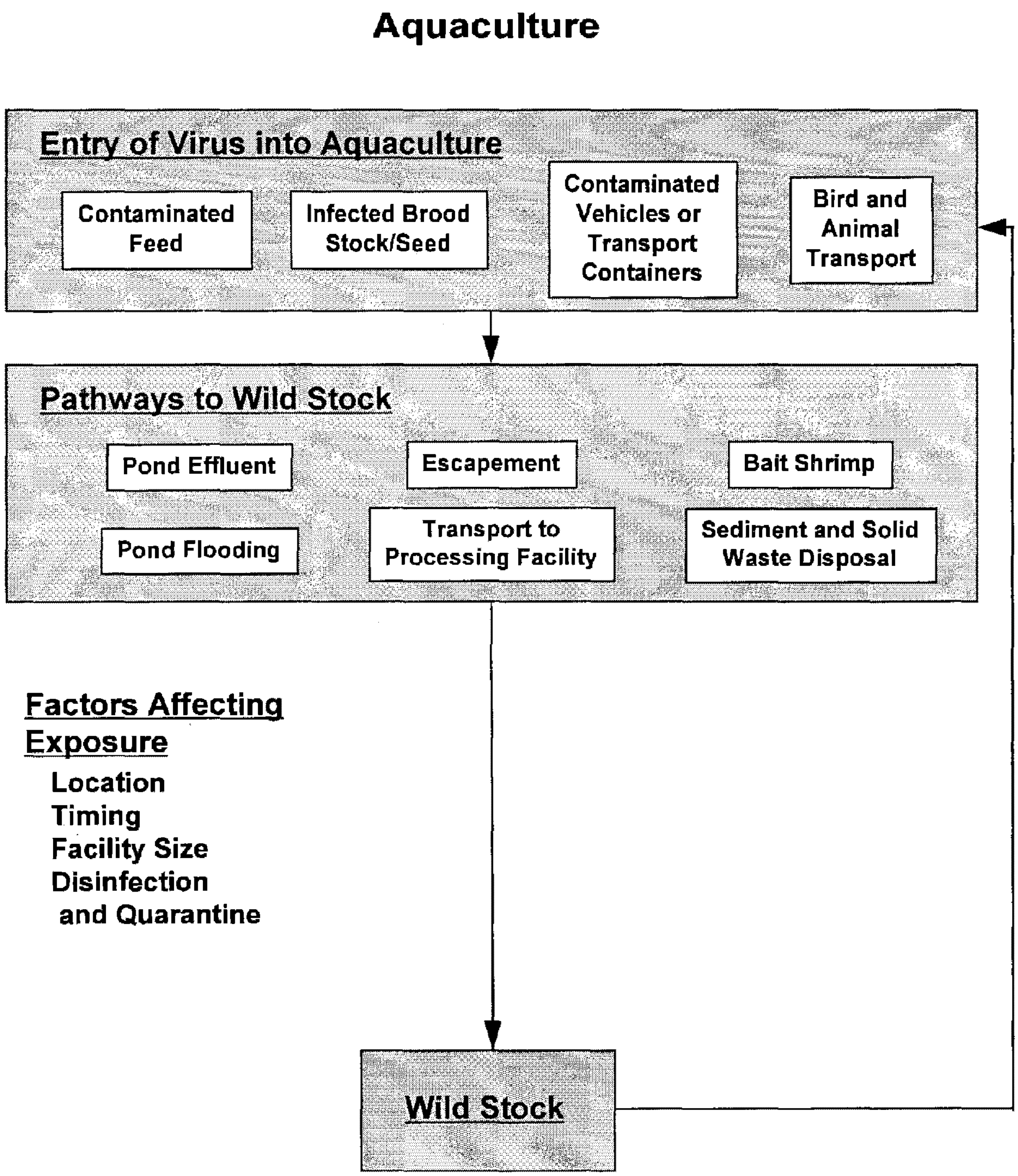

Figure A-1. Conceptual model: Virus sources and pathways for aquaculture (JSA, 1997)

Table A-1. Summary of Aquaculture Breakout Group risk rankings

Refer to supporting discussion in the text to properly evaluate information presented in this table. The risk assessment process is described in Section 2.1 and Appendix G.

	Pathways to the Environment				
Probability of Establishment	**Escapement**	**Pond Flooding**	**Pond Effluent**	**Transport to Processing Facility**	**Sediment and Solid Waste Disposal**
Association with Pathway	High/very certain	High/very certain	High/very certain	High/very certain	High/very certain
Entry Potential	High/very certain or low/reasonably certain[1]	Low/ very certain	Medium/very certain	Low/ reasonably certain	Low/ reasonably certain
Colonization Potential	Low (or medium to high)[2]/very certain	Low (or medium to high)[2]/very certain	Low (or medium to high)[2]/very certain	Low (or medium to high)[2]/very certain	Low (or medium to high)[2]/very certain
Spread Potential	Low/relatively uncertain to high/very uncertain[3]	Low/relatively uncertain to high/very uncertain[3]	Low/relatively uncertain to high/very uncertain[3]	Low/relatively uncertain to high/very uncertain[3]	Low/relatively uncertain to high/very uncertain[3]
Overall Probability of Establishment	Low to high	Low	Low to medium	Low	Low
Consequences of Establishment	Low to medium/very uncertain	Low to medium/very uncertain	Low to medium/very uncertain	Low to medium/very uncertain	Low to medium/very uncertain
Overall Risk Estimate	Low to high	Low to medium	Low to medium	Low to medium	Low to medium

[1] High if pond is infected and shrimp escape from pond; low otherwise.

[2] Some breakout group members believed that the potential was medium and would be high if the aquaculture industry expands significantly along the Gulf Coast.

[3] The breakout group could not reach consensus; opinions on entry potential ranged from low to high.

A.1.2 PROBABILITY OF ESTABLISHMENT OF VIRUSES IN AQUACULTURE

A.1.2.1 Probability of Nonindigenous Viruses Being in the Aquaculture Pathway

The occurrence of nonindigenous viruses in U.S. aquaculture operations is well documented. The breakout group concluded that the probability of nonindigenous viruses being in the aquaculture pathway is **High (Very Certain).** As summarized in the JSA report, TSV has been identified in disease outbreaks in Hawaii, Texas, and South Carolina (Lightner, 1996a, 1996b). IHHNV was first identified in Hawaii (Lightner et al., 1983a, 1983b) and was subsequently observed in farms in South Carolina, Texas, and Florida (Fulks & Main, 1992). WSSV and YHV also have been documented at a shrimp farm in Texas (Lightner, 1996a, 1996b), and a WSSV-like particle has been identified in South Carolina (P. Sandifer, personal communication). Breakout group members noted that the origins of these viruses are not always traceable to their ultimate sources, but it was suggested that their introduction to the United States may have resulted from importation of infected shrimp from other regions of the world (e.g., Latin America and Asia). The breakout group questioned the frequency of virus occurrence in U.S. aquaculture operations due to the lack of well-established monitoring programs and detection protocols; the group concluded, however, that, given the time course of disease progression and the nature of current shrimp farming practices (e.g., high shrimp densities), it is very certain when viruses are present.

A.1.2.2 Probability of Nonindigenous Viruses Surviving in Transit in the Aquaculture Pathway

To determine the probability of nonindigenous viruses surviving in transit, the breakout group considered the six subpathways from aquaculture to wild shrimp stocks, as shown in Figure A-1. The group initially attempted to rate survival in transit for each subpathway in an effort to provide complete information for management consideration; however, there was insufficient time for this task and the group determined that the probability of surviving in transit is primarily a function of the most likely subpathway. Given the lack of information and high uncertainty for subpathways such as pond effluent and sediments, the breakout group tabled discussions of these and other pathways and focused much of their discussion on one remaining subpathway (escapement), which includes both accidental and intentional releases, as well as "escape" via transport of shrimp tissue by the predatory activities of other animals. (However, as discussed in the following, opinions diverged on this topic. Some breakout group members believed that the sediment and effluent pathways, which the group tabled because of a lack of crucial data, may also be important.)

A.1.2.2.1 Escapement Subpathway

Information relevant to this rating includes documented cases of shrimp escapement in South Carolina (South Carolina Department of Natural Resources, C. Browdy, personal communication) and capture of cultured species in Texas waters by shrimp trawlers (R. Goldburg, personal communication). The frequency of escapement is said to be low and infrequent because of engineering controls, such as the use of screens in effluent streams (F. Jaenike, personal communication). However, the breakout group recognized that the release of viruses to the environment via this subpathway is dependent on the life stage of the infected shrimp (e.g., larval stages may be more likely to bypass engineering controls). Professional judgment suggests that all life stages are capable of escape under favorable conditions.

The breakout group agreed that viruses would survive shrimp escapement. The group acknowledged that the probability of release of viruses to the environment is a function of the probability that a pond is infected and the probability of shrimp escaping from that pond. The breakout group concluded that the probability of surviving in transit would be **High (Very Certain)** if these two conditions were met but would be **Low (Reasonably Certain)** if they were not met.

A.1.2.2.2 Pond Flooding Subpathway

The breakout group concluded that the probability that nonindigenous viruses could escape aquaculture operations via pond flooding was **Low (Very Certain)**, based on the judgment that ponds are unlikely to flood to overflowing. For example, ponds did not overflow during recent hurricanes in South Carolina, although the intensity of a storm event, its point of impact, and the specific location of aquaculture ponds would all influence the likelihood of flooding and the potential for escapement.

A.1.2.2.3. Pond Effluent Subpathway

Due to the lack of data and consensus among breakout group members, the breakout group did not complete an evaluation of this pathway, although a rating of **Medium (Very Uncertain)** was assigned. The primary uncertainties are the presence, viability, and infectivity of viruses in effluent waters. It was noted that TSV has been documented in water but not necessarily in effluent waters. There is suggestive evidence about this potential pathway. A workshop observer (R. Laramore) communicated results of an experiment that suggest that caged shrimp exposed in infected ponds developed disease. (Shrimp developed disease when exposed within 1 to 2 days to experimentally inoculated water, but they did not develop disease when exposed within 3 to 5 days of the water's inoculation [R. Laramore]). In 1995, HSF, Ltd., and the Arroyo Aquaculture Association conducted several trials in which cages were floated within a shrimp growout pond that had experienced a TSV epidemic and with pond water in tanks. The cages were suspended above the pond bottom and stocked with juvenile *P. vannamei.* No TSV was detected in shrimp exposed for 30 days under these conditions (F. Jaenike, personal communication). These results suggest that TSV may be transmitted during the acute but not the chronic stages of the disease. An unsubstantiated statement was made that viruses sorb quickly to particulate matter and, by so doing, may reduce their potential for future infection. The breakout group concluded that experiments critical to addressing this subpathway have not been conducted.

Some members of the breakout group offered a dissenting opinion about the potential for virus transmission in effluent waters. They believed that the group had not adequately evaluated this pathway. It was noted that data from J. Lotz suggest that IHHNV and TSV can survive in an infective state for a minimum of 28 days. D. Lightner suggested that IHHNV can survive in sediments for up to 24 days; however, he had not evaluated the virus's infectivity during that period. Waters in the Gulf of Mexico typically have high particulate loads; therefore, once particulate matter is suspended, it represents a viable route of exposure to *P. setiferus* (which is primarily pelagic) and *P. aztecus* (which is both demersal and pelagic over the course of a day). Some participants felt that this information suggests that effluents released from infected farm ponds could represent a viable pathway for exposure to native populations.

A.1.2.2.4 Transport to Processing Facility Subpathway

The breakout group assigned a rating of **Low (Reasonably Certain)** for this pathway, because cases of accidental shrimp escapement by this route have not been documented and are believed to be virtually nonexistent.

A.1.2.2.5 Sediment and Solid Waste Disposal Subpathway

The breakout group assigned a rating of **Low (Reasonably Certain)** for this pathway, assuming that pond dredging activities do not occur within 30 days of disease outbreak. This judgment is based on the relatively short half-lives of viruses in sediments (estimates of viability ranged from 1 to 2 days for WSSV to 30 days for IHHNV) and also on the knowledge that disposal of solid wastes into the ocean is not permitted under U.S. regulation.

A.1.2.2.6 Bait Shrimp Subpathway

This pathway was evaluated by the "Other Pathways" Breakout Group.

A.1.2.3 Colonization Potential for the Aquaculture Pathway

In evaluating the potential for virus colonization, the group concluded that the probability of nonindigenous viruses successfully colonizing and maintaining a population where introduced is **Low (Very Uncertain).** Some breakout group members expressed concern about the rating of **Low** for colonization potential and offered a dissenting opinion. These individuals believe that the rating should be changed to **Medium**, based on information communicated during plenary discussions and the judgment that pond effluent might provide a continuous input of virus to near-coastal systems. Furthermore, they believe that if the aquaculture industry were to expand significantly along the Gulf coast, this potential might more appropriately be rated as **High.**

Nonetheless, the breakout group concluded that the potential for colonization from U.S. aquaculture sources is **Low**, because of the lack of evidence suggesting establishment of viable virus populations in wild U.S. shrimp stocks introduced via the aquaculture pathway and because virus outbreaks in farm ponds have not been correlated with similar outbreaks in local wild stock. This may not be true in other areas of the world, where past practices have involved the "dumping" of entire ponds when outbreaks have occurred. The breakout group recognized, however, that colonization potential is likely to be virus specific and dependent on the specific shrimp species and its life stage susceptibilities.

A.1.2.4 Spread Potential for the Aquaculture Pathway

After considerable discussion, the breakout group was unable to reach consensus on the potential for the spread of viruses once the viruses had colonized. The group ultimately concluded that the potential ranges from **Low (Relatively Uncertain)** to **High (Very Uncertain).**

Workshop participants suggested that stocks of *P. setiferus* in the Atlantic are genetically homogeneous (Mark Frischer, Skidaway Institute of Oceanography, personal communication), as are the northern and southern populations in the Gulf of Mexico (D. Boudreaux, observer, personal communication). Thus, there is the potential for substantial interaction over broad geographic regions, which could promote the spread of viral infection. However, other penaeid species may not be genetically homogeneous.

During its deliberations, the breakout group considered whether experiences with viral disease in aquaculture farms could be extrapolated to field situations. Participants noted that when an outbreak occurs at a facility, viral infection spreads fairly rapidly within individual ponds and can spread beyond the originally infected pond. The mechanisms of transmission between individuals and from pond to pond remain unknown. The breakout group recognized that disease transmission in aquaculture may not be analogous to transmission in wild populations, due to differences in the relative stress experienced by farm shrimp (e.g., crowding, nutrition, predation).

The breakout group agreed that the potential for spread depends in large part on the time course of the disease and the density of shrimp in wild populations (and therefore the rate of individual encounters). For example, low shrimp densities are likely to hinder disease spread, whereas high densities are likely to promote transmission. The breakout group recognized that spread potential is virus specific as well as host dependent. (TSV and IHHNV are thought to have low spread potential, while the spread potential of YHV and WSSV is currently unknown). Additionally, WSSV, when detected in wild stocks in Asia, is distributed over wide geographic areas. This supports the conclusion that viral disease can spread readily from its original locus of colonization. As noted in the JSA Report, other stressors (such as low dissolved oxygen and extreme salinity) are likely to influence the potential for spread of the disease. The mechanisms of virus transmission and infectivity remain major data gaps with respect to spread potential.

A.1.3 CONSEQUENCES OF ESTABLISHMENT OF VIRUSES FROM AQUACULTURE

To assess the consequences of establishment, the breakout group made the assumption that nonindigenous shrimp viruses are established. However, this assumption does not reflect a belief on the part of the breakout group that viruses have indeed been established in U.S. waters.

The breakout group's evaluations focused on the two assessment endpoints articulated in the JSA report: the direct effects on the survival, growth, and reproduction of wild penaeid shrimp populations and the effects on ecological structure and function of marine communities as they affect wild shrimp populations. The breakout group gave primary attention to the first assessment endpoint.

A.1.3.1 Direct Effects on Wild Shrimp Populations

The breakout group concluded that direct effects on wild shrimp populations are **Low to Medium (Very Uncertain).** Participants noted that penaeid shrimp can be characterized as "r-selected" organisms because they display an annual life history pattern with high reproductive output and high mortality during early life stages. In reviewing the existing information, the breakout group concluded that mass mortalities of adult shrimp typically have short-term repercussions on standing shrimp stocks. For example, the suspected 1987 IHHNV-induced mortality event in the Gulf of California (Pantoja-Morales, 1993) was associated with reductions in *P. stylirostris* population abundances for approximately 6 to 7 years, but stocks are reported to be returning to preoutbreak levels. (No specific references were offered in support of this contention, and considerable doubts remain about the role that IHHNV played in the observed population declines.) Additionally, participants noted that because of high fecundity and migratory behavior, *P. setiferus* is capable of rebounding from a very low population size in one year to high abundances in the next year, if environmental conditions are favorable. This has been observed off the South Carolina coast several times in the past 50 years (Linder & Anderson, 1956; McKenzie, 1981). A reported increase in reproductive output of wild shrimp populations in Honduras during the 1994 outbreak of TSV provides additional support for demographic compensatory responses (R. Laramore, observer, personal communication), although it was noted that other factors may have contributed to these population changes. Along with anecdotal information regarding the possible long-term effects of viral infections in Latin American and Asian shrimp populations, the breakout group determined that these observations suggest that direct mortality effects would be relatively transitory. Also, it was suggested that initial outbreaks could lead to enhanced resistance to future viral infection, based on the observation that resistance to IHHNV appears to have increased in all populations tested since the identification of this virus in Hawaiian stocks (Lightner, personal communication).

In addition to direct mortality effects, the breakout group discussed the potential for sublethal effects of viruses on shrimp reproduction and growth. The breakout group was aware of no information describing adverse viral effects on reproductive potential of infected individuals. One expert noted that reproductive output of infected *P. vannamei* brood stock appears to be unaffected by viral infection (F. Jaenike, personal communication). However, in contrast to the previous statement, individual growth impairment in offspring of *P. vannamei* infected with IHHNV has been documented (Fulks & Main, 1992). Assuming that fecundity of female *Penaeus* is an increasing function of size (a phenomenon common in other invertebrate species), breakout group participants considered that stunted growth of offspring could result in reduced reproductive output of the second generation. The breakout group concluded that individual growth impacts could therefore cause population-level effects, although an analysis of the importance of reproduction to shrimp population dynamics would be required to support this conclusion.

To complete its evaluation of direct consequences of viruses to shrimp populations, the breakout group considered a scenario in which a shrimp population experiences a 50 percent decrease in abundance for 5 years as a result of viral outbreak. (This scenario is similar to the Gulf of California situation described by Pantoja-Morales.) By extrapolating from the information summarized previously, the breakout group suggested that the direct consequences on population abundance might be short lived and that stocks would rapidly recover to historic abundances; therefore, the environmental impacts would be low to medium for the immediate population. The breakout group recognized, however, that the genetic consequences of rapid reductions in population abundance (the so-called "founder effect") are unknown but potentially important. Substantial uncertainty surrounds this rating due to the lack of information regarding analogous situations in actual wild populations and the lack of direct experimental evidence.

A.1.3.2 Effects on Ecological Structure and Function

The breakout group did not rate this element due to insufficient data and a lack of time for a thorough evaluation. The breakout group identified examples in which other invertebrate species have experienced severe disease consequences:

- The near decimation of oysters (*Crassostrea virginica*) by the protozoan pathogens *Haplosporidium nelsoni* and *Perkinsus marinus*, called MSX and dermo disease respectively (Haskin & Andrews, 1988; Andrews, 1996; Burreson & Ragone-Calvo, 1996), has resulted in significant changes in the oyster reef habitat throughout Chesapeake Bay and dramatically reduced the rate at which bay water was filtered by feeding bivalves (Kennedy, 1996).

- Insect/virus associations in which high abundances of the host species promote rapid outbreaks of viral disease, followed by dramatic declines in the host, near-disappearance of the virus, and reestablishment of the host (S. Thiem, personal communication).

- The introduction into Scandinavia of North American crayfish that were carriers of the freshwater crayfish plague *Aphanomyces astaci* (Unestam & Weiss, 1970).

Some breakout group members believed that these examples might serve as models for extrapolating potential consequences of viral establishment in aquatic systems as they affect shrimp populations. These examples may show how ecological systems might be affected by viral outbreaks in shrimp. The breakout group recognized that careful analysis of these examples would be needed to identify similarities and differences relative to the shrimp virus situation.

The aquaculture breakout group did not discuss the effects of viral disease on other components of the ecosystem that might influence dynamics of shrimp populations. Subsequent plenary discussion, however, suggested that other crustaceans (notably paleomonids or "grass shrimp") might suffer negative impacts with potentially severe consequences to the ecological system as a whole. The breakout group suggested that fish catch data maintained by Mexico during the Gulf of California shrimp decline might help provide insight on possible impacts of shrimp viruses on nonshrimp species.

The breakout group agreed that development of an epidemiological model describing virus-shrimp interactions and subsequent sensitivity analyses of its results would be useful for identifying critical areas of uncertainty and prioritizing research needs. Such a model would permit initial quantitative assessments of the potential consequences of viral infection on wild shrimp populations.

A-2. Report Of The Shrimp Processing Breakout Group

A.2.1 INTRODUCTION

This breakout group was charged with assessing the risk associated with introduction of nonindigenous virus to wild shrimp populations from the shrimp processing pathway (see Figure A-2).

Currently, over 60 countries export both pond-raised and wild shrimp to the United States Over one-half of the shrimp processed in the United States is imported from foreign countries, where viral diseases may be a problem. To minimize disease effects on cultured shrimp yield, some countries harvest shrimp during the early stages of a disease outbreak. This strategy avoids high mortality and catastrophic economic losses, but it increases the likelihood that shrimp imported to the United States will be contaminated with viable viruses (Lightner, 1996a). Shrimp infected with WSSV, YHV, and TSV have been identified in retail stores in the United States (D. Lightner, unpublished); therefore, the importation and processing of infected shrimp may increase the potential for the introduction of pathogenic viruses into coastal waters adjacent to processing plants. This pathway may thus pose a threat to wild shrimp populations (JSA, 1997).

The breakout group reviewed the steps in shrimp processing to identify the potential pathways for the release of virus-contaminated material into the environment. This information was used to examine the conceptual model contained in the JSA report (Figure A-2) to ensure the model's completeness and to evaluate the probability of establishment, impact, and risk for each of the pathways.

The steps in the commercial processing of shrimp are described in Figure A-3. Of the shrimp processed in the United States, 80 percent of total crop is foreign and 20 percent is domestic in origin. Of the imported shrimp, 50 percent is farm raised and 50 percent is wild catch. Most foreign shrimp arrives frozen and generally without heads. Approximately 50 percent of domestic landings arrive at processing plants frozen, and the remainder is fresh. Therefore, only about 10 percent of the total shrimp processed in the United States is actually fresh. The breakout group estimated that up to 40 percent of the total shrimp processed in the United States arrives at processing plants without heads. Because shrimp heads can carry a high concentration of some viruses, the presence or absence of heads on shrimp arriving in the United States is significant.

Processing involves several steps, including thawing (if the shrimp arrive frozen), grading, peeling, and culling (see Figure A-3). Participants noted that no water is transferred when foreign, frozen shrimp arrives in the United States on container ships. Liquid effluent produced from thawing, culling, and washing is either sent to wastewater treatment facilities or is discharged into the coastal environment without treatment. Participants noted that the level of treatment varies according to state requirements. For example, Florida requires treatment of all

Shrimp Processing

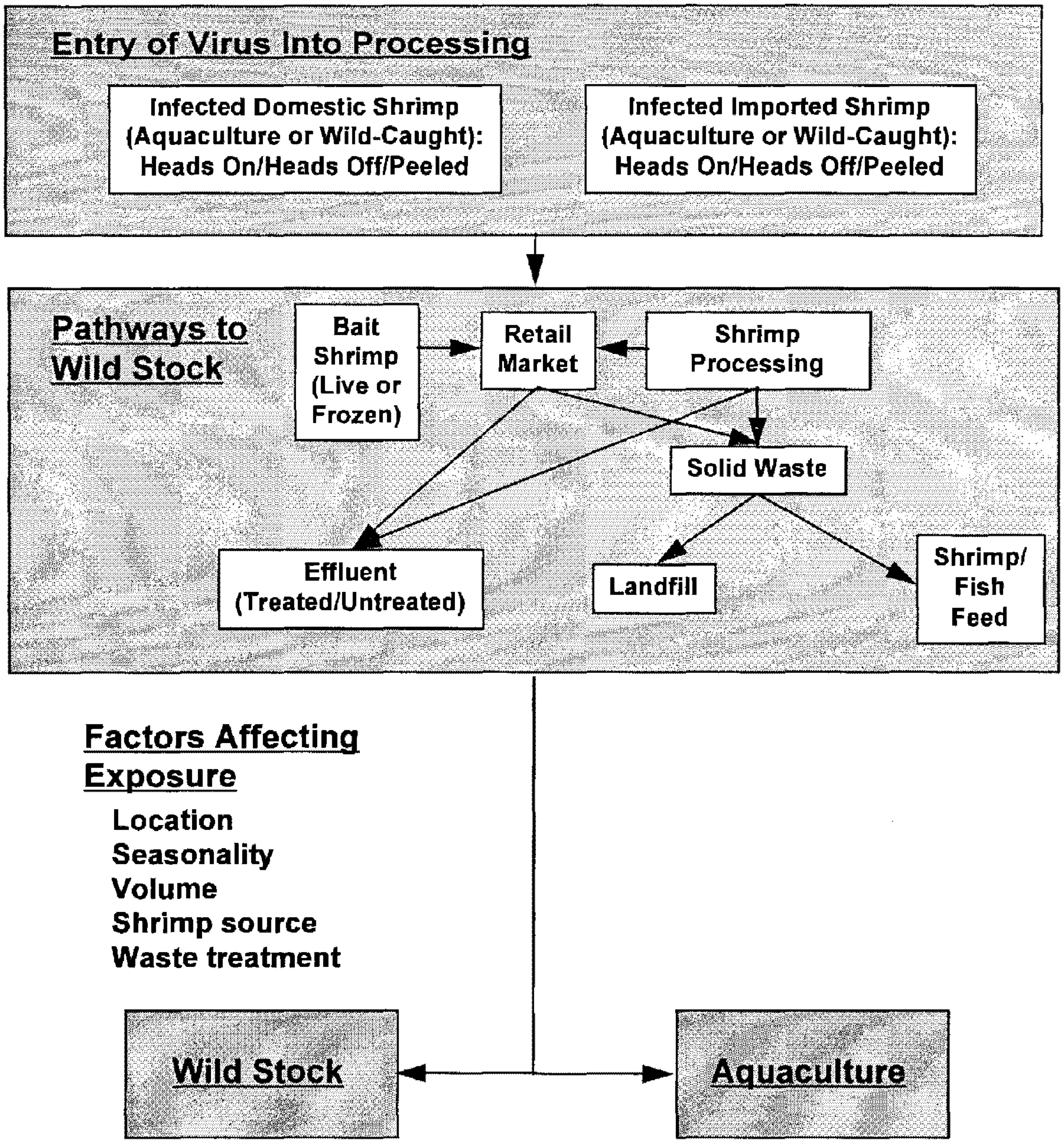

Figure A-2. Conceptual model: Virus sources and pathways for shrimp processing (JSA, 1997)

IQF Cooked Shrimp Production Flow

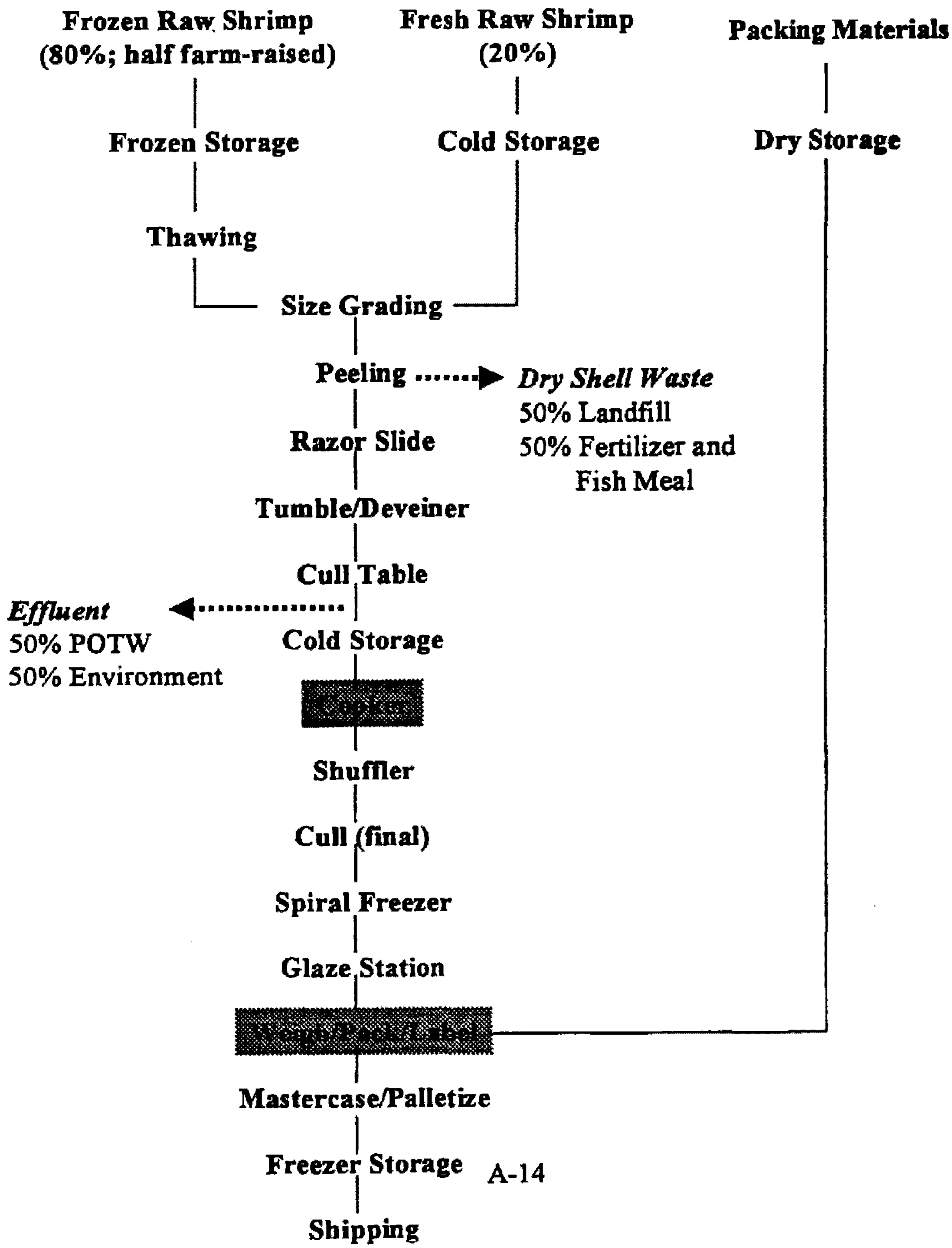

Figure A-3. Flow diagram for shrimp processing.

effluent from shrimp processing. Breakout group members estimated that, nationally, 40 to 50 percent of shrimp-processing effluent is treated. Breakout group members concluded that the discharge of processing effluent from wastewater treatment facilities poses no potential risk because it is believed that the disinfection process is likely to kill viruses. The direct discharge of processing effluent into estuarine waters, however, may represent an important pathway for the establishment of viruses in the environment. Breakout group members felt that this pathway may represent a frequent or continuous source of virus into the environment, thereby increasing the probability of establishment.

Solid waste is generated primarily during peeling, when shrimp shells are removed and sent to landfills or processed for fish feed or fertilizer. Breakout group members noted that, in general, landfills are covered in 24 hours; however, seagulls and land crabs (*Sesarma*) have been reported to immediately descend on shrimp heads once they reach the landfill. There is evidence that TSV can survive intact in seagull feces (D. Lightner, personal communication), thereby providing a potentially important pathway for viruses to contaminate both aquaculture facilities as well as nearshore bays and estuaries.

Processing operations have an effect on the viability of some viruses. For example, breakout group members reported that the viability of WSSV (and YHV, by analogy) declines with increasing frequency of freeze/thaw conditions, but this is not the case for either IHHNV or TSV. This difference in persistence may result from the size and structure of the viruses; IHHNV and TSV are small virus particles whereas WSSV and YHV are larger, more complex viruses that may be more labile (Table A-2). Similarly, breakout group participants noted that experimental evidence shows that IHHNV and TSV have longer half-lives (28 days) in open water than do WSSV and YHV (7 days) (J. Lotz, personal communication, for TSV and WSSV; Flegel et al., 1995, for YHV).

The breakout group noted that effluent from shrimp boats is of minimal concern, because it represents such a small amount of the total potential pathways of virus introduction into the system.

Based on its analysis of shrimp processing, the breakout group decided that the basic elements of the conceptual model presented in the JSA report adequately represent the major pathways associated with processing. For the purposes of this exercise, the breakout group selected four pathways for evaluation: treated effluent, untreated effluent, solid waste in landfills, and shrimp feed/fish feed.

A summary of risk ratings discussed by the shrimp processing breakout group is provided in Table A-3.

Table A-2. Virus persistence, virulence, and infectivity

	IHHNV	TSV	YHV	WSSV
Persistence (1 = least, 4 = most)	**3.5**	**3.5**	**1.5**	**1.5**
Virulence to Gulf Species (1 = least, 4 = most)	1	2	3	4
	Relative Infectivity			
Penaeus setiferus				
Larvae	—	—	ND	ND
Post-larvae	—	++	—	++
Juvenile	+	+	++	++
Adult	ND	+	ND	ND
Penaeus duorarum				
Larvae	—	—	ND	ND
Post-larvae	—	—	—	++
Juvenile	+	+	++	+
Adult	ND	ND	ND	ND
Penaeus aztecus				
Larvae	—	—	ND	ND
Post-larvae	—	+	—	++
Juvenile	+	+	++	+
Adult	ND	ND	ND	

INFECTIVITY

ND = No data
+ = Infectious
++ = Mortality
— = Tried but negative

Table A-3. Summary of shrimp processing breakout group risk rankings

Refer to supporting discussion in the text to properly evaluate information presented in this table. The risk assessment process is described in Section 2.1 and Appendix G.

Probability of Establishment		Pathways to the Environment: Treated Effluent	Untreated Effluent	Landfill	Shrimp/Fish Feeds
Association with Pathway		High/ very certain	High/ very certain	High/ very certain	High/very certain
Entry Potential		Low/ very certain	High/ very certain	Medium/ reasonably certain	Low/ very certain
Colonization Potential		Low/ very certain	Medium/ moderately certain	Low/ reasonably uncertain	Low/ very certain
Spread Potential		Low/ very certain	Medium/ moderately certain	Low/ reasonably uncertain	Low/ very certain
Overall Probability of Establishment		Low	Medium	Low	Low
Consequences of Establishment	Local	Low-medium/ reasonably uncertain	Low-medium/ reasonably uncertain	Low-medium/ reasonably uncertain	Low-medium/ reasonably uncertain
	Large Scale	Low/highly uncertain	Low/highly uncertain	Low/highly uncertain	Low/highly uncertain
Overall Risk Estimate	Local	Low-medium	Medium	Low-medium	Low-medium
	Large Scale	Low	Medium	Low	Low

A.2.2 PROBABILITY OF ESTABLISHMENT OF VIRUSES FROM SHRIMP PROCESSING

A.2.2.1 Factors Influencing Colonization and Spread from Shrimp Processing

To accurately assess the probability of establishment of viruses released from shrimp processing, the breakout group reviewed the concepts of colonization/infectivity potential and spread potential, which are two key elements of the establishment process that may ultimately influence environmental impact. Participants noted that implicit in any discussion of risk is the issue of co-occurrence between the stressor (viruses) and the receptor (wild shrimp populations). Exposure to the shrimp virus, therefore, depends not only on the spatial and temporal patterns of viral entry into coastal and marine systems but also on the movements and life-history patterns of the shrimp (JSA, 1997). To help understand this concept, the breakout group discussed the spatial and temporal distribution of the shrimp populations.

A.2.2.1.1 Life-History/Behavior

Breakout group members noted that shrimp populations move into the nearshore regions as postlarvae and as juveniles during the spring. During these life stages, shrimp may be more likely to be exposed to viruses entering from onshore processing discharges or from landfills via avian and crustacean vectors. It was also noted that prior to leaving the estuaries in late summer and early fall, many shrimp undergo a "staging period" in which different species commingle and aggregate in high densities in the nearshore environment for 1 to two months.

The breakout group hypothesized that this behavior increases the likelihood for exposure and subsequent transmission and spread of disease. It was suggested that this hypothesis is probably valid for IHHNV and possibly for WSSV, but not for TSV. A breakout group member also noted that the spread of virus is a function of the different susceptibilities of shrimp species, their life stages (Table A-2), and the ways in which the shrimp are distributed. For example, if shrimp are homogeneously distributed throughout the Gulf, they act as one population. However, participants noted that a more likely scenario is that there are localized areas with high shrimp densities and other areas where there are no shrimp. Even within good habitat, populations are likely to be patchy.

A.2.2.1.2 Population Density

To determine if population density affects disease outcomes, the breakout group discussed whether experiences in aquaculture can be related to field populations. Breakout group members noted that the virus will create a long-term problem in aquaculture when densities are high. For example, in 1996, South Carolina farms experienced widespread infection with TSV. Not all ponds, however, became infected; ponds stocked less densely appeared to avoid the disease. Breakout group members suggested that densely populated conditions create a stressful environment that makes shrimp more susceptible to the spread of disease. This hypothesis is supported by observations from more "natural" impoundments in South Carolina coastal waters, where shrimp densities are reported to be lower and no disease was found.

A.2.2.1.3 Persistence and Virulence

Knowledge of the persistence and virulence of viruses in various environmental media is important to predicting the probability of infection/colonization in wild shrimp populations. Breakout group participants cited data suggesting that persistence in water is virus dependent. IHHNV and TSV persist for weeks to a month, and WSSV and YHV persist for days (Table A-2). Virulence of the four viruses was considered by breakout group participants and ranked in decreasing order as follows: WSSV, YHV, TSV, IHHNV. In addition, there is a wide range of sensitivity among species and among life-history stages within a species. Participants noted that IHHNV, though very persistent, is not particularly virulent to Gulf species. It has only been detected within juveniles and has not been known to cause mortality. WSSV is least persistent but appears to be very virulent (Lightner, 1996), causing mortalities to the postlarvae of all three Gulf species in laboratory experiments (Lightner et al., in press). Variations in persistence, virulence, and life-stage sensitivity underscore the uncertainties associated with determining colonization potential.

A.2.2.1.4 Routes of Infection

To determine the potential for viral establishment, the breakout group also considered the primary routes of infection. There are four plausible pathways: exposure to water (in particular, contact with respiratory surfaces), ingestion of water and associated particles, ingestion of other infected shrimp, and transmission from infected spawning adults through gametes to larvae. It was noted that this last pathway is limited to the offshore stage of the shrimp's life history, while the other three pathways are of greater significance during nearshore stages. Breakout group participants further suggested that animal vectors such as sea gulls and land crabs could represent plausible routes of exposure from solid waste disposal of processed shrimp. It was proposed that sea gulls, which eat potentially infected carcasses disposed at landfills, could disperse virus through their excrement, thereby infecting coastal ponds. Some breakout group members cited reports from Thailand that land crabs (*Sesarma sp.*) feeding on infected matter in landfills could be infected with WSSV and carry the virus back to coastal environments.

A.2.2.1.5 Spatial Scale

Breakout group participants considered that spatial scale is an important factor in both the spread and the probability of environmental impacts. The breakout group generally agreed that local discharges of virus-laden effluents have a reasonable likelihood of infecting a local population of shrimp, particularly in a closed embayment with restricted exchange. Participants hypothesized that such a localized population would be likely to extinguish itself as a result of disease and thus have little or no effect on the population as a whole. Participants noted, however, that there is no evidence to support such a hypothesis.

Similar scenarios can be constructed for large-scale impacts. For example, the "staging" and "aggregating" behavior discussed previously provides an opportunity for a locally infected population to commingle with other species at high densities, thereby increasing the likelihood of transmission and the spread of the virus. Furthermore, the subsequent offshore migration provides a vector for the virus to reach other populations, thereby potentially transmitting viruses through the reproductive cycle. Participants noted that, while scenarios such as these may be plausible, they tend to have very high uncertainty.

A.2.2.2 Pathway Analyses for Shrimp Processing

Breakout group participants observed that several potential exposure pathways can be developed for the conceptual model for shrimp processing. Both shrimp processing plants and retail outlets produce liquid effluent. The proportion of untreated effluent from the retail sector is likely to be relatively unimportant, because most effluent is directed to municipal treatment facilities. Breakout group members recognized that this is not the case in processing, where the volumes of liquid effluent are quite large. The breakout group estimated, however, that at least 50 percent of liquid effluent from processing passes through a municipal treatment facility, which potentially reduces the total risk from this pathway. In addition to effluents, both the retail and processing sectors produce solid waste in the form of shells and heads, which are disposed of either in landfills or used in the production of shrimp or fish feed.

The breakout group qualitatively estimated the probability of establishment of the virus in wild shrimp populations for the following pathways: treated effluent from shrimp processing and retail, untreated effluent from shrimp processing and retail, solid wastes to landfills, and solid wastes to shrimp feed. The breakout group generally agreed that there is a very high probability that wild and farmed foreign shrimp in each of the four pathways are contaminated with viruses. Because foreign shrimp compose 80 percent of the total shrimp consumed in the United States, they represent a major source of potential infection of U.S. farmed and wild shrimp populations. The breakout group therefore agreed that there is a **High** probability of viruses being associated with the pathways leading from both processing and retail to the environment. The breakout group was **Very Certain** of this ranking. These rankings are based on general knowledge, with empirical data for the presence of both WSSV and YHV in foreign products.

A.2.2.2.1 Treated Effluent

Because of the high likelihood that virus-infected shrimp may be in this pathway, the primary effluent emanating from plants and retail markets is very likely to carry viruses; however, both retail and processing effluent treated at municipal treatment plants are highly unlikely to retain live viruses because of the rigorous disinfection practices used. As a result, the breakout group was **Very Certain** that the entry potential is **Low**. The breakout group was also **Very Certain** that there was a **Low** risk of colonization and subsequent spread of infection. In this case, the breakout group based its rankings on a general knowledge of virus disinfection and survival in municipal treatment plants and professional judgment regarding its colonization and spread.

A.2.2.2.2 Untreated Effluent

Untreated effluent from retail and, more important, from shrimp processing poses the greatest potential risk for disseminating shrimp viruses to wild shrimp populations. The breakout group estimated that approximately 50 percent of liquid effluent from shrimp processing is untreated and that potentially virus-laden discharges could be released regularly into the environment. The breakout group was **Very Certain** that the probability of the organism surviving in transit and the potential for entry into the environment is **High.**

Breakout group participants noted that the persistence, infectivity, and virulence of the virus in the receiving waters is somewhat more uncertain and is a function of the type of virus, the distance from the receiving waters, the properties of the receiving waters, the stage in the shrimp life cycle, and time of year. Consequently, the breakout group judged the potential for colonization to be **Medium (Moderately Certain).** Because spread of the infection within the wild shrimp population is also dependent on a variety of factors, the breakout group estimated that the potential for spread of the virus once initial colonization has occurred to be **Medium (Moderately Certain).**

A.2.2.2.3 Solid Waste in Landfills

Because of the uncertainties associated with the amount of material reaching landfills, the types of vectors, and the threshold amount of virus required to infect the wild and aquaculture populations, the breakout group found it more difficult to assess the probability of establishment of shrimp virus in the wild population from the solid waste in landfills pathway. The breakout group was **Very Certain** that the shells and particularly the heads of foreign farmed and wild shrimp are highly likely to contain viruses (**High**) and that these viruses are likely to persist for some time in landfill settings. However, the persistence of infectivity of these viruses is unknown.

Participants noted that land crabs (*Sesarma*) and sea gulls are two primary vectors thought to move viruses from the landfills to estuarine waters. Both of these vectors are known to carry viruses. The breakout group also noted that WSSV and YHV are not known to pass through these animals' digestive systems in an infective state; however, TSV is known to pass through the guts of seagulls in an infectious state. At issue is whether the concentrations and frequency of virus introduction from these vectors is sufficient to exceed the threshold level required to infect wild and aquaculture shrimp populations.

An important factor in virus transmission is that the virus is concentrated in the heads (specifically, the lymphoid organ) of shrimp that survive TSV infection. The virus is systemic in the bodies of shrimp at the early stages of TSV and YHV infection. Breakout group members observed that, because shrimp from Asia are being harvested at the onset of infection so that the harvest is not lost, some imported shrimp are now noticeably smaller. The breakout group was **Reasonably Certain** that there is a **Medium** probability of entry potential from landfills to estuaries. Primarily because of the absence of virus-to-shrimp dose-response data and the uncertainties (**Reasonably Uncertain**) associated with frequency and concentration of viruses being introduced by these vectors, the group believed that there is only a **Low** likelihood of colonization within the wild population. Participants noted that dose-response data are critical in defining potential threshold levels for colonization. The breakout group also expressed caution in evaluating the potential for spread or viruses in the wild populations (**Low [Reasonably Uncertain]**).

Although the breakout group did not explicitly discuss the solid waste in landfills pathway in terms of effects to aquaculture, participants hypothesized that there is a greater likelihood of colonization and spread in closed ponds than in open circulating estuaries. Participants noted that there is a higher probability of establishment from repeated small inocula from seagulls and crabs in small ponds than in estuaries. Because of the increased density of organisms in aquaculture systems, breakout group members concluded that the potential for spread is likely to be very high. The critical uncertainties remain (e.g., persistence of virus long enough for wild shrimp to become infective, retention of its virulence, and exceedence of threshold dose). Therefore, the breakout group determined that colonization in aquaculture settings from this pathway is ranked **Medium (Moderately Certain).** However, participants recognized that once the virus has colonized, the probability of spread is ranked as **High** with a fair amount of confidence (**Reasonably Certain**).

A.2.2.2.4 Shrimp and Fish Feeds

One of the important markets for shrimp by-products (e.g., heads and shells) is the shrimp and fish feed processing industry. The breakout group did not have information about the volume of shrimp by-products that contribute to this pathway, but the group was very confident that shrimp by-products can be virus contaminated (**High [Very Certain]**). However, because shrimp and fish feed are processed at very high temperatures, there is little chance that the virus can survive and be a threat to the environment. The breakout group was therefore **Very Confident** that the entry potential of viruses into the environment through this pathway is very **Low.** The group was **Very Certain** that the colonization and spread potentials are **Low.** Overall, the breakout group considered the potential risk of establishment from the shrimp feed/fish feed pathway to be very low to nonexistent with very little uncertainty. The "Other Pathways" Breakout Group also evaluated shrimp feed as a source of virus introduction but came to somewhat different conclusions (see Section 3.2.3).

A.2.3 CONSEQUENCES OF ESTABLISHMENT FROM SHRIMP PROCESSING

The breakout group identified three approaches that could be used to estimate the magnitude and probability of environmental impacts from processing discharges into the environment:

- Field studies that associate virus incidence with disease or effects
- Experimental data that link viruses to biological effects such as mortality, reproduction, and growth
- Modeling studies that explore scenarios of virus exposure

A.2.3.1 Field Evidence for Environmental Impact Potential

The breakout group considered whether field observations have been made on the association and/or co-occurrence of viruses and environmental impacts. It also considered whether empirical data exist that associate viral infection with effects on wild shrimp populations. Workshop participants noted that a crayfish introduced from California to Europe may likely have initiated and served as a carrier to spread the freshwater crayfish plague throughout Scandinavia (Unestam & Weiss, 1970). The Gulf of California shrimp declines described by Pantoja-Morales provide another example (Lightner et al., 1992); however, the population declines were not conclusively demonstrated to result from the virus. There is also evidence of WSSV-like infections in wild populations of shrimp from a South Carolina estuary; however, it is not known how long the virus has been in these waters. Data exist on the South Carolina *P. setiferus* catch during the development of shrimp aquaculture in the state (Figure A-4). These data appear to reflect the natural variability of the populations. This variability is largely related to annual spawn success, which is controlled, at least in part, by winter temperatures. Participants emphasized that there is no evidence to suggest that WSSV has affected wild shrimp populations in South Carolina or anywhere in the world. Despite a serious outbreak of TSV in South Carolina in 1996, the 1996 and 1997 crop harvests were near or above the historical mean (Figure A-4). *Baculovirus penaei* (BP) has also been detected in the mysis stage of brown shrimp in the Gulf of Mexico, which suggests that the virus may have been transmitted via gametes from infected parent stock that spawned in open Gulf waters. However, one workshop participant noted that there is no evidence in the literature to suggest that BP can be transmitted via gametes.

Studies of viral infections in populations of shrimp in Honduras suggest that endemic virus has not had an impact on population levels (Laramore, observer comment, also in JSA, 1997).

Finally, workshop participants noted that there is evidence that IHHNV has become established in aquaculture and that stunted growth in *P. vannamei* has occurred. These data suggest that some viruses (e.g., IHHNV and TSV) exist in wild populations of shrimp; however, there is currently no evidence (based on shrimp landings) that these infections have caused or are causing impacts.

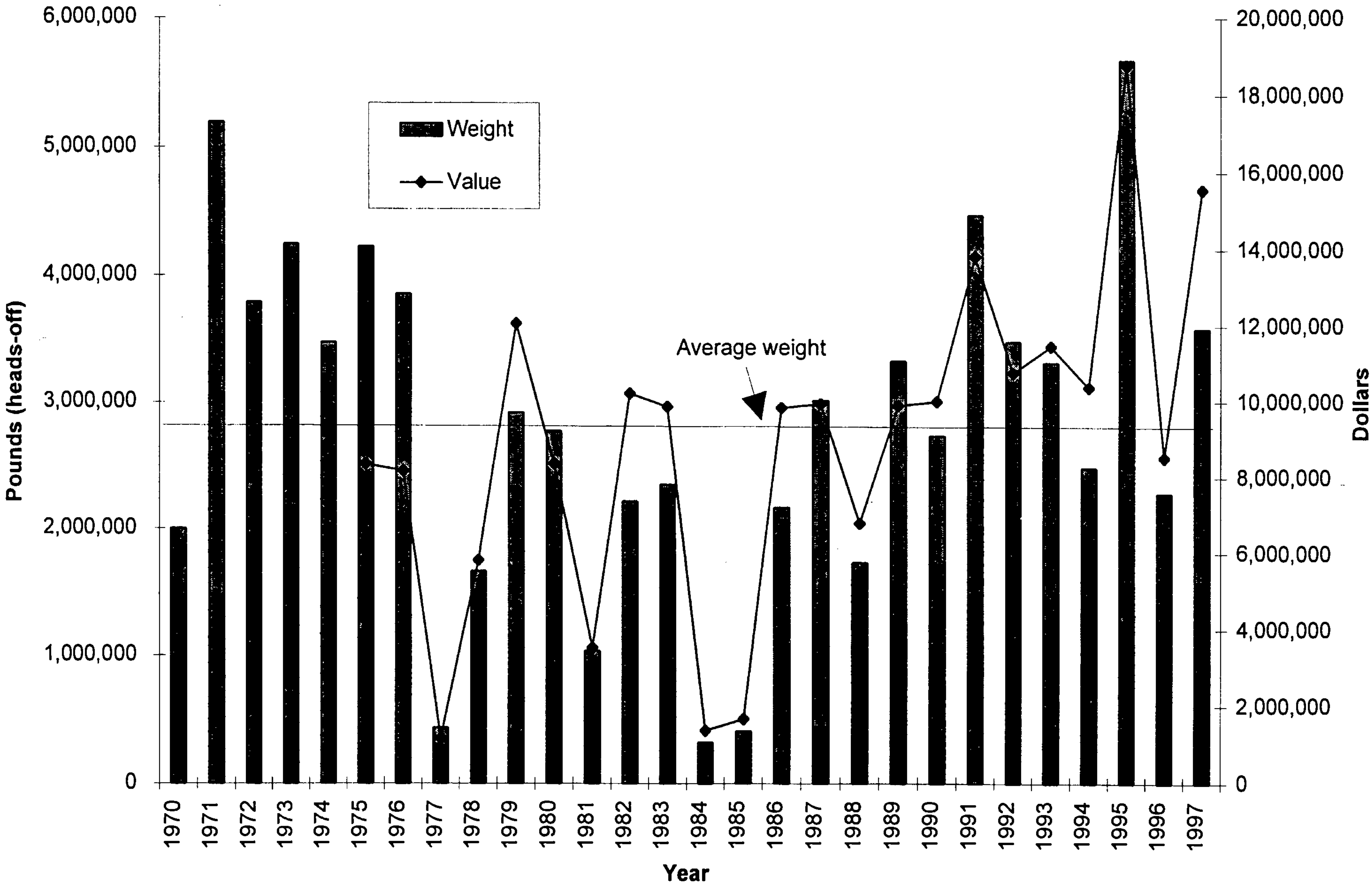

Figure A-4
South Carolina Commercial White Shrimp Landings and Values
(South Carolina Department of Natural Resources, 1998)

It should also be noted that shrimp landings do not necessarily correlate well with shrimp reproduction. The breakout group identified several remaining questions:

- Are chronically infected wild populations at greater risk to challenge from other stressors?
- Is there a delayed expression of chronic viral infection to the populations?
- Have these populations developed resistance to the virus?
- Has the virulence of the virus attenuated so that an equilibrium has been established between virus and host?

A.2.3.2 Experimental Data for Environmental Impacts

Workshop participants noted that laboratory or field experimental data provides another line of evidence for determining the probability of environmental impacts from virus infection of shrimp populations. Studies in aquaculture facilities indicate that virus exposure, infection, and mortality are strongly associated. However, no experimental studies have been conducted on any species that can be used to establish any of the following:

- Dose-response relationships
- Virus transmission rates
- Virus-induced impairment of reproduction
- Virus infection rates
- Transmission between life history stages or species

Therefore, breakout group members concluded that the lack of threshold information makes it impossible to develop infection/colonization estimates with any degree of certainty.

A.2.3.3 Population Modeling

The breakout group briefly discussed the potential use of shrimp population models to estimate impacts from various virus-induced mortality or reproductive impairment scenarios. Because of the commercial importance of shrimp, participants believed that it is highly likely that population models exist for these species. Additionally, participants felt that a large body of catch statistics could be subjected to time series analysis in concert with known periods of virus outbreaks. These types of data may be available for foreign fisheries as well. A modeling framework could be created to examine specific hypotheses by using population, transport, and fate models that incorporate appropriate constants for infection and transmission. Sensitivity analyses could then be performed to determine which parameters are most important and contribute the most uncertainty. Participants concluded that research could then be directed to reduce uncertainty.

A.2.3.4 Summary

A.2.3.4.1 Local Impacts

The breakout group determined that there is a **Low** to **Medium** probability that local impacts will occur from the discharge of untreated liquid effluents from processing plants discharging into coastal waters. The breakout group assigned a medium ranking because of the large amount (e.g., one-half million pounds per day) of contaminated foreign farm-raised shrimp that are routinely processed with untreated effluents (Dunkelberger, personal communication). Sources of uncertainty in this assessment include the virulence and persistence of the virus and the susceptibility of the life stage of the host species. As a result, the breakout group was **Reasonably Uncertain** about the likelihood that local impacts would occur. Furthermore, participants concluded that the infrequency of local impacts to wild shrimp populations supports a **Low** to **Medium** rating for impact.

A.2.3.4.2 Large-Scale Impacts

The breakout group determined that, for several reasons, it is more problematic to estimate the consequence of establishment of virus diseases at large scales than at local scales. In addition to the sources of uncertainty described for local impacts, mechanisms are required to explain a broad-scale transmission of the virus. Breakout group members noted that, while the pre-migration "staging" behavior could serve as a plausible mechanism, its validity has not been demonstrated. The breakout group concluded that there is a **Low** probability of widespread impacts from viral disease in shrimp, but they were **Highly Uncertain** about this rating. To date, however, no evidence from field studies or catch statistics suggests large scale impacts to wild shrimp populations from virus infection.

A-3. Report of the "Other Pathways" Breakout Group

A.3.1 INTRODUCTION

The "Other Pathways" breakout group was charged with assessing the risk associated with introduction of nonindigenous virus to wild shrimp populations from pathways other than shrimp aquaculture or shrimp processing operations. The group first itemized potential pathways and then placed them in two categories: likely pathways and secondary or incidental pathways.

Likely pathways were identified as the following:

- Ballast water
- Bait shrimp
- Shrimp feed
- Animal vectors

Secondary or incidental pathways included:

- Natural spread
- Research and display facilities
- Human sewage
- Fishing vessels
- Hobby and ornamental displays
- Live seafood distribution
- Other crustacean aquaculture
- Incidental introductions

The group also discussed transplantation of wild shrimp from one location to another as a potential source of viruses, but this pathway was dismissed because such activity is illegal in all southeastern Atlantic and Gulf Coast states.

A.3.2 PROBABILITY OF ESTABLISHMENT—LIKELY PATHWAYS

The breakout group discussed and rated, using a qualitative approach, the four likely pathways for their probability of establishment. In addition to compiling their ratings, this breakout group also noted whether their supporting information came from general knowledge, judgmental evaluation, extrapolation, or cited literature (see pp. 22–24, Appendix G). Because time was limited, individual breakout group members rated the secondary or incidental pathways individually, without group discussion. A summary of risk ratings discussed by the "Other Pathways" Breakout Group for likely pathways is provided in Table A-4.

A.3.2.1 Ballast Water

Following the ANSTF approach, the breakout group estimated the probability of the organism being on, with, or in the pathway to be **High (Moderately Certain;** professional judgment). The breakout group defined the ballast water pathway to include the water itself, free virus in the water, invertebrate organisms that might or might not carry the virus (either alive or dead), and viruses associated with inorganic particulate material in the water. The breakout group considered that ballast water is used on very large container ships and oil tankers and that therefore discharges from these vessels represent a large volume to the nearshore or offshore environments. The breakout group noted that no one has ever investigated whether ballast water or any of its components contain shrimp viruses. Nonetheless, it is known that many large organisms are discharged routinely with ballast water (e.g., Carlton & Geller, 1993; Williams et al., 1988). These include species of mysid shrimp, some of which have colonized bays and estuaries with devastating effects, and the zebra mussel, which has recently colonized the Great Lakes after frequent discharges in ballast water over an extended period.

The breakout group estimated the probability of the organism surviving in transit in ballast water to be **High (Very Certain;** extrapolation from other organisms). Participants concluded that many other organisms are known to survive transit in ballast water, so there is every reason to believe that shrimp viruses could do so as well.

The breakout group estimated the probability of the organism successfully colonizing and maintaining a population where introduced to be **Low (Moderately Certain;** extrapolation from

Table A-4. Summary of other pathways breakout group risk rankings for likely pathways to the environment

Refer to supporting discussion in the text to properly evaluate information presented in this table. The risk assessment process is described in Section 2.1 and Appendix G.

Probability of Establishment	Ballast Water	Bait Shrimp		Shrimp Feed		Animal Vectors
		Foreign	Domestic	No Heat	Heat-Treated	
Association with Pathway	High/moderately certain	High/ moderately certain	Low/very certain	Medium/ moderately certain	Medium/ moderately certain	High/very or reasonably certain[1]
Entry Potential	High/very certain	High/very certain	High/very certain	High/ very certain	Low/ very certain	High/reasonably certain
Colonization Potential	Low/moderately certain	High/very uncertain	High/very uncertain	Medium/very uncertain	Medium/very uncertain	Medium to high/ relatively uncertain
Spread Potential	Medium/very uncertain	Medium/very uncertain	Medium/very uncertain	Medium/very uncertain	Medium/very uncertain	Medium/very uncertain
Overall Probability of Establishment	Low	Medium	Low	Medium	Low	Medium

[1] Very certain for gulls and freshwater and marine invertebrates; reasonably certain for other vertebrates.

other organisms). Breakout group participants noted that many organisms are introduced into exotic environments but few survive to colonize. For example, the group noted that only after 70 years of ballast water introductions did the zebra mussel successfully establish itself in the Great Lakes. For penaeid shrimp, however, colonization potential of virus discharged with ballast water will depend on whether the discharge occurs in the open ocean or in nearshore estuarine environments and on contact of the discharges with shrimp. Breakout group members recognized that neither the transmission rates of viruses in open oceans nor the infectivity of the viruses to wild populations is known; only information about laboratory infectivity rates is currently available. A breakout group member provided one example: John Couch, using a baculovirus model, had great difficulty in getting infections to transmit among shrimp. Field surveys of wild shrimp populations in Texas suggest that colonization potential is not high. Studies for the past 25 years on shrimp and other crustacean species have not revealed any new species that have colonized as a result of ballast water discharges. However, the breakout group noted that the volume of ballast water discharged into the Gulf of Mexico along the Texas and Louisiana coasts is low compared to levels discharged into California or the Great Lakes.

The breakout group estimated the probability of the organism to spread beyond the colonized area to be **Medium (Very Uncertain;** professional judgment). They believed that the virus could be spread from a small focus of live shrimp that feed on dead infected shrimp discharged with the ballast water. The spread from that focus is dependent on the infectivity threshold of the virus, the transmission rate, and the density of susceptible host species. Breakout group participants determined that each of these factors is dependent on the specific virus and may also be dependent on life stage.

The breakout group concluded that the overall probability of establishment by the ballast water route is **Low** because of the low colonization potential.

A.3.2.2 Bait Shrimp

The breakout group estimated the probability of the organism being on, with, or in the bait shrimp pathway as **High (Moderately Certain;** general knowledge) for foreign (frozen) shrimp, and **Low (Very Certain;** general knowledge) for domestic (live) shrimp. Anglers use shrimp as bait when fishing for species that naturally eat shrimp. They purchase bait from bait shops or they use shrimp sold in grocery stores for human consumption. It was noted that bait shrimp generally are smaller than those sold for human consumption and are considered substandard. It was suggested that they may originate from aquaculture facilities that have harvested their shrimp prior to full growout because of a viral outbreak. Breakout group participants noted that Latin American and Asian producers may freeze these small shrimp and ship them to the United States for sale as bait, while the larger, uninfected shrimp will be sold at premium prices for human consumption. Therefore, there is a high probability that these smaller, frozen shrimp may contain virus.

Some states (e.g., South Carolina) do not allow the use of nonnative farm shrimp as bait, but domestic aquaculture shrimp may be harvested and sold as live bait. Breakout group participants said that, although it is known that these domestic shrimp carry indigenous viruses (e.g., BP, another baculovirus), there is no evidence to date that these shrimp carry nonindigenous viruses such as those considered by the workshop. Participants noted that domestic shrimp harvested early because of virus problems are likely to be frozen, so there is a low probability that live domestic shrimp bait carry nonindigenous viruses.

The breakout group estimated the probability of the organism surviving in transit to be **High (Very Certain;** general knowledge). Participants based this determination on the knowledge that shrimp viruses would be carried in shrimp tissues. It is not likely that the freezing process will significantly reduce the virulence and infectivity of the virus. Instead this may be virus specific.

The breakout group estimated the probability of viruses from bait shrimp successfully colonizing and maintaining a population where introduced to be **High (Very Uncertain;** professional judgment). Breakout group members recognized that bait shrimp are deposited in areas where native shrimp are known to occur. Anglers fish in these spots because there is a greater likelihood of catching shrimp-feeding fish in such areas. Therefore, participants noted that the virus has a greater potential to be placed directly into a viable shrimp population. The greatest potential for colonization occurs when an angler disposes of leftover bait by dumping all remaining bait shrimp overboard and into the estuary. These shrimp will sink to the bottom and may be eaten by the native shrimp, thereby creating a direct exposure route.

The breakout group estimated the probability of viruses from bait shrimp to spread beyond the colonized area to be **Medium (Very Uncertain;** professional judgment). The virus could be spread from a small focus of shrimp feeding on discarded and infected dead shrimp. As with ballast water discharges, participants noted that the spread from this focus depends on the infectivity threshold of the virus, the transmission rate, and the density of susceptible host species. Each of these factors is dependent on the specific virus and may also depend on shrimp life stage.

The breakout group estimated the overall probability of establishment by the bait shrimp route to be **Medium** for imported foreign frozen bait shrimp and **Low** for domestic bait shrimp.

A.3.2.3 Shrimp Feed

The breakout group estimated the probability of the organism being on, with, or in the shrimp feed pathway as **Medium (Moderately Certain;** professional judgment). Shrimp feed is made from soy protein, fish protein (including anchovies and menhaden), shrimp heads, and other types of shrimp and crustaceans (e.g., *Artemia*). The breakout group agreed that some shrimp parts have a high probability of carrying viruses.

The breakout group estimated the probability of the organism surviving in transit as **Low to High (Very Certain;** extrapolation from other organisms). The probability of survival in transit depends on whether or not the feed meal is heat treated to a temperature sufficient to kill all viruses. Participants noted that some of the viruses (e.g., TSV) may survive and maintain infectivity even when heated to temperatures greater than 100 °C. While most of the fish meal produced in the United States is subjected to heat treatment that appears to be sufficient to kill the viruses, it is not known for certain that this is the case. Furthermore, workshop participants stated that other countries, such as Mexico, do not heat their meal. The breakout group determined that transit survival probability is **Low** (for heat treated) to **High** (for no treatment).

The breakout group estimated that the probability of the organism successfully colonizing and maintaining a population where introduced as a result of this pathway to be **Medium (Very Uncertain;** professional judgment). Breakout group participants noted that virus may be introduced into the environment either through use of the feed in aquaculture or through chumming, which is the dumping of feed into the marine environment to attract other shrimp or fish for easy harvest. The group estimated the risk from chumming to be **Medium** (assuming that live virus is present), because relatively large quantities of material could be dumped within a small area.

The breakout group estimated the probability of the organism to spread beyond the colonized area to be **Medium (Very Uncertain;** professional judgment). The spread of virus from the focus of introduction depends on the infectivity threshold of the virus, the transmission rate, and the density of susceptible host species. Each of these factors is dependent on the specific virus and may also depend on shrimp life stage.

As a result of their discussions, the breakout group estimated the overall probability of establishment by the shrimp feed route to be **Medium** to **Low** (depending on whether heat treatment is successful or not).

A.3.2.4 Animal Vectors

The breakout group estimated the probability of the organism being on, with, or in the animal vectors pathway to be **High (Very Certain;** published data) for gulls and freshwater and marine invertebrates and **High (Reasonably Certain;** extrapolation from other organisms) for other vertebrates. Published data indicate that TSV in shrimp consumed by gulls can be passed through the digestive tract and discharged in fecal matter. Participants noted that gulls and other scavengers (e.g., raccoons) are often seen feeding on dead shrimp and other organic matter associated with aquaculture facilities that have undergone a viral outbreak. Other data demonstrate that water boatmen (Corixids) may pick up virus from aquaculture ponds and then move to nearby natural bodies of water. It was also noted that the viruses WSSV and YHV are carried (as silent carriers, with no infection) by marine invertebrate species in Asia.

The breakout group estimated the probability of the organism surviving in transit to be **High (Reasonably Certain).** Published data have shown that these viruses can survive transmission by at least some of the pathways described previously. Survival may be virus specific, because avian guts have low pH and relatively high temperatures that could inactivate some viruses.

The breakout group estimated the probability of the organism successfully colonizing and maintaining a population where introduced to be **Medium** to **High (Relatively Uncertain**; professional judgment). As detritivores, shrimp are likely to feed on bird fecal matter. Participants observed that the potential for colonization would increase in areas where vector density is high (e.g., when a shrimp die-off occurs in an aquaculture facility, particularly if the facility is near an area that supports wild shrimp populations). Breakout group members noted that genetic variability of shrimp in Asia varies among regions. Areas with less genetic variability may be more susceptible to disease.

The breakout group estimated the probability of the organism to spread beyond the colonized area to be **Medium (Very Uncertain;** professional judgment). The virus could be spread from a small focus of infected shrimp. Breakout group members acknowledged that the spread from that focus depends on the infectivity threshold of the virus, the transmission rate, and the density of susceptible host species. In addition, these factors are very dependent on the specific virus and may also depend on shrimp life stage.

The breakout group estimated the overall probability of establishment by the vector route to be **Medium,** depending on the density of vectors and their proximity to wild populations of shrimp or the genetic diversity of the shrimp.

A.3.3 PROBABILITY OF ESTABLISHMENT—SECONDARY OR INCIDENTAL PATHWAYS

Due to time constraints, secondary or incidental pathways were not discussed during the breakout group meeting. Instead, breakout group members rated these pathways individually, using worksheets. No discussion was recorded, and any comments reflect those written on the individual participant's worksheets. A summary of risk ratings developed by the "Other Pathways" breakout group for secondary or incidental pathways is provided in Table A-5.

A.3.3.1 Natural Spread

Estimate the probability of the organism being on, with, or in the pathway: **Medium (Very Uncertain;** professional judgment). This pathway includes the spread of virus from one shrimp population in the Gulf of Mexico to other native populations through natural means, such as movement of infected shrimp or movement of viruses by hurricanes or currents.

Estimate the probability of organism surviving in transit: **High (Very Uncertain;** professional judgment).

Estimate the probability of the organism successfully colonizing and maintaining a population where introduced: High (**Very Uncertain;** professional judgment).

A.3.3.2 Research and Display Facilities

Estimate the probability of the organism being on, with, or in the pathway: **High** (**Very Certain**; published data); **Low** (**Moderately Certain**; professional judgment); **High (Very Uncertain**; professional judgment). Inoculum to the environment would usually be very small. Research facilities tend to take greater biosecurity precautions than many commercial ones.

Estimate the probability of the organism surviving in transit: **High** (**Very Certain**; published data); **High** (**Moderately Certain**; professional judgment); **High** (**Very Uncertain**; professional judgment).

Estimate the probability of the organism to spread beyond the colonized area: **High (Very Uncertain**; professional judgment).

The overall probability of establishment through natural spread is estimated to be **Medium.**

Table A-5. Summary of other pathways breakout group risk rankings for secondary or incidental pathways to the environment

Refer to supporting discussion in the text to properly evaluate information presented in this table. These pathways were rated individually by breakout group members, and there was no group discussion of these ratings. Consequences of establishment were not rated for these pathways. The risk assessment process is described in Section 2.1 and Appendix G.

Probability of Establishment	Natural Spread	Research and Display Facilities	Human Sewage	Fishing Vessels	Hobby and Ornamental Displays	Live Seafood Distribution	Other Crustacean Aquaculture	Incidental Introductions
Association with Pathway	Medium/ very uncertain	Low/ moderately certain to high/very certain	Medium/ very uncertain	Low to medium/ moderately certain	Low/ moderately certain	Low/ reasonably uncertain	Low/very uncertain to medium/ moderately certain	Low/very uncertain
Entry Potential	High/very uncertain	High/ moderately to very certain	Medium/ very uncertain	High/ reasonably certain	High/ moderately certain	High/ moderately certain	Low/very uncertain to medium/ reasonably certain	Low/very uncertain
Colonization Potential	High/ very uncertain	Low/very certain to high/very uncertain	Medium/ very uncertain	Medium/ reasonably uncertain	Low/ moderately certain	Low/ reasonably uncertain	Low/very uncertain to medium/very uncertain	Low/very uncertain

Table A-5. Summary of other pathways breakout group risk rankings for secondary or incidental pathways to the environment (continued)

Spread Potential	High/ very uncertain	Low/ relatively certain to high/very uncertain	Medium/ very uncertain	Medium/ very uncertain	Medium/very uncertain	Medium/very uncertain	Low/very uncertain to medium/very uncertain	Low/very uncertain
Overall Probability of Establishment	Medium	Low to medium	Medium	Low to medium	Low	Low	Low to medium	Low

Estimate the probability of the organism successfully colonizing and maintaining a population where introduced: **Low (Very Certain**; published data); **Low (Moderately Certain to Very Uncertain;** professional judgment**); Medium to High (Very Uncertain**; professional judgment). This estimate assumes that the research facility is working with organisms that have not been tested to ensure they are Specific Pathogen-Free (SPF) before introduction to the lab. For labs that are specifically involved in research on SPF organisms, the probability would be rated as low.

Estimate probability of organism to spread beyond the colonized area: **Low (Relatively Certain;** general knowledge); **Medium (Very Certain to Very Uncertain;** professional judgment); **Medium to High (Very Uncertain;** professional judgment).

The overall probability of establishment through research and display facilities is estimated to be **Low to Medium.**

A.3.2.3 Human Sewage

Estimate the probability of the organism being on, with, or in the pathway: **Medium (Very Uncertain;** professional judgment).

Estimate the probability of the organism surviving in transit: **Medium (Very Uncertain;** professional judgment).

Estimate the probability of the organism successfully colonizing and maintaining a population where introduced: **Medium (Very Uncertain;** professional judgment).

Estimate probability of organism to spread beyond the colonized area: **Medium (Very Uncertain;** professional judgment).

The overall probability of establishment through human sewage is estimated to be **Medium.**

A.3.2.4 Fishing Vessels

Estimate the probability of the organism being on, with, or in the pathway: **Low to Medium (Moderately Certain;** professional judgment).

Estimate the probability of the organism surviving in transit: **High (Reasonably Certain;** professional judgment).

Estimate the probability of the organism successfully colonizing and maintaining a population where introduced: **Medium (Reasonably Uncertain;** professional judgment).

Estimate probability of organism to spread beyond the colonized area: **Medium (Very Uncertain;** professional judgment).

The overall probability of establishment through fishing vessels is estimated to be **Low to Medium.**

A.3.2.5 Hobby and Ornamental Displays

Estimate the probability of the organism being on, with, or in the pathway: **Low (Moderately Certain;** professional judgment).

Estimate the probability of the organism surviving in transit: **High (Moderately Certain;** professional judgment).

Estimate the probability of the organism successfully colonizing and maintaining a population where introduced: **Low (Moderately Certain;** professional judgment).

Estimate probability of organism to spread beyond the colonized area: **Medium (Very Uncertain;** professional judgment).

The overall probability of establishment through hobby and ornamental displays is estimated to be **Low.**

A.3.2.6 Live Seafood Distribution

Estimate the probability of the organism being on, with, or in the pathway: **Low (Reasonably Uncertain;** professional judgment). There is very little live seafood imported into the United States.

Estimate the probability of the organism surviving in transit: **High (Moderately Certain;** professional judgment).

Estimate the probability of the organism successfully colonizing and maintaining a population where introduced: **Low (Reasonably Uncertain;** professional judgment).

Estimate probability of organism to spread beyond the colonized area: **Medium (Very Uncertain;** professional judgment).

The overall probability of establishment through live seafood distribution is estimated to be **Low.**

A.3.2.7 Other Crustacean Aquaculture

Estimate the probability of the organism being on, with, or in the pathway: **Low (Very Uncertain**; professional judgment); **Medium (Moderately Certain**; professional judgment); **Low (Very Uncertain**; professional judgment).

Estimate the probability of the organism surviving in transit: **Low (Very Uncertain;** professional judgment); **Medium (Reasonably Certain**; professional judgment); **Low (Very Uncertain;** professional judgment).

Estimate the probability of the organism successfully colonizing and maintaining a population where introduced: **Low (Very Uncertain;** professional judgment); **Low (Reasonably Certain;**

professional judgment); **Medium (Very Uncertain;** professional judgment). Crayfish are freshwater species and crayfish farms are not as close to coastal waters as shrimp farms.

Estimate probability of organism to spread beyond the colonized area: **Low (Very Uncertain;** professional judgment); **Medium (Very Uncertain;** professional judgment); **Low (Very Uncertain;** professional judgment).

The overall probability of establishment through other crustacean aquaculture is estimated to be **Low to Medium.**

A.3.2.8 Incidental Introductions

Estimate the probability of the organism being on, with, or in the pathway: **Low (Very Uncertain;** professional judgment).

Estimate the probability of the organism surviving in transit: **Low (Very Uncertain;** professional judgment).

Estimate the probability of the organism successfully colonizing and maintaining a population where introduced: **Low (Very Uncertain;** professional judgment).

Estimate probability of organism to spread beyond the colonized area: **Low (Very Uncertain;** professional judgment).

The overall probability of establishment through incidental introductions is estimated to be **Low.**

A.3.4 CONSEQUENCES OF ESTABLISHMENT IN "OTHER PATHWAYS"

To begin its discussions of the consequences of establishment, the breakout group was presented with the assumption that it is difficult to start an epizootic but eventually one will occur, given continued input of virus to the estuarine or marine environments.

The group agreed that this basic premise is valid, although some members preferred to say that an epizootic "might" rather than "will" occur. The breakout group noted that it is very difficult to infect animals, even in laboratory settings. In some studies, attempts to infect *P. vannamei* postlarvae with WSSV by feeding resulted in 100 percent survival (Overstreet et al., 1997). Many factors influence the susceptibility of shrimp to experimental virus infection, including host species, the manner in which the virus is prepared and stored, and environmental conditions in which the shrimp are maintained. In addition, an infected shrimp may or may not exhibit clinical signs of infection and may or may not die from the disease. The group briefly discussed which of the four viruses of concern would be most likely to cause a natural epidemic. The group thought that WSSV and YHV are more likely than IHHNV or TSV to cause acute mortality but that IHHNV and TSV are more likely to become endemic.

A breakout group member stated that genetic resistance is likely to differ among populations. Without further knowledge of this variability among Gulf Coast shrimp, for example, it is difficult to make accurate predictions about which area has the highest potential for an epizootic. An individual also noted that a published paper from Thailand shows that southern populations of shrimp are much less genetically diverse than those from the northern part of the country. Participants noted that it has been hypothesized that these differences are due to release of shrimp from aquaculture into the wild.

Breakout group members observed that if a virus is successfully introduced into an estuary and wipes out the entire local shrimp population, the effects are likely to be short-term. They noted that repopulation could occur in 3 to 5 years, or perhaps sooner (see Figure A-4). This estimate is based purely on professional judgment and not on any hard data. Similar population impacts and recoveries have been observed from natural stressors such as low temperatures or freshwater flooding. Breakout group members pointed out that recovery from winter kills may occur within 1 year (Figure A-4). The group indicated that information is needed to better determine whether the shrimp that recolonize an area differ genetically from the original stock.

The breakout group also discussed the shrimp-virus interaction, noting that the target organ of the virus may influence its infectivity and be dependent on the life stage of the shrimp. For example, juvenile shrimp have a larger gut-to-body mass ratio than older shrimp and are therefore more susceptible to the viruses (such as TSV) that replicate in gut epithelium. Participants recognized that much more information is needed on the shrimp immune response to viral infection. Some noted that viruses are typically able to escape cellular immune mechanisms such as hemocytes or macrophages, by moving from cell to cell rather than through the hemolymph. Participants concluded that coupling the understanding of target-organ sensitivity with information about resistance will improve the ability to predict which shrimp are likely to become carriers. Virus carriers may have active infections (perhaps systemic) and continuously shed virus, or they might be silent carriers with the virus sequestered in particular organs and expressed only during times of stress.

The breakout group also discussed whether a shrimp population would develop tolerance following a major virus disease outbreak. If the population were to develop tolerance, the virus could remain endemic in the population, and disease outbreaks could occur cyclically. Population numbers may be stable but at a lower level than would be present in the absence of the virus. One individual suggested that information on wild shrimp population levels before and after the introduction of TSV into Honduras and Ecuador may provide insight into this hypothesis.

The breakout group briefly discussed cross-species transmission (shrimp-to-shrimp or shrimp-to-other-crustacea) and speculated that virulence may change during such a passage. Some evidence exists that these viruses can replicate in crabs or other shrimp without causing disease symptoms; however, it is unknown whether this would increase or decrease virulence, although one individual pointed out that all viruses change genetically over time.

The breakout group noted that it would be very difficult to diagnose the cause of a decline in a population of shrimp because many factors interact to cause natural population fluctuations of up to 25 percent per year. They concluded that identification of virus in the shrimp would indicate that the virus may have played a part in the change, but it would not establish a cause-and-effect relationship.

The potential impact of viruses on the entire shrimp population is unknown. Some participants suggested that natural mortality rates in shrimp approach 100 percent and that approximately 90 percent of the shrimp are harvested before they die. Virus-induced mortality, therefore, should not be biologically significant. Virus-induced mortality, however, may have economic significance if the shrimp are killed before reaching harvestable size. One breakout group member pointed out that although the mortality in the postlarval shrimp that leave the estuary is naturally high, it must be less than 100 percent or there would be no shrimp left to reproduce. Participants suggested that complete mortality of a single estuary's shrimp (which may occur following a virus outbreak) may not have a significant impact on the overall population. Recolonization of the estuary would occur as shrimp from nearby locations drift in on currents in subsequent years; however, as stated previously, recolonization may take from 3 to 5 years (or less if the population responds in a similar fashion as it does to natural stressors, such as temperature). The breakout group concluded that, in the short term, the alteration of the estuarine ecosystem could be substantial.

The breakout group also discussed the potential for viruses to affect estuarine ecology by infecting other species of shrimp, such as grass shrimp. Participants noted that grass shrimp (*Paleomonetes sp.*) are an important part of the estuarine food web. Many species of fish (and penaeid shrimp) rely on grass shrimp as an important prey item. Data from Thailand suggest that grass shrimp may be carriers of one or more of these viruses, but data on infectivity rates and effects are lacking.

The breakout group acknowledged that an important area of uncertainty is whether viruses that are endemic in shrimp populations have the potential to change the population's reproduction rate. A change in the reproduction rate could occur either by directly affecting the number or viability of gametes produced or by reducing growth and subsequent reproduction of offspring of infected individuals. Without this information, the breakout group concluded that it will not be possible to make any statements about population consequences beyond the educated guesses outlined previously.

A.3.5 RESEARCH NEEDS IDENTIFIED BY THE "OTHER PATHWAYS" BREAKOUT GROUP

The breakout group identified the following important research needs:

- Tests for virus identification are critical. Tests must have specificity for the virus and be standardized across labs. Tests should be useable on different shrimp species, live shrimp, dead shrimp (frozen or fresh), and pieces of shrimp tissue.

- Tests for infectivity are needed to establish the threshold number of viruses that would be required for colonization potential. At least two tests such as a PCR and ELISA or a PCR and a bioassay, should be employed. Natural susceptibility of native shrimp to nonindigenous viruses needs to be documented better, including looking for differences among genetic strains or within populations with more or less genetic diversity.

- Virus inactivation parameters should be better identified. The amount of duration of heat treatment for reactivation of the various viruses should be studied systematically. Also, other environmental factors that could inactivate the virus (e.g., dryness and ultraviolet light) should be elucidated to understand how long a virus can persist outside its host.

- A map of the known locations around the world of virus prevalence in shrimp should be created so that potential sources can be identified. Because general surveys have not been done widely, areas in which the virus is not identified as prevalent may or may not be infected.

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APPENDIX B

PEER REVIEW EXPERTS AND BREAKOUT DISCUSSION ASSIGNMENTS

United States
Environmental Protection Agency
National Center for Environmental Assessment

Shrimp Virus Peer Review Workshop

Crystal Gateway Marriott Hotel
Arlington, VA
January 7-8, 1998

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Shrimp Virus Peer Review Workshop

Crystal Gateway Marriott Hotel
Arlington, VA
January 7-8, 1998

Breakout Discussion Assignments

Aquaculture	Shrimp Processing	Other Sources
Wayne Munns, Leader	Jack Gentile, Leader	Anne Fairbrother, Leader
Kay Austin, Notetaker	Scott Warner, Notetaker	Bill van der Schalie, Notetaker
Bill Fisher	Ned Alcathie	Acacia Alcivar-Warren
Rebecca Goldburg	Dwaine Braasch	Mark Berrigan
Howard Harder	Dana Dunkelberger	Larry McKinney
Donald Lightner	Jeff Lotz	Paul Sandifer
Gary Pruder	Roy Martin	Max Summers
Suzanne Thiem		Shiao Wang
Gerardo Vasta		
Fritz Jaenike		

APPENDIX C

PREMEETING COMMENTS PREPARED BY EXPERTS

Shrimp Virus Peer Review Workshop

Premeeting Comments

Arlington, VA
January 7-8, 1998

Prepared and compiled by:
Eastern Research Group, Inc.
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Table of Contents

Peer Reviewer Comments

Charge to Experts

Please prepare your written comments to address the questions posed below. The first 18 questions are organized based on elements of the ecological risk assessment process, as described in the shrimp virus report (which is located in the Minutes of the Stakeholder Meetings on the Report of the JSA Shrimp Virus Work Group). Questions 19 to 22 ask your opinion about the need for a comprehensive risk assessment; this topic will be discussed during the last half-day session at the workshop. You may also address other issues that you feel are important. All written premeeting comments will be distributed to other experts prior to the workshop and may be included as an appendix to the final workshop report.

Management goals, assessment endpoints, and the conceptual model

1. How well does the management goal reflect the dimensions of the shrimp virus problem?

2. Some have suggested modifying the assessment endpoints to emphasize potential risks of shrimp viruses to non-shrimp organisms and the larger estuarine ecological system or, alternatively, to the aquaculture industry. Please comment on the assessment endpoints as the focal point for the ecological risk assessment.

3. It has been suggested that the scope of the proposed risk assessment is too narrow and that it should be broadened to consider the impacts of such stressors as alternative land uses and seafood production methods in coastal areas. Please comment on this suggestion.

Viral stressors and factors regulating shrimp populations

This topic includes basic information about shrimp viruses as well as the full range of natural and anthropogenic factors that regulate shrimp populations. Questions for consideration:

4. How relevant to virus effects on wild populations is information on infectivity and effects that is derived from laboratory or intensive aquaculture operations?

5. How likely is it that exposure of wild shrimp populations to viral diseases could lead to the development of immunity and reduced effects on population survival over time?

6. How can the strong influence of both natural and non-viral anthropogenic factors on shrimp populations be separated from risks associated with viral stressors?

7. Can human health effects from shrimp viruses be ruled out as a concern? Why or why not?

8. Are the available identification techniques for shrimp viruses reliable enough to allow definitive conclusions to be drawn about the occurrence of viruses in shrimp and environmental media?

Viral pathways and sources

The shrimp virus work group considered aquaculture and shrimp processing to be the primary pathways of concern leading to exposure to pathogenic shrimp viruses, but it also identified a number of other potential pathways. Some related questions are listed below.

Aquaculture

9. U.S. aquaculture operations have had problems with viral diseases for several years. How does information from local wild shrimp populations support or refute the importance of aquaculture operations as a source for the virus?

10. It has been widely held that it is highly unusual for domesticated animals to infect wild animal populations; usually it is the other way around. How well does this observation apply to the relationship between shrimp in aquaculture and wild shrimp populations, with regard to shrimp viruses?

Shrimp processing

11. Some believe it likely that shrimp processing operations have processed virus-infected shrimp from foreign sources for several years. How does information from local wild shrimp populations support or refute the importance of shrimp processing as a potential source for the virus?

12. Should the retailers who distribute (rather than process) shrimp products receive additional evaluation as potential sources of exposure?

Other potential sources and pathways

13. After considering the sources addressed in the shrimp virus report, what sources other than aquaculture and shrimp processing are most critical for evaluation in a risk assessment of shrimp viruses? Given time constraints, which of these should be the focus of discussion at the workshop?

14. Is manufactured shrimp feed a potential virus source, or is the processing temperature sufficient to rule this source out?

<u>Stressor effects</u>

These next questions concern the possible consequences to wild shrimp populations and marine communities from exposure to pathogenic shrimp viruses.

15. How should the available evidence concerning the effects of introduced viruses on wild shrimp populations be interpreted? (For example, what was the role of IHHNV in the decline of shrimp populations in the 1980's in the Gulf of California? What about TSV release from aquaculture into the wild in South America?)

16. There is presently a lack of basic data on background levels of pathogenic shrimp viruses in wild shrimp populations in U.S. waters. How should this data gap be evaluated in a risk assessment?

17. How can changes in wild shrimp populations be used to interpret the effect (or lack of effect) of introduced shrimp viruses? How could shrimp population models be used in the future?

18. How important are potential viral effects on non-shrimp species?

Comprehensive risk assessment and research needs

19. How will a comprehensive risk assessment contribute to management of the shrimp virus problem, i.e., will it add significantly to the information presently available?

20. What type of assessment should be conducted next (e.g., quantitative risk estimates using shrimp populations models), and what would be the likely time frame and cost?

21. Should a future risk assessment consider the risk reduction potential of a range of treatment options associated with specific exposure scenarios?

22. Summarize the critical research needs for completing such a risk assessment.

Peer Reviewer Comments

Ned Alcathie

NED ALCATHIE

12/17/97

Responses to Charge to Panel members (Shrimp Virus Workshop):

Management goals, assessment endpoints, and conceptual model

1. The management goal appears to adequately reflect the shrimp virus problem.

2. Modifying the assessment endpoints to emphasize potential risks to non-shrimp organisms, the estuarine ecological system or the aquaculture industry appears to be very wide in scope. However, information on these areas of concern may be useful during determinations of final endpoints.

3. In order to have for the risk assessment to be manageable I feel it should remain narrowly focused. Seafood processing in coastal areas should be considered since this may be possibly a significant source of introduction of viruses into the wild shrimp population.

Viral stressors and factors regulating shrimp populations

4, 5, 6, 7, 8 - unable to answer with any degree of certainty, best left to those with backgrounds in virology.

Viral pathways and sources

Aquaculture

9, 10 - unable to answer

Shrimp processing

11. As far as I know little if any information exists which would support or refute the importance of shrimp processing as a potential source of the virus. It is possible that the processing industry is contributing to the introduction of viruses since many facilities in coastal areas discharge untreated water used in processing directly into rivers, bays and the Gulf of Mexico.

12. It is doubtful that retailers would constitute more than a minimal risk.

Other potential sources and pathways

13. Unable to answer

14. The only processor of shrimp plant wastes (shells, heads, etc.) that I am familiar with uses a drying process that begins at approximately 1000 deg F, 20-30 minutes later the end product exits the dryer at approximately 200 deg F, with a moisture content of 8-9%. This would seem to rule out a potential source of the virus.

Stressor effects

15, 16, 17, 18 - unable to answer

Comprehensive risk assessment and research needs

19, 20, 21, 22 - unable to answer

Acacia Alvicar-Warren

Management goals, assessment endpoints, and the conceptual model

1. How well does the management goal reflect the dimensions of the shrimp virus problem?

The shrimp virus problem is a very broad problem with many dimensions both in and outside the shrimp industry. As long as the stated management goal of "Prevent the establishment of new disease-causing viruses in wild populations of shrimp" is interpreted very broadly, I agree with most of it with two exceptions. First, the geographic coverage should be enlarged to include the US Pacific coast. Second, because contributing factors to the shrimp virus problem may reside in many industries and activities seemingly unrelated to the shrimp industry, the portion of the management goal that refers to "minimizing possible impacts" should not be limited to "shrimp importation, processing, and aquaculture operations" but instead should be broadened to include minimizing the impacts on all industries and activities that are found to contribute to the shrimp virus problem. For example, the destruction of estuarine habits and environmental degradation might prove to be a significant source of new viruses.

2. Some have suggested modifying the assessment endpoints to emphasize potential risks of shrimp viruses to non-shrimp organisms and the larger estuarine ecological system or, alternatively, to the aquacultture industry. Please comment on the assessment endpoints as the focal point for the ecological risk assessment.

The two assessment endpoints suggested by the Shrimp Virus Work Group should be the focal points for the ecological risk assessment:

1. "Survival, growth, and reproduction of wild penaeid shrimp populations", and

2. "Ecological structure and function of coastal and near-shore marine communities as they affect wild penaeid shrimp populations"

Point 1, however, should be broadened to include the US Pacific coast.

Point 2, I would agree that there is a need to emphasize potential risks of shrimp viruses to non-shrimp organisms and the larger estuarine ecological system. However, a comprehensive epidemiological / genetic study should first be performed in order to obtain baseline information on both the genetic structure and the prevalence of the viruses in the natural penaeid shrimp populations.

A healthy estuarine ecological system will supply the virus-free wild shrimp stocks needed to support the aquaculture industry.

3. It has been suggested that the scope of the proposed risk assessment is too narrow and that it should be broadened to consider the impacts of such stressors as alternative land uses and seafood production methods in coastal areas. Please comment on this suggestion.

I agree with this statement and recommend that it should be broadened to include all stressors associated with "alternative land uses and seafood production methods in coastal areas" including (1) habitat destruction, (2) chemicals and environmental contaminants, and (3) introduction of exotic species and release of cultured stocks.

For point (1), the impact of habitat (mangrove) destruction on the production rate of wild shrimp is well documented (Jothy, 1984, Lahman *et al.*, 1987; Paw and Chua, 1991). The presence of mangroves has been found positively correlated with nearshore yield of shrimp (Paw and Chua, 1991). The loss of mangroves translates into a direct loss of habitat and species diversity of an unknown magnitude and has been suggested as the dominant cause of the decline in the abundance of wild shrimp postlarvae in Ecuadorian estuaries (Lahman *et al.*, 1987; Twilley, 1989; Parks and Bonifaz, 1994).

For point (2), intensive levels of industrial shrimp farming has also brought about an increased use of chemicals and other products which can cause marine pollution (Primavera, 1993). Mortalities and morphological deformities in shrimp larvae caused by the widespread use of such chemicals as oxytetracycline, nitrofurans, chloramphenicol, malachite green and copper sulfate have been reported (ibid.).
Pathogenic bacteria causing luminous vibriosis in shrimp larvae were found to be resistant to antibiotics and it is now a serious problem in various countries in Southeast Asia. The direct effects of these chemotherapeutants and antibiotics on humans constitute a public health concern.

For point (3), exotic shrimp species have been introduced to various countries for many decades with ecosystem-wide repercussions. The problems include hybridization, competition, introduction of new diseases, or lead to genetic changes in the wild population (Rosenthal, 1980; Brock, 1992; Sinderman, 1992). The release of exotic shrimp from cultured populations has been documented in the Atlantic coast of the United States (Wenner and Knott, 1992) where native Pacific stocks of *P. vannamei* and presumably escapees from a shrimp farm, were found in offshore samples. The *P. vannamei* in the Atlantic coast was estimated to be at ~7% of the total shrimp sampled. The presence of a sexually mature *P. vannamei* males off South Carolina suggested the potential for interbreeding (Wenner and Knott, 1992). Moreover, considering that some cultured stocks are potentially inbred and genetically susceptible to viral diseases (Alcivar-Warren *et* *al.*, 1997) there is a possibility that they could also serve as a reservoir for rapid multiplication of the viruses and spread of diseases.

Viral stressors and factors regulating shrimp populations

4. How relevant to virus effects on wild populations is information on infectivity and effects that is derived from laboratory or intensive aquaculture operations?

Is the only way to measure viral threats to date. Research is needed to demonstrate virus infectivity in samples from aquaculture and wild shrimp populations.

5. How likely is it that exposure of wild shrimp populations to viral diseases could lead to the development of immunity and reduced effects on populations survival over time?

Basic research on the immune system of shrimp needs to be performed before this question can be addressed.

Research funds should be directed to study both immunology and genomics of shrimp. Studies on the molecular biology and evolution of shrimp viruses as well as the cellular mechanisms involved in the recognition and interaction of the virus with the host genome will help to understand species-specific disease expression.

It is possible that because of the apparent lack in shrimp of the major immune (T and B) cells present in fish and other vertebrate species, a mechanism of "adaptive immunity" has evolved in shrimp species which may reduce the effects of viruses on population survival over time. This hypothesis need to be tested first.

6. How can the strong influence of both natural and non-viral anthropogenic factors on shrimp populations be separated from risks associated with viral stressors?

I doubt that the influence of both natural and non-viral anthropogenic factors on shrimp populations can be separated from risks associated with viral stressors.

The possibility exists that the environmental pollutants (e.g. heavy metals and pesticides) present in the estuarine ecosystem are of such magnitude that they also weaken the shrimp immune system making the animals even more susceptible to a viral pathogenic attack. Pollutants like the heavy metals mercury and cadmium are also known to accumulate in marine organisms, including shrimp, and cause rapid genetic changes (Nevo *et al.*, 1986). Moreover, the impact to the natural populations caused by the release of cultured stocks also need to be considered in the risk assessment. Some cultured stocks are potentially inbred and genetically susceptible to viral diseases (Alcivar-Warren *et al.*, 1997) and may serve as a reservoir for rapid multiplication of the virus and disease transmission.

7. Can human health effects from shrimp viruses be ruled out as a concern? Why or why not?

Nothing should be ruled out pertaining to virus diseases. More basic research is needed in order to understand the biology and mutation rate of the viruses.

Viral samples should be stored to maintain a shrimp virus database for future studies on infectivity, mutation rates and potential transmission to other species.

Government agencies should begin monitoring / inspecting shrimp imported for human consumption. Other shrimp diseases (vibrios in particular) should not be ignored as they represent a real threat to human health.

8. Are the available identification techniques for shrimp viruses reliable enough to allow definitive conclusions to be drawn about the occurrence of viruses in shrimp and environmental media?

More research is needed to develop sensitive molecular (quantitative RT-PCR) and immunological (antibodies) techniques to screen for the viruses (particularly TSV and YHV) in various samples including tissues from wild populations, manufactured feed and environmental media. This is an important issue for the risk assessment as viral detection can be tissue-specific and various tissues may need to be tested from each animal. For example, sensitivity of detection of WSSV by PCR depends on the tissue selected, being more sensitive in hepatopancreas and pleopods than in hemolymph of *P. monodon* DNA (Lo, personal communication).

Viral pathways and sources

9. US aquaculture operations have had problems with viral diseases for several years. How does information from local wild shrimp populations support or refute the importance of aquaculture operations as a source for the virus?

Though it appears that the guidelines recommended by the US Marine Shrimp Farming Program have not always been followed by the aquaculture industry, there is no published data to support or refute the importance of aquaculture operations as a source for the virus, nor do I believe that we would be able to document it with the current detection technologies and lack of information about the prevalence of the viruses in the wild populations.

I recommend that the analysis of the natural population be performed first. The first step should be to develop epidemiological and genetic baseline information. See my comments under questions 4, 15, 20 and 22.

Also, we need to study the possibility that cultured stocks, if released into the estuarine environment, may transfer these and other unidentified viral pathogens and may influence the fitness of the natural shrimp populations. See my comments under question 10 below.

10. It has been widely held that it is highly unusual for domesticated animals to infect wild animal populations; usually it is the other way around. How well does this observation apply to the relationship between shrimp in aquaculture and wild shrimp populations, with regard to shrimp viruses?

This is perhaps one of the most important questions that remain to be answered. If it proves to be the case the wild populations are immune or much less susceptible or able to recover on their own from viral attacks, then that would seem to argue strongly that either shrimp farming procedures or shrimp broodstock breeding programs need to be changed.

No scientific research has been performed to date to document the impact of domesticated populations into the natural populations. It is possible that viral diseases may spread if cultured stocks are accidentally or intentionally released into the wild. Even if these cultured stocks are free of the virus, their susceptibility could make them a reservoir for the virus to multiply even faster.

International efforts should be made to help other countries to properly discard diseased shrimp from viral epidemics, effluent from aquaculture facilities, waste from processing plants and untreated human sewage from local communities surrounding the estuary ecosystem.

Shrimp processing

11. Some believe it likely that shrimp processing operations have processed virus-infected shrimp from foreign sources for several years. How does information from local wild shrimp populations support or refute the importance of shrimp processing as a potential source for the virus?

Unable to make an statement at this time. We need a baseline epidemiological / genetic study on he natural population first.

Research funds are needed to examine the impact of releasing cultured stocks (potentially inbred and genetically susceptible to viral diseases) into the natural population. The American industry should also take precautions when exporting these stocks to other countries in Latin America or across continents.

A genetic risk assessment from the other country should be required first. This movement of shrimp needs to be more closely monitored.

If we are really serious about preventing viral and other diseases in the US wild shrimp populations, and aprotect human health, we should begin immediately a federal monitoring program aimed at screening for the presence of the viruses in the shrimp food and in live and frozen shrimp brought into the US.

12. Should the retailers who distribute (rather than process) shrimp products receive additional evaluation as potential sources of exposure?

Yes - at least with a small study that would make a reliable estimate as to whether they are a large or small contributing factor. If large, continue study. If small, stop.

May need international cooperative agreements with exporting countries.

Other potential sources and pathways

13. After considering the sources addressed in the shrimp virus report, what sources other than aquaculture and shrimp processing are most critical for evaluation in a risk assessment of shrimp viruses? Given time constraints, which of these should be the focus of discussion at the workshop?

Other sources most critical for evaluation in a risk assessment of shrimp viruses are:

- habitat destruction and environmental contaminants
- impact of potentially inbred and genetically susceptible cultured stocks on the wild populations
- international trade in brood and seed stocks
- manufactured feed - fish meal from South American countries, is used to prepare shrimp feeds in Southeast Asia - is the food processed at >100°C?
- what about other species (including humans) as sources?
- for a pathway, what about other vehicles such as human sewage or the wastes of other industries from countries surrounding US coastal waters?

14. Is manufactured shrimp feed a potential virus source, or is the processing temperature sufficient to rule this source out?

Don't know. Additional research is needed to demonstrate that manufactured shrimp feed is a potential virus source and should be further investigated, particularly considering the preliminary information about the possibility that infectivity of TSV is maintained after boiling at 100°C (Lotz, USMSFP Progress Report, preliminary information). This is important because a large percentage of the supply of fish meal and other ingredients used by the shrimp feed industry originates from South American countries where TSV disease is endemic.

Stressor effects

15. How should the available evidence concerning the effects of introduced viruses on wild shrimp populations be interpreted? For examples, what was the role of IHHNV in the decline of shrimp populations in he 1980's in the Gulf of California? What about TSV release from aquaculture into the wild in South America?)

There is not nearly enough evidence yet on the effects of introduced viruses on wild shrimp populations for valid conclusions to be drawn.

This is probably the most important area for research and the following should be addressed:

- Publish yearly census of wild shrimp populations.
- Save virus samples year by year in order to determine if the viruses have mutated or if shrimp really have developed immunity - need to develop monoclonal antibodies to help differentiate virus strains.
- Regarding "the role of IHHNV in the decline of shrimp populations in he 1980's in the Gulf of California", how can a population come back from a virus attack such as IHHNV after 7 years? Are there good records of the yearly census of the wild populations in the area? May be other non-virus, environmental, anthropogenic factors (e.g. destruction of the mangrove habitat, weather parameters, salinity, El Nino, etc.) influenced the population decline.
- The statement about "TSV release from aquaculture into the wild in South America" should be considered with caution. While some cultured shrimp stocks are known to have low levels of genetic diversity (Garcia *et al.*, 1994; Sunden and Davis, 1991) and are genetically susceptible to most viruses (Alcivar-Warren *et al.*, 1997) a proper monitoring of the industry trade activities and the epidemiology/genetic structure of the wild South American shrimp have not been done. The potential impact of environmental degradation on the health of wild shrimp populations on the Gulf of Guayaquil, Ecuador is well documented (see my comments under question #3 above). It is possible that environmental stressors (water quality and toxicants like heavy metals and PCBs) affected the immune system of the wild penaeid populations making them susceptible to Taura Syndrome epidemics and other viral and bacterial diseases.
- The possibility that cultured stocks released into the marine environment may impact the natural population and should be included as an endpoint of the risk assessment.

- The impact of human activities (sewage treatment, etc.) and industrial toxicants (oil and agricultural runoffs) in both US and Mexico communities surrounding the Gulf of Mexico and Pacific coastal waters.

16. There is presently a lack of basic data on background levels of pathogenic shrimp viruses in wild shrimp populations in US waters. How should this data gap be evaluated in a risk assessment?

This is a key gap and should be evaluated first through a comprehensive epidemiological / genetics study that includes the participation of research teams that include epidemiologists, virologists, immunologists, veterinarians, marine biologists and populations geneticists.

The lack of basic data on background levels of pathogenic shrimp viruses and the genetic structure of the shrimp natural populations should be the first issue to be addressed in the risk assessment.

17. How can changes in wild shrimp populations be used to interpret the effect (or lack of effect) of introduced shrimp viruses? How could shrimp population models be used in the future?

Until we have a baseline information on the genetic structure of the wild shrimp populations and the presence / absence of different viruses, we will not be able to make this interpretation.

18. How important are potential viral effects on non-shrimp species?

Research is needed and baseline information on the presence of the viruses on non-shrimp species should be obtained first.

19. How will a comprehensive risk assessment contribute to management of the shrimp virus problem, i.e., will it add significantly to the information presently available?

Yes - if it is done broadly enough. If the risk assessment is done in a narrow fashion (i.e., concentrating only on the aquaculture shrimp industry), then it will likely not be very useful.

20. What type of assessment should be conducted next (e.g. quantitative risk estimates using shrimp populations models), and what would be the likely time frame and cost?

A more holistic approach to quantitative risk assessment is needed. At all costs, the risk assessment should be performed immediately and focus first on the development of baseline information on epidemiology and genetic structure of the natural populations. The following goals should be addressed:

1. study the genetic structure and effective population size of the wild penaeid species in US coastal waters (*P. aztecus, P. setiferus* and *P. duorarum*) as well as the species used by the aquaculture industry (*P. vannamei* and *P. stylirostris*).

2. determine the prevalence of viral (DNA and RNA) sequences in the same samples of wild shrimp from which the genetic data is derived.

3. maintain a genetic database of shrimp viral sequences obtained from different geographic regions representatives of different estuarine habitats.

This will be a long-term and expensive project aimed at documenting population changes in time and space but it should be performed if we are really serious about preventing diseases and protecting the wild shrimp populations. It t will be impossible to tell if you are having success in managing disease without the baseline information.

In the meantime, government agencies should join efforts to put a moratorium on the importation of foreign shrimp (for all uses, food and aquaculture) until exporting countries agree that their frozen shrimp products need to be tested (similar to the current practices with cattle diseases).

At all costs, the industry should also be proactive regarding environmental issues and controlling spread of diseases by stopping the movement of shrimp species across regions. For example, *P. stylirostris* has been moved from the Pacific coast to the Atlantic coast of Venezuela. The stocks used by the industry should also be genetically diverse and free of diseases, pond by pond.

With high fecundity species such as shrimp, immunity may be on a population basis rather than an individual basis. If this is the case, then it might mean that we need to fundamentally change the approaches that we use in developing shrimp breeds for use in acquaculture. For example, it might be better to use tagged offspring (using molecular markers) from a large group of genetically different individuals/species in a pond rather than from a few.

21. Should a future risk assessment consider the risk reduction potential of a range of treatment options associated with specific exposure scenarios?

Yes.

22. Summarize the critical research needs for completing such a risk assessment.

The most critical areas of research needed are:

1. Perform an epidemiological / genetics study to develop baseline information on the wild shrimp population. Yearly census of the wild populations is needed.
2. Asses the risk posed to the wild shrimp populations because of accidental or intentional release of cultured stocks. The first step is to know the structure of the wild populations in their natural range.
3. Research the impact of other stressors (e.g. habitat destruction, PCBs and heavy metals, exotic introductions, weather changes, El Nino, gene flow, salinity, processing plants and pond wastes, infected bait shrimp, human waste, non-shrimp hosts/carriers) which may affect the health of natural shrimp populations.
4. Fund studies on shrimp immuno-genetics.
5. May need to fund Mexican participation on the first three issues above. A "fortress America" approach will not work.

Finally, consumers should be reassured that the food we are eating is properly inspected. Federal agencies should better define and coordinate their activities on importation, interstate movement, release of live animals and waste management in order to prevent future threats to wild shrimp populations, aquatic ecosystems and aquaculture; and to protect human health.

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Mark Berrigan

COMMENTS:SHRIMP VIRUS WORK GROUP
Mark Berrigan

1. The draft management goal reflects the ecological and economic elements associated with the potential establishment of marine shrimp viruses. The draft management goal does not include scientific confirmation that a specific problem exists or its specific ecological consequences.

2. The proposed endpoints are very broad. Without previous understanding of the potential problems associated with introducing shrimp viruses, the reader might not make a connection between the potential risks of disease and the proposed endpoints. Should the stressor of concern be incorporated in the assessment endpoint?.

3. Initially, the risk assessment should focus on potential ecological implications. Broadening the scope of the risk assessment should be a consequence of preliminary analyses, findings and recommendations.

4. Relevance is an important consideration, and should be determined through good science.

5. Uncertain -

6. Uncertain -

7. Uncertain -

8. Uncertain -

9. There is little scientific information to confirm or refute the occurrence of epizootics among wild shrimp populations associated with naturally occurring or introduced viruses. Obviously, resolving this issue is problematic. However, this is a critical element in determining the direction of the risk assessment. Assessing the likelihood of potential epizootics should be an assessment endpoint, at least in the initial phases of the risk assessment plan

10. Uncertain -

11. As far as I know, there is no strong link between processed shrimp or process wastes and shrimp epizootics. However, since the imports of shrimp compromised by viral diseases has probably increased, and new viral diseases have been manifested in the last several years, it may not be appropriate to suggests that "if a problem existed, it would have been identified by now".

Also, it becomes important to determine if it is common practice for growers to harvest shrimp that manifest diseased conditions and export them to particular markets. It is understandable that certain international markets would not accept diseased shrimp when quality and appearance have been compromised. Other markets may not make this distinction.

12. Quality control and quality assurance will be problematic in the import, processing, distribution and marketing sectors. Retailers probably make the assumption that the product is safe if nothing of public health significance is associated with the product.

13. Although other pathways may be plausible, focusing on alternative sources will detract from the critical issues- "does importing shrimp (dead or alive) pose a threat to natural populations and aquaculture?" While these other pathways are realistic - management will be problematic.

14. Supplemental feeds that incorporate processing by-products, such as solar-dried shrimp exoskeletons, may be a potential pathway. Certain extruded rations may be produced at low temperatures and pressures that might not destroy pathogenic viruses.

15. Anecdotal information should be considered carefully, and should not be treated as fact. However, this type of information may be useful in identifying underlying problems. For example, reports from fishermen may be helpful in identifying fishery trends. Likewise, scientific information should also be carefully scrutinized and interpreted before specific results are applied to different scenarios.

16. The lack of scientific data associating viral diseases with epizootics among natural shrimp population is a major shortcoming in establishing what risks actually exist: for example, identifying threatened populations, determining exposure levels, and characterizing ecological consequences remains a problem.

17.

18.

19. Aquaculture: A comprehensive risk assessment will assist resource managers in developing best management practices for marine shrimp aquaculture operations. Types of aquacultural practices, site selection, and regulations could be developed based on known risks. For example, low-risk aquacultural activities in low-risk locations could be conducted under substantially different criteria than culturing a high-risk species in a high-risk location. Obviously, aquacultural activities in the high-risk scenario would not be suitable in all circumstances, while implementing specific management practices could allow aquacultural activities in low-risk scenarios under a much broader set of circumstances. Management decisions for activities that fall between the low and high risk scenarios should be based on best available information.

20. An assessment of natural shrimp in areas where aquacultural activities are concentrated would be useful, but perhaps difficult. For example, models of shrimp populations on the coasts of Ecuador, Honduras, and Panama might shed some light on the threat to these populations from concentrated shrimp aquaculture. I understand that natural populations in these countries are thriving, and it would be interesting to determine: 1) if taura virus is present in the wild populations, and 2) if affected populations manifest any level of resistance to taura virus. Obviously, there is scientific and anecdotal information that may be relevant, but is not currently being used in a risk assessment format.

21. I think that risk reduction through a range of options is the logical outcome of a risk assessment when a risk can be identified and characterized.

22.

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1. How well does the management goal reflect the dimensions of the shrimp virus problem? While the goal specifically addresses the prevention of new disease-causing viruses, it does not suggest a goal of further understanding and prevention of recurrent virus epizootic events. Further, it was suggested in the report to include considerations of alternative marine hosts such as crab, crayfish etc., but no suggestion has been made to address related arthropod viruses such as those infecting insect populations which could readily enter the food chain of shrimp.

2. The tiered method of risk assessment is applicable here as described in the review materials. The focus should remain on preserving the wild populations of shrimp. The corollary to include non-shrimp host susceptibility should remain a concern, but should be placed in the second tier of concerns along with the aquaculture industry and estuarine ecological impacts.

3. Consideration of any and all pathways that may impact the spread and resilience of viruses should be accepted and given adequate attention. Though the ultimate decisions will be based on what is practical, all potential areas that may initiate an epizootic event need to be identified and considered for future assessment if necessary.

4. Results on virus infectivity gained from laboratory or aquaculture facilities are valuable, but need to be tempered by the artificial conditions and or animal densities that are typically maintained. The spread of virus in these populations proposes to be far more rapid than would occur naturally in wild populations. Further, in a natural environment, a continual dilution of virus due to wave action and tidal exchange would reduce the potential for a localized concentration of virus to occur. An underestimation of virus concentration may also occur in experimental infections due to the protection offered viruses by sediments and or secondary hosts. The relevant value of experimental infections is that it targets specific tissues of infection and the nature of the lesions produced, which may assists in initial diagnosis in wild populations.

5. Given the high mortalities associated with the four viruses specifically addressed in the JSA report, it would seem highly unlikely that an intentional infection of wild populations would result in an overall immunity conferred upon survivors. Moreover, it would seem likely that survivors would be carriers and represent potential vectors that may introduce a virus to a different population leading to sustained infections across many populations as they interact. The only evidence (though not specifically identified as such) of this technique is an extrapolation of the events that transpired from 1990-1994 California with *P. stylirostris*.

6. The non-viral and natural factors affecting shrimp can be separated from viral stressors only after there has been substantial monitoring and evaluation of these non-viral factors. Much remains unknown as to the impact of effectors such as salinity, temperature etc. in regard

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immigration during postlarval stages of development. A more comprehensive model based on additional research efforts at this basal level are required prior to fully understanding the causal relationships between specific viruses and shrimp. Until such a time, the best we can do is to state as many relationships between viral and non-viral associated risks and be aware of the potential consequences of these interactions.

7. Most human health effects can be removed, except for very isolated incidents. The treatment of waste in a municipal waster treatment system is generally more that adequate to dispose of any threat of shrimp viruses. Most wastewater treatment plants implement several stages to handle such threats of reintroduction of human pathogens back into the population. Steps in processing and disposal have been implemented in most communities to include a series of effluent treatments such as ozonation and aeration prior to discharge into receiving streams. Solids disposal has been addressed in these facilities as well in that many facilities incinerate the solid waste materials. If any threat remains it is in locations where solid waste is landfilled and water run off could reenter a water system co-occupied by shrimp.

8. No. Additional research is required to develop reliable diagnostic detection of viruses in shrimp stocks and further techniques need to be developed for the testing of pond effluents or other waters suspected of containing shrimp viruses. The ideal system of diagnosis would be a cell culture system hat would allow the testing of a range of sources from a single suspected animal to the determination of virus presence in specific pond. To date the efforts to establish a cell line for diagnostic viral detection have been hampered by limited availability of significant quantities of tissues from specific-pathogen-free animals. Other resources that would permit a consistent concentrated effort have also been lacking.

9 &10. As of this report, no direct causal relationship has been established between outbrakes of virus in aquaculture facilities and the transmission of the virus to a wild population. Though there has not been a direct link established it does not rule out that it has occurred in the past or will occur in the future. As was mentioned in the JSA report, wild populations have not been adequately monitored. As the capabilities become available to accurately monitor wild populations, and detect viruses in aquaculture discharge, we may be able to track the movement of potential virus infection. This goal would best be achieved in trials employing biomarkers.

11. This is an ever-increasing potential problem especially in light of the fact that Asian markets are exporting ponds after initial traces of virus infection. The disposal of wash waters form port-side processing facilities directly into receiving waters that support any phase of shrimp

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development should be of great concern. Additional solid waste provides a protective cover for virus propagation and entry into the shrimp food chain.

12. No. Aside from increasing demand for ultraviolet (UV) treatment of potential infectious agent in other markets, no additional evaluation is warranted. It should be noted that the additional push for UV treatment could also diminish the negative public opinion.

13. The two potential sources or pathways most critical after processing and aquaculture are that of ballast water discharge and secondary or alternative hosts which can harbor the shrimp viruses. The potential impact of the former may be diminished or eliminated if ballast discharges were properly filtered and the filter dried and incinerated.

14. The temperature obtained during feed production, is adequate to eliminate it as a source of virus. However, is a cell culture system were available, soluble extracts could be prepared and checked for active virus.

15. Evidence concerning the introduction of viruses into wild shrimp populations should be interpreted with caution and reserve. No definitive association has been made between the incidents.

16. Though no data is available for background levels of virus in the wild populations of shrimp, it would be better to error on the side of caution. In a risk assessment, it would be better to presume that a wider variety and higher numbers of viruses exist in the wild populations. The reason that the viruses have not resulted in epizootic events is due to proper timing of environmental and physical conditions. Furthermore, the lack of adequate monitoring of wild populations may have precluded us from characterizing these events.

17. Monitoring of changes in the wild shrimp populations can be used to interpret the impact of introduced populations by characterizing the latent period of the virus in a population after infection in their natural environment. Additionally, if there is an advantage to propagating animals that survive an infection due to some immune advantage, this would allow the true determination of this phenomenon. The immune resistance conferred by an initial infection of this manner at low MOI (multiplicity of infection) could potentially be explained as the population recovery curve were established and animals were randomly screened for viruses. What this scenario does not consider is a multiple pathogen infection.

18. Shrimp virus effects on non-shrimp species is of significant concern as a protected latent storage potential, as well as the possibility of infecting a non-shrimp species through mutation. The ability of a non-shrimp species to harbor a shrimp virus that may re-infect on a recurring basis either seasonally or any time in which the two species interact.

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19. A comprehensive risk assessment will outline the primary, secondary and tertiary factors affecting both wild and cultured shrimp. This format will also point out the research data lacking and potentially bring to light the avenues of research that need to be developed. The assessment will bridge gaps in communication between interested parties to the shrimp economy and will hopefully result in the cooperative exchange of ideas and information toward a common goal.

20. Data gaps are to staggering to proceed directly to a quantitative risk assessment. Which research redirection and monitoring of wild populations for a minimum of two complete developmental cycles for shrimp, population models could then begin to be developed. The cost of such an endeavor would likely cost several hundred thousand dollars, but would add immensely to the scientific integrity of the model development.

21. As technology is developed to reduce the risk of virus introduction from processing and aquaculture wastewater disposal, a future risk assessment should reward these efforts by factoring in a risk reduction element to the formula similar to the risk assessment model, figure 2 from the Report to the Aquatic Nuisance Species Task Force.

22. In reviewing the materials presented, four key points in research come to mind. First, there is a great need to implement a monitoring program of wild shrimp populations for virus presence and genetic diversity. Second, determination of specific non-shrimp harboring species needs to be looked at. This should include not only other marine species such as the aforementioned crab and crayfish, but also in non-marine arthropods. Third, a key element in the advancement of our ability to understand and characterize shrimp viruses is the development of an *in vitro* cell culture system. Fourth, aquaculture pond effluents could be disinfected by through treatment with ozone and or permanganate to neutralize viruses. Solid waste could be incinerated. Both procedures serve to reduce the potential of accidental infection of wild populations through receiving waters.

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Charge to Panel Members- Shrimp Virus Peer Review

l. How well does the management goal reflect the dimensions of the shrimp virus problem?

The focus of the management goal should include the aquaculture industry in order to reflect the true dimensions of the virus problem. Viral impacts to cultured shrimp throughout the world are known to be substantial and widespread, while evidence of impacts to wild shrimp populations is lacking.

Some have suggested modifying the assessment endpoints to emphasize potential risks of shrimp viruses to non-shrimp organisms and the larger estuarine ecological system or, alternatively, to the aquaculture industry. Please comment on the assessment endpoints as the focal point for the ecological risk assessment.

The assessment endpoints should reflect and emphasize the substantial potential risks of shrimp viruses to the aquaculture industry and should not emphasize risks that have no basis or have not been demonstrated.

It has been suggested that the scope of the proposed risk assessment is too narrow and that it should be broadened to consider the impacts of such stressors as alternative land uses and seafood production methods in coastal areas. Please comment on this suggestions.

A broader based input system would make the task of completing the proposed risk assessment unexceptably more difficult due to the extremely large data gaps that exist.

How relevant to virus effects on wild populations is information on infectivity and effects that is derived from laboratory or intensive aquaculture operations?

Information on viral infectivity and effects derived from laboratory or intensive aquaculture operations is not highly relevant to effects on wild populations. There are numerous examples in the literature of infection forced in the laboratory by, for example, injection of live virus that did not produce unusual moralities or is not exhibited in pond conditions. There is no good scientific evidence of any abnormal wild population declines due to viral effects although the aquaculture industry worldwide has a well known history of viral problems. For example, Ecuador's wild population of P.vannamei continues to prosper although most of its 250,000 acres of shrimp ponds have been devastated by TSV and continue to discharge into the coastal waters. Furthermore, as Laramore (1997) reported, there was actually an increase in wild postlarvae over the next three years after TSV first appeared in aquaculture ponds in Honduras. The physiological stress and crowding of intensive aquaculture conditions may potentiate the development and spread of disease that may not happen in the wild or less crowded conditions. The 1995 TSV outbreak that devastated South Carolina did not effect any impoundments that were stocked at lower densities, although they received seedstock from the same hatchery as those that exhibited disease, which supports the observation that crowding may significantly influence the expression of disease that may not be relevant in wild populations.

5. How likely is that exposure of wild shrimp populations to viral diseases could lead to the development of immunity and reduced effects on population survival over time?

It's very likely that both host and virus will adapt to coexistence. Historically, the exposure of populations to viral epidemics does not do permanent damage because of the development of immunity. For example,
we now have populations of P. stylirostris that are resistant to the IHHN virus and P.vannamei coexist with IHHN. Laramore (1997) gives good evidence for the emergence of

a wild population of P.vannamei having increased resistance to the lethal effects of TSV. The tremendous fecundity of shrimp helps insure any potential negative environmental effects on populations survival over time.

6. How can the strong influences of both natural and non-viral anthropogenic factors on shrimp populations be separated from the risks associated with viral stressors?

Without additional data it's extremely difficult at present to separate the influence of natural and anthropogenic factors on shrimp populations from risks associated with viral stessors. Influences of individual stessors including those of combinations of stessors must be first quantified in controlled laboratory settings to demonstrate possible cause and effect (those factors that may predispose shrimp to disease.)

Can human health effects from shrimp viruses be ruled out as a concern? Why or why not?

Human health effects from shrimp viruses can be ruled out since there is no evidence or suggestion of any effects to justify this.

Are the available identification techniques for shrimp viruses reliable enough to allow definitive conclusions to be drawn about the occurrence of viruses in shrimp and environmental media?

Identification techniques are only available for three of the four viruses that were focused on by the JSA workgroup and the complex nature of this testing may not allow for definitive conclusions to be made about the occurrence of viruses. For example, it is almost impossible to rule out the occurrence of viruses in large volumes of water or soil with these techniques.

U.S. aquaculture operations have had problems with viral diseases for several years. How does information from local wild shrimp populations support or refute the importance of aquaculture operations as a source for the virus?

There is no evidence from local wild populations to suggest that domestic aquaculture may be a source of virus. TSV that devastated Texas and South Carolina has not been identified in domestic wild populations. Also IHHN has not been identified in any wild populations. In South Carolina WSSV was first diagnosed Jan. 1997 in wild caught P.setiferus, and only later in Oct. 1997 showed up in one companie's ponds.

It has been widely held that it is highly unusual for domesticated animals to infect wild animal populations; usually it is the other way around. How well does this observation apply to the relationship between shrimp in aquaculture and wild shrimp populations, with regard to shrimp viruses?

Despite the use of certified Specific Pathogen Free shrimp, the aquaculture industry continues to experience viral infections in which the sources may be of an external origin. There is no evidence to suggest that shrimp in aquaculture have infected wild populations, but there is some suggestion of wild populations infecting shrimp ponds. For example in South Carolina WSSV was identified first in wild stock prior to it appearing for the first time in an aquaculture growout pond (see above #9).

Some believe it likely that shrimp processing operations have processed virus-infected shrimp from foreign sources for several years. How does information from local wild shrimp populations support or refute the importance of shrimp processing as a potential source for the virus.

As in aquaculture, there is no evidence of infectivity or declines in the wild population due to shrimp processing.

12. Should retailers who distribute (rather than process) shrimp products receive additional evaluation as potential sources of exposure?

Shrimp viruses have been positively identified in imported farm-raised shrimp. Since retailers handle potentially contaminated imports and may distribute these, for example, as bait, they should receive additional evaluation as potential sources of exposure.

After considering the sources addressed in the shrimp virus report, what sources other than aquaculture and shrimp processing are most critical for evaluation in a risk assessment of shrimp viruses? Given time constraints, which of these should be the focus of discussion at the workshop?

An average of approximately one million pounds of farm raised-shrimp is imported into the domestic market daily. Probably a significant portion of this product goes directly to the retail and restaurant business without being touched by the processors.

Is manufactured shrimp feed a potential virus source, or is the processing temperature sufficient to rule this source out?

The usual processing temperatures that most shrimp feeds are subjected to are most probably sufficient to render any harmful virus inactive. I have consulted one viral expert who suggests we should not be comfortable at the lower processing temperatures mentioned (170-180 degrees F) without knowing the length of time the food is at this temperature during processing . He suggests hours rather than minutes.
However, if feed was a source of virus the effects probably would have shown up in the various diagnostic labs that must be using it in their challenge studies during bioassays.

How should the available evidence concerning the effects of introduced viruses on wild shrimp populations be interpreted? (For example, what was the role of IHHNV in the decline of shrimp populations in the 1980's in the Gulf of California? What about TSV release from aquaculture into the wild in South America?)

The Gulf of California information originates from a Masters thesis and represents the best piece of epidemiological information available world-wide to suggest a link between introduced viruses and declines in wild shrimp populations. I have reviewed a translation of this and find no sound evidence that the decrease in catch observed was due to IHHN. Further, there is no evidence that the IHHN found in wild stock originated in shrimp ponds - the opposite is just as likely. Decrease in catch followed a gradient with the lowest numbers found towards the blind northern end of the Gulf. With the atypical geography of the area there appears to be other stessors, such as pollution and low dissolved oxygen, that could have contributed to the decline observed. The decline observed in other species as well supports the possibility that other stessors may have influenced this decline. After the TSV outbreak in South America catch data indicates the population not only did not decrease but actually increased in later years .

There is presently a lack of basic data on background levels of pathogenic shrimp viruses in wild shrimp populations in U.S. waters. How should this data gap be evaluated in a risk assessment?

It's difficult to assess the risk of pathogenic shrimp viruses in wild populations when there has been little monitoring or data to determine what is already present. For example, a pathogen already present in a wild population would represent a much lower risk to that particular wild stock than to Specific Pathogen Free shrimp stocked in aquaculture facilities that have no developed resistance.

17. How can changes in wild shrimp populations be used to interpret the effect (or lack of effect) of introduced shrimp viruses? How could shrimp population models be used in the future?

Normal fluctuations occur in wild shrimp populations. There is already good documentation of catch data available for domestic species that currently show no unusual or unexplained declines in wild shrimp populations which is interpreted as a lack of evidence of a negative effect of possible introduced shrimp viruses. Population models, environmental data, and background levels of pathogenic shrimp viruses should be monitored in the future in order to spot and explain unusual population declines.

How important are potential viral effects on non-shrimp species?

Non-shrimp species are ecologically important however pathogenicity of viruses is usually species specific.

How will a comprehensive risk assessment contribute to management of shrimp virus problem, ie., will it add significantly to the information presently available?

It will not add significantly to information presently available. The best outcome of a tiered approach will be the organization of data needed to stimulate sound scientific information on viral epidemiology.

What type of assessment should be conducted next (e.g., quantitative risk estimates using shrimp populations models), and what would be the likely time from and cost?

A quantitative risk assessment with numerical estimates of the risks to shrimp populations would provide the best basis for making risk mitigation decisions. However, the extremely large data gaps at present will not support this. We must have a sound basis for such an assessment that will require a large amount of critical additional research. Good population models must be developed and a determination must be made on what viral diseases, either native or introduced, are present in these populations.

21. Should a future risk assessment consider the risk reduction potential of a range of treatment options associated with specific exposure scenarios?

Yes, but treatment options associated with specific exposure scenarios would be valuable only if based on good new research data.

22. Summarize the critical research needs for completing such a risk assessment.

Critical initial research needs must include the following:

1. Development of definitive diagnostics

The lack of necessary tools as well as inconclusive and subjective tests make it difficult to test for possible pathogens.

2. Monitoring of wild populations

We need to know what is out there. We must determine what diseases are native to our populations and what the background levels are.

3. Monitoring of imports

Imports of farm-raised shrimp average approximately one million pounds each day, and based on volume, this potential source of viral introduction overwhelms all others. We need to know what's being brought in, how it's handled, and where it's going.

Anne Fairbrother

Management goals, assessment endpoints, and the conceptual model

1. *Prevent the establishment of new disease-causing viruses in wild populations of shrimp in the Gulf of Mexico and southeastern U.S. Atlantic coastal waters, while minimizing possible impacts on shrimp importation, processing and aquaculture operations.*

   This management goal is a little too narrow for the risk assessment, as it does not include the goal of keeping shrimp aquaculture virus-free as well. In fact, the viruses appear to have the potential to have a devastating effect on this industry, either through direct mortality of a year's worth of shrimp or through restrictions on exportation of the animals, regardless of their role in infecting wild populations. While the last clause of the goal statement may be interpreted to include this additional goal, it would be helpful to have it stated more explicitly, such as (bold text is suggested addition):

   Prevent the establishment of new disease-causing viruses in wild populations of shrimp in the Gulf of Mexico and southeastern U.S. Atlantic coastal waters ***and the shrimp aquaculture industry****, while minimizing possible* ***economic*** *impacts on shrimp importation, processing and aquaculture operations.*

2. The assessment endpoints should be modified for two reasons: 1) they are too broad for the current exercise and 2) they do not include the aquaculture industry (see comment #1). In regard to the first point, the assessment endpoints suggest that the risk assessment will look at all possible causes of changes in survival, growth and reproduction of wild penaeid shrimp, including indirect effects of ecological structure and function. In reality, the current risk assessment is focused only on assessing the risk that introduced viruses pose to the wild shrimp populations and shrimp aquaculture. Therefore, the assessment endpoints for this risk assessment should be narrowed; later, they can be expanded to examine all other potential environmental stressors and their interactions.

   It is perfectly acceptable to ask a narrowly focused risk question, particularly in a case such as this. If it is determined that the nonindigenous viruses do not pose a risk to shrimp, then there is no need to go any further. If, on the other hand, it is determined that there is a high probability that the viruses could severely reduce the wild shrimp populations or make aquaculture economically infeasible, then there may be a reason to look at all the potential stressors on shrimp and determine the relative risk from viruses as compared to other environmental degradation processes.

In particular, the second assessment endpoint listed on page 18 of the report could be deleted. An additional assessment endpoint should be added to address the concern that introduced shrimp viruses may have a broad host range and affect other marine organisms (e.g., clams or fish) as well. Suggested wording would be:

Maintenance of viable populations and communities of marine organisms other than penaeid shrimp, free of virus-induced effects.

Note that what this does is to remove the endpoint of ecological structure and function and specify the more narrow assessment goal of the maintenance of populations of marine organisms. This allows the current risk assessment to be focused on effects of introduced pathogens, and does not imply that the assessment will include such things as coastal development, water diversion projects, etc.

Finally, an additional endpoint should be added to address the aquaculture issues, for example:

Economic viability of the shrimp aquaculture and processing operations.

3. The above comments suggest that I believe that it is useful to keep the scope of the current risk assessment narrowly focused on the question of the potential risk of introduced virus. There may be a need to do a comprehensive risk assessment at some point, but that does not preclude asking this particular question about the potential effects of viral introductions.

Viral stressors and factors regulating shrimp populations

4. Information on infectivity and effects of viruses derived from laboratory or aquaculture operations is very relevant to the *potential* for effects to occur in wild populations. In fact, it is only through laboratory studies that Koch's postulates can be fulfilled, thus proving that a particular pathogen is the causative agent of an observed disease. Most of the known diseases have been studied in the laboratory at some time. Lab studies are particularly useful for establishing potential host range (i.e., susceptibility of various species to the virus) and an idea of how much virus must be present to initiate an effect in an exposed organism.

Of course, laboratory studies only identify the *potential* for effects in wild populations, as they do not account for all the exposure factors. Assuming that all environmental conditions are exactly the same as the laboratory, one could predict with a great deal of certainty what the effects would be. Given that this is never the case, uncertainty in extrapolating lab results to the probability that effects will occur in the field increases. However, laboratory studies also can provide information that can be used to extrapolate lab results to field situations, such as the range of environmental conditions tolerated by a virus (e.g., pH, temperature, water quality), the transmissibility of the agent (e.g., how close together do hosts need to be in order to become infected), how the agent passes from one generation of hosts to another (vectors, transovarial transmission, water dispersal, etc.).

5. I am not qualified to speak authoritatively about development of immunity to viral infections in shrimp, as I am not familiar with shrimp immunology. If they are similar to shellfish (e.g., clams), then they would have the capability to develop immunity (also known as "resistance"), provided the virus is not 100% lethal with a high transmissibility rate. It is to the advantage of both the host and the virus to become more commensalistic through time, i.e., for the host to develop resistance and for the virus to become less virulent. There are numerous examples of this occurring in vertebrates, the most well-known being the introduction of myxomatosis virus to Australian rabbits. The one notable example where this has not occurred is rabies, which is nearly always fatal to the host so natural immunity (i.e., development of antibodies) does not occur. However, it is noteworthy that the virus has adapted to this by initiating a behavior prior to death (e.g., salivation for virus shedding, aggression, and biting) that nearly guarantees transmission should another susceptible host be nearby. Rabies also has a relatively low transmission rate since it requires direct contact of an infected and susceptible host.

6. The risk from viral stressors should first be assessed as if the virus was the only stressor present. Then, modifying factors would be added that could potentially change the host-virus interaction. For example, changes in hydrology of the aquaculture system of the nursery marshes, changes in density of the shrimp due to harvesting or natural factors, etc. The viral risk to the shrimp under these modifying

conditions then could be assessed. If one wishes to compare the risk from viruses to the risk from other environmental stressors (i.e., do a comparative risk assessment of viral risk versus risk of overharvesting or risk of reduction of nursery areas or risk from bacteria and parasites, etc.), then each of the potential stressors would need to be assessed both individually and in appropriate combinations as modifying factors of each other. This would be a very long and intricate process, but could be done.

7. There was insufficient information provided in the report to rule out potential human health effects from all the viruses. The white spot syndrome virus (WSSV) is a Baculovirus, a virus group which has no known vertebrate hosts (non-occluded baculoviruses such as WSSV cannot tolerate the acidity of the GI tract or the relatively high body temperature of vertebrates). Therefore, this virus could be ruled out as a potential human pathogen. Two of the virus groups have known human pathogens: polio belongs to the picornavirus group along with taura syndrome virus (TSV) and rabies is a rhabdovirus similar to yellow head virus syndrom (YHV). Infectious hypoderma and hematopoietic necrosis virus (IHHNV) is a parvovirus, a group that primarily infects animals (e..g., canine parvovirus or feline panleukopenia). These groups also include viruses pathogenic to domestic and wild animals, some of which have great economic concerns, should they affect livestock. Note, in particular, that there are 5 pathogenic rhabdoviruses in fish, affecting rainbow trout, carp and pike in Europe, salmonids in the Pacific Northwestern US and the American eel. Therefore, there should be discussion about potential pathogenicity in *any* vertebrate, particularly when discussing the possibility of birds or other animals to act as vectors of transmission.

   It should be noted, however, that many of the viruses in these three groups have restricted host ranges, so there is an equal possibility that humans and other vertebrates would *not* be susceptible to the viruses. None of the viruses in these groups have been known to infect *both* vertebrates and invertebrates (the only viruses that do this routinely are the arboviruses, a group comprised mainly of encephalitic viruses that infect and are transmitted by arthropod vectors), so the probability of human infection is remote. However, until more information is provided about host range and environmental tolerances of these viruses, it is not possible to make *a priori* predictions about transpecific susceptibility. Some simple cell culture laboratory studies could provide a great deal of reassurance in this regard, while simultaneously providing information about the environmental persistence of these viruses.

8. The report states that a gene probe is available from commercial sources for IHHNV and WSSV, which would suggest that reliable identification methods are available for drawing definitive conclusions about the occurrence of these viruses in shrimp, other organisms, or the environment. The other two viruses do not have such reliable identification tools, and epidemiology must rely on bioassays or electron microscopy. While these more traditional methods can provide a great deal of information, they are neither as definitive nor as quick as a gene probe.

Viral pathways and sources

Aquaculture

1. The report identifies several potential routes for introduction of exogenous pathogens into the populations of wild shrimp in the Gulf of Mexico or the Atlantic Ocean off the southeastern US coast. These were detailed in Figure 8 of the report and include: water discharges from aquaculture ponds; sludge dumping from aquaculture ponds; escape of infected shrimp; spills or losses during transport to the shrimp processing facilities; or through use of infected shrimp as bait. Page 25 provides further discussion of shrimp phenology that appears to support the possibility of aquaculture to wild shrimp virus transfer. However, no data were presented that would substantiate a conclusion about the actuality of such a transfer. What would be needed would be isolation of similar viruses from an aquaculture facility and a geographically connected wild shrimp population. Using gene probe technology, it should be possible to determine if the viruses were, indeed, the same agent. Without such information, the role of aquaculture in infection of wild shrimp remains speculative.

   It should also be noted that infection of a local population of shrimp as a result of aquaculture practices might or might not result in pathogenic infections of the entire population through the Gulf. More information is required about pathogenesis, carrier states, and transmissibility before such conclusions could be drawn. For example, if the virus is very pathogenic, it may wipe out the local population before it has time to come in contact with other subpopulations. If the virus persists in the environment of the local nursery marsh, any shrimp coming in to breed in subsequent years may be infected and die, making the marsh unsuitable to continued shrimp production. But the population as a whole might remain uninfected.

2. The observation that domesticated animals rarely infect wild animals while the converse frequently happens is not true. Avian cholera (*Pasteurella multocida*) is a devastating disease of wild waterfowl, killing as many as hundreds of thousands every year in North America. This disease was introduced to waterfowl from the poultry industry in Texas in the 1940s. Duck viral enteritis, a herpesvirus, was introduced to North American waterfowl from the domestic duck industry on Long Island, NY in the 1960s. Brucellosis (*Brucella abortus*, *B. canis*, and *B. suis*) was introduced to the American bison, various wild cervids (deer and elk), wild canines (coyotes), and wild pigs from domestic livestock. Tuberculosis (*Mycobacterium bovis*) occurs in many species of cervids and bovids where they come in contact with domestic livestock. Mycoplasmas (e.g., *Mycoplasma gallisepticum* or *M. synovium*) are picked up by wild turkeys that intermingle with domestic turkey flocks. There are many, many other such examples of domestic animal to wildlife transfers of disease agents.

   Transmission of diseases from wild animals to domestic livestock or pets is less well documented. Rabies and rinderpest (a paramyxovirus) are perhaps the best known examples of wild animal reservoirs with direct transmission to domestic animals. Foot-and-mouth disease (a picornavirus) and other vesicular diseases (in the rhabdovirus group) may be endemic in wild hoofed stock in Africa, providing a reservoir for infection of range cattle. Myxomatosis virus (an arbovirus) is endemic in wild rabbits in California, and occasionally infects domestic herds. Other organisms that persist well in the environment may infect both wild and domestic animals equally, and include diseases such as anthrax, leptospirosis, and tularemia. Other groups of organisms that cycle regularly between wild and domestic animals are the arboviruses and rickettsial diseases that are maintained in wild vertebrate hosts with transmission through arthropod vectors. Occasional epidemics of disease occur in domestic livestock or humans, including such devastating diseases as yellow fever and dengue fever. Other agents have a lower, more endemic pattern such as Lyme disease or Rocky Mountain spotted fever.

In sum, there is ample evidence that domestic animals (e.g., shrimp aquaculture) may infect wild animals (e.g., wild shrimp populations) should there be appropriate co-occurrence of infected and susceptible populations or contamination of the environment.

Shrimp processing

3. As with the shrimp aquaculture industry, the shrimp processing industry has the *potential* to discharge virus-contaminated materials into waters inhabited by wild shrimp, particularly due to the practice of receiving shrimp from other countries that harvested shrimp during the early states of a disease outbreak (page 26 of the report). Section 3.7.1 of the report describes what is known about infection of wild shrimp by IHHNV, TSV, WSSV, and YHV. Based on this information, there is little evidence to either support or refute the hypothesis that the processing industry is a source of infection.

4. Whether or not retailers who distribute (rather than process) shrimp products should receive additional evaluation as potential sources of exposure to wild shrimp depends upon whether they discharge any shrimp products to marshes, shorelines, or oceans. As they likely do not, it would not seem necessary to investigate them further.

Other potential sources and pathways

5. The most critical additional sources and pathways of infection of wild shrimp and aquaculture include: bait shrimp and ballast water discharges. Research and display aquaria would have similar issues to aquaculture and so need not be considered separately. Non-shrimp translocated animals (e.g., shellfish, crabs, etc.) may be important, but since we do not know anything about host range of the viruses it would be difficult to evaluate this pathway. Indeed, ballast water discharge includes the potential for translocation of infected organisms as well as contaminated water. Vector transport by nonsusceptible hosts (e.g., birds) has a low probability. Natural

spread should be considered, again within the context of little knowledge about environmental persistence or host transmission rates.

6. There is no information presented in the report about the composition of manufactured shrimp feed or the temperature to which it is subjected. However, if the temperature is high (>100 ^{o}C), then it is likely that the viruses would be killed.

Stressor effects

7. The available evidence concerning the effects of introduced viruses on wild shrimp populations should be interpreted with caution. The role of IHHNV in the decline of shrimp population in the 1980's in the Gulf of California is speculative – correlation does not equal cause-and-effect. I believe the points made on pages 42 and 43 of the report about why viruses (and related effects) have not been detected in the U.S. wild stocks is right on target. Collection of TSV-infected shrimp from near-shore or off-shore fisheries in Ecuador, El Salvador, and the southern Mexican state of Chiapas suggests that the virus might exist in these free-living populations, but insufficient data are presented in the report to determine if this is a conclusive statement.

8. The limited data on background levels of pathogenic shrimp viruses in wild population in U.S. waters must be evaluated cautiously. Pages 42-43 of the report suggest that we really do not know whether or not these viruses currently are present. Until more information is made available, the risk assessment should assume that they are not endemic as a worse-case scenario.

9. Shrimp population numbers suggests that there are forces in the environment that can control shrimp populations. Correlational studies can suggest what some of these factors might be. For example, comparing climate cycles, hurricane incidence rate, ocean temperatures, harvest rates, or known viral introductions with population numbers can suggest which one(s) may have the greatest potential for effect. In order to quantitatively model the relationship of viruses and shrimp population numbers, information on the age-class specific infectivity rate, transmission rate, mortality rate, and immunity rate needs to be made available, none of which appear to be very well known.

10. The importance of potential viral effects on non-shrimp species is not known, as there is no information on whether or not other species are susceptible to these viruses. If they are, then the same suite of information outlined in the previous comment would need to be understood for these species as well, in order to derive a definitive answer. However, the evolutionary pattern appears to be that a newly introduced pathogen may be extremely virulent initially, killing a large percentage of the host population. Eventually, either one of two outcomes occurs: the host population is completely destroyed (rare occurrence) or the host-virus association is modulated towards co-adaptation, with the host becoming less susceptible and the virus becoming less pathogenic. The population may, however, become stabilized at a lower density that previously. Both the initial population depression and the subsequent reduced equilibrium numbers may put an industry, such as the wild shrimp or shellfish harvesters, at an economic disadvantage.

Comprehensive risk assessment and research needs

11. A comprehensive risk assessment will not add to the available information. The risk assessment process uses information and synthesizes it to generate a risk statement, it does not develop new information. In the process, however, information gaps are identified and new information may be gathered prior to a second iteration of the risk assessment. This helps to focus research into areas that will immediately result in a reduction in the uncertainty associated with the risk prediction. Therefore, the risk assessment process can be very useful in identifying data gaps and prioritizing research needs.

12. Following the qualitative risk assessment, a quantitative risk assessment using shrimp models could be done but *only* if additional information about viral pathogenesis (transmission, immunity, mortality rates; see above comment) is provided. Additional information that would be required is persistence of the virus under various environmental conditions.

13. Risk reduction potential of various treatment options should eventually be considered, once more information is available about the virus (see previous comment).

14. Critical research needs for conducting a quantitative risk assessment include (at a minimum): viral pathogenesis; viral resistance/susceptibility to environmental conditions; endemnicity of virus in U.S. coastal populations, interspecific susceptibility and transmissibility; identification of virus in possible vectors and sources. The list of data gaps presented on page 49-50 is fairly complete. A reasonable first step towards assessing risk would be a well-conducted survey of the U.S. coastal shrimp populations to determine if these (or other) pathogenic viruses are endemic in the wild populations. If they are, the risk from further introductions might be considerably less than if the populations are naïve.

William Fisher

William S. Fisher

1. The management goal falls short by focusing only on four shrimp viruses, which are of current concern, but may only be the tip of the iceberg. This does not account for other micro-organisms and small eukaryotes (such as isopods), that are parasites, pathogens, commensals, and symbionts of imported shrimp, regardless of whether they are imported for aquaculture or food processing. Nor does it account for populations of indigenous organisms that can be exacerbated by the high-density conditions of aquaculture. Focus on the four current viruses assumes that no other viruses (either latent or undetected) and no other organisms will disrupt the wild populations of shrimp. South Carolina apparently monitors for at least nine different organisms ... where are all of those included in this management goal?

2. A third assessment endpoint should focus on ecological aspects NOT NECESSARILY related to wild shrimp populations and harvest. Society has many different values for estuarine resources and these require an estuarine infrastructure (= integrity) that sustains those values. If any organism brought into the estuary alters that infrastructure, then values other than wild shrimp harvests may suffer. For example, imported penaeid shrimp may carry organisms that are not harmful to wild penaeid populations, but do impact grass shrimp populations. The many commercial fish species that rely on grass shrimp during their estuarine nursery life stages would be affected, as would the harvests of these fish; additional social values at risk.

3. It is important to remember that wild shrimp harvesting techniques are very destructive to coastal habitats and several different marine organisms, and that one important value of aquaculture is the potential to develop non-destructive, or minimal impact, food production capabilities. Regardless of how important it is to reduce the impact of wild shrimp harvests, this issue does not help to focus on risks and consequences of nonindigenous introductions.

4. Obviously any extrapolations must be verified. Certainly the highly contagious conditions of high-density, high nutrient aquaculture will not reflect a natural condition.

5. Over time, and assuming that there is reasonable genetic vitality and cross-breeding, this is a reasonable scenario. But there are a minimum of 4 (probably many more to appear in the future) organisms that may have to go through this selection process and mortalities will be high during the period of development of resistance. Crustacea are not vertebrates, so they do not have antibodies to provide specificity and memory in an immune response. Protection, or resistance, usually comes from selection pressure exerted over many generations that ultimately allows host and parasite to reach an equilibrium that is not as destructive to the host.

6. It is usually difficult to distinguish the actions of single stressors when the occurrence of disease requires a suitable juxtaposition of host, parasite and environmental conditions (Snieszko paradigm). However, if non-indigenous viruses are associated with disease, then their introduction should be considered a highly significant factor.

7. Zoonoses are rare. In most cases, parasites survive because of their ability to use a unique or unused resource; consequently they develop close associations of dependence on specific host populations.

8. No response.

9. The fact that there have not been reports of massive epizootics (or only one reported) is not sufficient to suggest that local wild populations have not been infected. In the wild, infected shrimp may not die, may not die immediately, or may not die *en masse* to be detected. It may be that responses are less acute than observed in high density and high nutrient conditions of aquaculture. Or that on or more of these viruses is not expressed unless environmental conditions are met. Therefore, only specific diagnostic techniques for the presence of the virus should be accepted as a measure of exposure (infection). Information developed using such techniques would also have to be based in a defendable monitoring effort, with appropriate frequency and timing of samples. Also, if viruses are detected near an aquaculture facility, this is not sufficient evidence to proclaim it the source; however, such a finding should instigate an investigation.

10. It's hard to think of shrimp in aquaculture as "domesticated". Mostly they are offspring of wild shrimp that have been penned and repeatedly spawned. Programs like the SPF broodstock development may begin to move shrimp toward natural and artificial selection that leads to domestication. Nonetheless, the question is valid. Unfortunately, the ease and ability to monitor diseases of domestic "penned" populations far outstrips our ability to monitor wildlife diseases, so this influences our observations of the rate of occurrence in or out of pens and corrals. It is possible that a wildlife disease expert may have many examples of agricultural plants or animals creating major impacts on natural populations.

11. See #9

12. Yes, depending on the status of the product (boiled? raw?).

13. There is some confusion here since it is not apparent that bait shrimp come from foreign sources, so the occurrence of these "exotic" (presumably meaning non-indigenous) viruses should not be a concern in bait shrimp. However, indigenous viruses and other organisms should certainly be a concern (see #1). Other concerns (ballast, research display, other translocated animals) may be valid concerns, but the potential for large inoculations is less. The larger the inoculation, the greater opportunity to become established.

14. No response.

15. From the summaries presented, it appears that they can only be interpreted as *potential evidence.* Without better documentation, they cannot be used to demonstrate source, direct cause, effect, lack of long-term effect, or development of resistance.

16. This lack of information is not particularly relevant if it refers to background levels of indigenous viruses, since the primary concern here is non-indigenous viruses. If we simply do not know whether these "exotic" viruses already exist in U. S. wild populations, then the lack of information becomes very important. If the viruses are

indigenous, then the wild shrimp population may not be as 'immunologically' naive as otherwise suspected and the primary concern should shift to the potential impact of additional dosage or stresses issuing from anthropogenic activities. This question seems to infer that effects of introduced organisms may be altered by existing disease conditions; if so, this is corollary, and not primary, to protecting wild shrimp populations from introduced viruses (we don't need to know how many people have pneumonia to protect the population from a new strain of pneumonia, or from influenza).

17. The most obvious scenario is massive mortalities of wild shrimp populations with clear evidence of viral infection from a previously unreported (and presumably nonindigenous) virus. Increased / decreased presence of virus in wild populations can also be used. Stock assessments are much more difficult to interpret.

18. Probably shrimp viral effects are not very great on non-shrimp species, but a major shortcoming of the report is the lack of concern over non-penaeid shrimp species. For example, grass shrimp (Palaemonidae) include species that are dominant (biomass) in many southeastern estuarine systems and serve vital ecological roles in nutrient cycling (detritovores) and as prey for important commercial and non-commercial fish species during their early developmental stages. Major losses of these organisms would severely impact many important sport and commercial fisheries and undermine the existing estuarine infrastructure. A second issue that this question raises is the importation of organisms unrelated to shrimp — microorganisms or small eukaryotes that are commensally or inadvertently associated with shrimp, on the gills or in the digestive glands, that are potentially harmful to other native organisms.

19. It should organize the information and create the dialogue to qualify the information available.

20. A conservative tiered approach. It would be unlikely to resolve many of the issues in a short period of time.

21. Yes, but not limited to treatment options — include prevention options such as location of aquaculture and processing plants away from estuaries.

22. Research needs are to determine:
- What organisms (virus, bacteria, fungi, eukaryotes, etc.) are imported with any foreign shrimp, whether for aquaculture or processing.
- Which of these survive and are present in effluent from aquaculture or processing
- Which surviving organisms are capable of infecting, infesting or associating with wild shrimp or other estuarine/ coastal inhabitants (particularly other shrimp species).
- What the consequences of such an association are on the organism, population and community.

Corollary question:
How do high-density, high-nutrient conditions of aquaculture exacerbate the proliferation and contagion of resting or latent microorganisms, indigenous and non-indigenous.

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Premeeting Comments: JSA Shrimp Virus Report

Management goals, assessment endpoints, and the conceptual model

1. The management goal should reflect the full scope of the problem. As such it is adequate if the scope is limited to estimating the risks to wild shrimp populations. Should the scope be widened to include non-shrimp species then the goal will need to be modified to reflect that change

2. My perspective has always been that the conceptual model should capture the full spectrum of probable risks and thus include a suite of assessment endpoints. This approach requires that one must include all the drivers, stressors, and possible interactions of importance operating on the assessment endpoints. If one accepts this strategy then if there is a probable risk to non-shrimp organisms and the larger estuarine ecological system then the suite of assessment endpoints should be expanded. It is important in conducting risk assessments to assure that potentially important interactions are identified and points of connectivity between endpoints or systems are represented. However, all risks within the conceptual model are not likely to have equal probabilities, that is, some are more important than others. Thus the risk assessor must rank the probable risks and provide a rationale for the decision to examine one risk rather than another. In this case, I would suggest that the full conceptual model be constructed and the probable risks weighted. This conveys the ideas that all risks were considered but these were the most important and selected for further study. Unless there is compelling evidence to suggest that there are no non-target species/system risks, that there are no plausible interactions with and connectivity to other systems then a broadening of the scope of the assessment should be considered

3. This follows logically from #2 above. Broadening the scope of the conceptual model logically requires addressing other drivers or sources of stress to the system. A point of clarification on terminology - a stressor must co-occur in space and time with the ecological endpoint/receptor - things like landuse changes, production methods are generally not stressors to aquatic systems rather they are drivers , sources, or agents that lead to stress. However, as part of expanding the conceptual model it will likely be necessary to expand the stressors and to identify specific interactions that may be important

Viral Stressors and factors regulating shrimp populations

4. The translation of laboratory to field is an issue that is common to most of the research we conduct. In general, laboratory studies permit the establishment of principles and pathways of causality under controlled conditions. Laboratory studies do establish the likelihood of realizing a particular stressor-response relationship often for optimum conditions. Translation to the field depends on the degree to which the laboratory conditions are realized in the field. If the laboratory study tests a range of response for what are considered critical variables then the likelihood of transference is enhanced. If the laboratory study is poorly designed then the uncertainty associated with transferring this data to the field would be so great as to be meaningless.

5. I have no comment on this question

6. Assigning relative importance of risks from multiple stressors is a generic problem in most risk assessments. Typically one looks for biological/ecological responses or markers that are specific and diagnostic for a particular stressor. If this relationship can be established the response must then be scaled to effects at the population level. In the case of shrimp, there are natural climatic factors influencing shrimp stocks, there is fishing pressures, as well as disease to name a couple. If there is a biological probe that can determine the proportion of a shrimp population that are infected it could be treated as mortality and then projected to a loss in population size.

7. This is certainly an important question and one for which I suspect there is insufficient evidence upon which to make a judgement with low uncertainty. This problem of animal diseases moving to humans seems to be becoming more and more of a concern with several incidence being documented lately. I suggest that the human health issue is never one that can be dismissed nor should it be without substantial evidence.

8. I have no knowledge regarding this question. It is important to have as many reliable diagnostic tools as possible for screening infection. Having such a tool would be invaluable for monitoring wild and cultured shrimp stocks as well as various steps in the process stream and field exposure pathways. I would suggest that this is an important data gap and research need.

Viral pathways and sources

Aquaculture

9. I have no experience with this topic. However, having a molecular probe or marker for the various virus types certainly would help to address this question.

10. Again this is not my field but a couple of questions come to mind, What is the evidence supporting the statement that it is unusual for domesticated stocks to infect wild animal populations and vice versa. Cultured shrimp are not really domesticated in the true sense of the term are they? More importantly if animal virus are now moving to human hosts with increasing regularity why should one not suspect that viruses from cultured populations can infect wild stocks all factors being equal?

Shrimp processing

11. This is an area with which I have no specific experience. However, I suspect monitoring

and experiments have been conducted that will address this question. Again it seems to be a question of having the appropriate monitoring methods for reliably detecting the viruses in the wild from those processed from cultured populations and being able to discriminate the origin of viruses from different sources (assuming they have unique markers).

12. I think this question falls more within the human health risk arena. From a health perspective shouldn't this be included within the rubric of "seafood safety" similar to concerns over bacterial contamination, biotoxins, and organic and metal contaminants. I think that the human health issue is particularly important for the retailing industry.

Other potential sources and pathways

13. No comment

14. The answer to this question depends on the process used and the viability of the virus under those conditions. If elevated temperatures (e.g., pasteurization of some type) could be used in the process without damaging the product then that would be a simple and inexpensive control mechanism that could be used in most countries.

Stressor effects

15. Though not familiar with the data from this field one general approach is commission a critical review of the data by a group of independent scientists.

16. The absence of natural background levels of shrimp virus pose at least two problem for the risk assessor. First, is the size of the natural source of the virus and thus its potential for causing effects. Without knowing background levels it is difficult to interpret what could be considered "normal" and whether management actions are having an affect. By knowing the controlling factors and the range of natural variability the risk assessor can then assess the efficacy or risk reduction efforts. Further, knowing the natural variability provides insight into potential impacts to the population. The incidence can be used as a variable in a population model to project the range of expected populations as a function infection

frequency. Thus when some anecdotal evidence is reported for the population or catch one can examine it within a context of the natural variability and degree of infection.

17. See #16 above

18. I do not know the answer to this question but suggest that it should be considered important until evidence proves otherwise. As discussed above under conceptual models, this is an important element that should be included in the conceptual model and as part of a comprehensive risk assessment.

Comprehensive risk assessment and research needs

19. A comprehensive risk assessment will put some real numbers on what now seems to be expert judgement. There is nothing wrong with the latter and in fact it is often as far as the risk assessor can go given available information. However, it doesn't treat uncertainty which is important for decision-making. At first glance, it may not add significantly to current information but it will put all the information within a systematic framework where it can be analyzed and evaluated. Further it will quickly identify critical data needs both in terms of quality and quantity. If nothing else it will tell you what you know and don't know and how confident you are with what you know and don't know.

20. I'd like to suggest that a full simulation model rather than just a shrimp population model. I say this because I don't know any other way to capture the full suite of variables and their interactions including multiple drivers, stressors, and modifying variables. Further the assessment endpoints should not be limited to only the shrimp population but should include other types of endpoints that could not be ascertained from just a shrimp model. However, in lieu of having a simulation model, the shrimp model can be used to test a variety of hypotheses or potential scenarios as long as one recognizes it limitations.

21. Absolutely. I'm a strong proponent of scenario-consequence analysis because it allows the risk assessor to play "what if games" without having to have every piece of information and know every uncertainty. In addition if scenario analyses are coupled with sensitivity

analysis valuable information is revealed that helps identify the most important variables contributing to the risks. This information can then be used to allocate research on obtaining those pieces of information that are most important and which contribute the most to reducing uncertainty.

22. I'd like to suggest that this is one of the outputs from the workshop.

Rebecca Golburg

Comments for the Shrimp Virus Peer Review Workshop, January 7-8, 1997

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I have structured the following comments as answers to questions in the "Charge to Panel Members" for the Shrimp Virus Peer Review Meeting, although I do not answer every question. In some cases I have drafted comments to answer two or more related questions.

My comments were prepared with input from Pam Baker, who works for the Environmental Defense Fund (EDF) in Texas, and from Dr. Cristina Tirado. My comments also draw on EDF's August 29, 1997, comments to the National Marine Fisheries Service concerning the JSA shrimp virus report. The August comments were prepared by Pam Baker, Dr. Doug Rader of EDF's North Carolina office, and me.

Questions:

1. How well does the management goal reflect the dimensions of the shrimp virus problem?

2. Some have suggested modifying the assessment endpoints to emphasize potential risks of shrimp viruses to non-shrimp organisms and the larger estuarine ecological system or, alternatively, to the aquaculture industry. Please comment on the assessment endpoints as the focal point for the ecological risk assessment.

18. How important are potential viral effects on non-shrimp species?

Answer/comment:

The management goal of the JSA report (p. 14) is generally appropriate. Nevertheless, I suggest the following additions (underlined), so that the management goal reads:

Prevent the establishment of new disease-causing viruses in wild populations of shrimp and other susceptible organisms in the Gulf of Mexico and southeastern US Atlantic waters while minimizing possible economic impacts on shrimp importation, processing, and aquaculture operations.

Add a statement following the goal stating that "When feasible, source reduction approaches will be the preferred methods for achieving the management goal."

The reasons for these three underlined changes are discussed below.

First, the goal should be broadened to include wild populations of susceptible organisms other than shrimp. The JSA report makes clear that some shrimp viruses may infect a range of invertebrates other than shrimp. Introduction of new shrimp viruses could therefore potentially lead to decreases in populations of a variety of organisms and even undermine marine food webs. The management goal should reflect these ecological concerns, as well as largely economic concerns about shrimp populations. Consideration of organisms other than shrimp may also be important to economic objectives. The health of marine food webs affects the health of fisheries and thus effects of new shrimp viruses on organisms other than shrimp could cause economic harm.

A challenge, of course, is to keep the risk assessment manageable: It is not a simple matter to fully assess the risks to marine ecosystems of new shrimp viruses. Nevertheless, in the short-term, the qualitative risk assessment could consider the limited information available about the host ranges of various shrimp viruses, and lay out the potential range of consequences establishment of new shrimp viruses could have for marine ecosystems. Over the longer term, research to better delineate the host ranges of new shrimp viruses should be a priority. Such additional information will almost certainly be necessary to judge the likely effects of shrimp viruses on marine ecosystems.

Second, the potential economic impacts should be minimized from any actions to prevent the establishment of shrimp viruses. Given the potentially devastating impacts of new shrimp viruses, the federal government should not shy away from working with or requiring the shrimp importation, processing, and aquaculture industries to make any changes necessary to protect wild populations of shrimp and other organisms. The management goal should be to keep the costs of any necessary changes as low as possible.

Third, source reduction should be acknowledged as the preferred means of addressing threats from new shrimp viruses. Over the past several decades, the strategic foundation for pollution control has evolved so that there is now a recognized spectrum of approaches to managing pollutants. The most preferred of these approaches is to prevent or reduce the production of pollutants in the first place. In decreasing order of preference, other approaches are to recycle and reuse wastes, waste treatment, and disposal of wastes in the environment. This ranking was written into law by the US Congress in 1990 under the Federal Pollution Prevention Act.[1] Although this spectrum of approaches most often is applied to manufacturing industries, it is applicable to terrestrial agriculture (Hoppin et al. 1997), and should be applicable to shrimp aquaculture.

Many shrimp aquaculture operations, particularly outside the United States, have poor environmental and other practices that lead to disease outbreaks on farms (for

[1] 42 U.S.C. Sec. 13101-13109

example, Clay 1996; Gujja and Finger-Stich 1996; Hopkins et al. 1995). These disease outbreaks are the root cause of current threats to the US shrimp fishery and US coastal ecosystems from new shrimp viruses.

Source reduction -- preventing imported and domestic farmed shrimp from becoming infected by new viruses -- should be the most preferred approach to preventing the establishment of new shrimp viruses in wild population of shrimp and other organisms in the United States. In a pollution prevention framework such an approach is clearly preferable to say, trying to stop introductions of new shrimp viruses by requiring on complete disinfection of effluents from coastal shrimp processing plants in the southeastern United States. Admittedly, there are hurdles to fully implementing a source reduction approach, and waste treatment approaches are likely to also be necessary. All the same, the management goal should make clear that risk management approaches to address threats from new shrimp viruses should be developed within a source reduction framework.

Question:

3. It has been suggested that the scope of the proposed risk assessment is too narrow and that is should be broadened to consider the impacts of such stressors as alternative land uses and seafood production methods in coastal areas. Please comment on this suggestion.

Answer/comment:

The scope of the proposed risk assessment should not be broadened to consider alternative land uses and seafood production methods (beyond alternative shrimp farming practices). It is possible to draw linkages from just about every environmental problem to a range of other problems and circumstances in our society. However, progress on any one environmental problem usually depends on sufficiently narrowing the scope of the issues considered in order to make the problem tractable. Broadening the scope of the risk assessment for new shrimp viruses to include land use and general seafood production issues would do just the opposite -- making the risk assessment process lengthy and possibly intractable. Particularly given the urgency of the potential threat from shrimp viruses, it would not be prudent to broaden the scope of the risk assessment to consider these issues.

Question:

4. How relevant to virus effects on wild populations is information on infectivity and effects that is derived from laboratory or intensive aquaculture operations?

Answer/comment:

Laboratory results can provide valuable information about viruses. Lab results concerning mode of transmission, virus viability, and the capability of survivors to become carriers are highly relevant to the risks that viruses pose to wild populations. Results from the aggressive challenges with a particular virus are valuable indicators of the relative susceptibility or resistance of wild individuals or populations to a the virus.

Nevertheless, a rule of thumb across many fields of biology is that pathogens more readily infect organisms under experimental conditions (in a laboratory, greenhouse, etc.) than they do in nature. Laboratory data concerning the effects of viruses provides an useful evidence about the potential effects of viruses on wild populations, but does not always predict how diseases affect wild populations.

There are several reasons why the infectivity and mortality observed in experimental infections of shrimp are likely to be more severe than what would probably be in the wild. For example, researchers often try to maximize the odds of infection by injecting shrimp with purified viral suspension or by feeding shrimp a diet with large amounts of infected material. In addition, lab animals are generally not subject to predation by other species. In contrast, wild animals weak from illness tend to suffer high rates of predation, reducing the chance that diseased individuals will transmit their infections.

Intensive shrimp aquaculture operations also have characteristics that tend to promote the spread of disease. High stocking densities and environmental and handling stresses in intensive systems increase the susceptibility of shrimp to disease and the chance that they will become infected. For example, shrimp in intensive systems are ften continuously exposed to virus-laden water. Infected animals tend to suffer high cannibalism rates, spreading disease. Some viruses may be vertically transmitted in spawning tanks. In contrast, the odds of horizontal transmission of viruses is lower in the wild, because populations are relatively sparse and cannibalism rates are relatively low.

Question:

5. How likely is it that exposure of wild shrimp populations to viral diseases could lead to the development of immunity and reduced effects on population survival over time?

Answer/comment:

It is difficult to speculate whether the exposure of wild shrimp populations to viral diseases would lead to the development of immunity and reduced effects on population survival. Information about immune mechanisms in shrimp is very limited and mostly concerns the response to commercial "immunostimulants" (cell-wall components from fungi or bacteria) and *Vibrio* vaccines, which do not necessarily provide complete or long-term protection against diseases. The relative protection provided by these vaccines may result from a general stimulation of cellular defense mechanisms rather than the development of immunity. Crustacea do not display long term specific immunological memory because they do not express specific antibodies (immunoglobulins).

Nevertheless, some scientific literature suggests that shrimp survivors of at least some viral infections are more resistant to challenges with that viral agent than shrimp that were not previously exposed to the virus. For example, Erickson et. al (1997) reported that *P. setiferus* and *P.vannamei* TSV survivors were relatively unaffected by a challenge with TSV (90% and 45% of individuals of each species, respectively, survived), while *P.vannemei* that were not previously infected were very sensitive to the challenge (only 7.5% of individuals survived).

In the wild, natural selection may have a greater effect than immunological mechanisms on reducing mortality rates from viruses. When virus is present, individuals with genetically-based resistance to a virus will tend to have more offspring that survive and reproduce than relatively susceptible individuals. Resistant genotypes may thus come to dominate a population. Of course, viruses also evolve, and they may mutate to becoem able to harm what were once relatively resistant genotypes. Of note, both YHV and TSV are RNA viruses, which are regarded as having rapid rates of evolution.

Question:

6. How can the strong influence of both natural and non-viral anthropogenic factors on shrimp populations be separated from risks associated with viral stressors?

Answer/comment:

Ecologists measure the effects of various factors on population density by performing controlled experiments. The effects of biological factors (for example, predators) are measured by excluding these organisms from some experimental plots. Experiments typically employ a factorial design if more than one factor is being studied.

Experiments to measure the effects of various factors, such as viruses, on shrimp populations would likely be impossible to perform with wild shrimp populations. However, small-scale lab experiments looking at, say, the effects of temperature and viral infection on fecundity may provide some clues to the relative importance of various factors.

Question:

7. Can human health effects from shrimp viruses be ruled out as a concern? Why or why not?

Answer/comment:

It is hard to imagine that viruses that infect as distantly a related organism as shrimp could harm the health of humans. However, some shrimp viruses come from groups of viruses that include strains which infect humans (Timmoney et al., 1992), so it may be incorrect to entirely rule out any possibility of human health effects under any circumstances.

IHHN (Parvovirus): There is no evidence that humans can be infected by Parvovirus strains that naturally infect other animals (e.g. Feline Panleukopenia, Canine Parvovirosis, Bovine and Porcine Parvovirosis, Aleutian Disease in Mink are not transmissible to humans) (Timmoney et al., 1992).

TSV (Picornavirus). There is no evidence that Picornavirus strains affecting other animals can be zoonotic (transmitted from animals to humans). However, there are two reports of humans becoming accidentally infected when manipulating vaccines (Timmoney et al., 1992).

YHV: (probably a Rhabdovirus, (Lightner 1996b)). Two diseases caused by Rhabdovirus are zoonotic, Rabies and Vesicular Stomatitis. Vesicular Stomatitis virus also infects arthropods and plants. Transmission to humans by ingestion of affected animals has not been demonstrated (Timmoney et al.1992).

WSBV: To the best of my knowledge, baculoviruses do not infect vertebrates.

Questions:

9. U.S. aquaculture operations have had problems with viral diseases for several years. How does information from local wild shrimp populations support or refute the importance of aquaculture operations as a source for the virus?

11. Some believe it likely that shrimp processing operations have processed virus-infected shrimp from foreign sources for several years. How does information from local wild shrimp populations support or refute the importance of shrimp processing as a potential source for the virus?

Answer/comment:

The scanty information currently available concerning viral infections of wild shrimp populations is completely inadequate to indicate the source of infection. Both aquaculture facilties and processing plants could be sources.

Evidence from the shrimp fishery demonstrates that farmed shrimp escape aquaculture facilities, potentially spreading disease. For example, in fall 1997 shrimp fishers harvested nonnative *P. vannamei* – almost certainly of farmed origin -- in Matagorda Bay, Texas. However, this evidence in no way negates the possibility that shrimp processing plants could also be a source of shrimp viruses.

On a related topic, disease outbreaks on US shrimp farms suggest that disease eradication on shrimp farms should be a vital element of efforts to prevent the establishment of shrimp viruses in wild populations of shrimp and other susceptible organisms. Although the JSA reports states that there are no reliable procedures for pond disinfection (p. 25), there are well-regarded procedures for cleaning up an aquaculture facility that has suffered a disease outbreak (Bell and Lightner 1992).

Question:

10. It has been widely held that it is highly unusual for domesticated animals to infect wild animal populations; usually it is the other way around. How well does this observation apply to the relationship between shrimp in aquaculture and wild populations, with regard to shrimp viruses?

Answer/comment:

Just because it appears unusual for domesticated animals to infect wild animals with disease does not mean that such disease transfers cannot have severe consequences and that the potential for disease transfers should not be of considerable concern. Consider the following relevant evidence:

•Aquaculture can be a source of new pathogens, parasites, and other organisms harmful to wild populations. The Japanese oyster drill (*Ocenebra japonica*) and a predatory flatworm (*Pseudosylochus ostreophagus*) were introduced with the Pacific oyster and have contributed to the decline of west coast oyster stocks (Clugston 1990).

•At least some experts consider the spread of exotic pathogens to wild fish to be the greatest threat to wild fish from salmon netpen farming (Kent 1994). Escaped farmed salmon may have been the source of the disease furunculosis in Norway, which has killed large numbers of wild fish (Heggberget et al. 1993). However, the evidence that farmed salmon have spread new diseases to wild salmon is not "airtight" (B.C. Environmental Assessment Office, 1997).

•The devastating spread of Asian chestnut blight to American Chestnut trees clearly demonstrates that introduced diseases can nearly eradicate a species (albeit a terrstrial plant species), radically change an ecosystem, and cause economic harm. American Chestnuts once dominated Appalachian forests (Keever 1953). Chestnuts were nearly eradicated following the inadvertant introduction early in this century of Asian chestnut blight on nursery stock of Asian chestnuts. Because of the introduction of this ascomycete-pathogen, Appalachian forests are now dominated by an oak-hickory complex instead of chestnuts (Keever 1953), and some researchers believe that ecosystem function (i.e. rates of nutrient cycling) may have changed in these forests (Shugart and West 1977). Moreover, the logging industry once supported by chestnuts – tall, straight hardwoods -- was ended by Chestnut blight.

Question:

12. Should the retailers who distribute (rather than process) shrimp products receive additional evaluation as potential sources of exposure?

Retailers and consumers should receive additional evaluation. Retailers and consumers may wash shrimp, using water that flows to municipal sewage and individual septic systems that may not deactivate viruses. Similarly, feces from consumers than have eaten uncooked shrimp (e.g., in ceviche) could contain active viruses that are not deactivated by sewage treatment.

Question:

13. After considering the sources addressed in the shrimp virus report, what sources other than aquaculture and shrimp processing are most critical for evaluation in a risk assessment of shrimp viruses? Given time constraints, which of these should be the focus of discussion at the workshop?

Answer/comment:

Given time constraints, bait shrimp are the most critical source for evaluation after aquaculture and shrimp processing. Shrimp are a popular form of bait in the southeastern Atlantic and Gulf of Mexico. Bait shrimp are often imported and are "released" directly into coastal waters.

Question:

14. Is manufactured shrimp feed a potential virus sources, or is the processing temperature sufficient to rule this source out?

Answer/comment:

The high temperatures at which shrimp and other animal feeds are typically processed are likely to greatly reduce, if not eliminate, the risk of transmission of shrimp viruses in feed. However, to the best of my knowledge there are not data to substantiate this assertion for all the shrimp viruses considered in the JSA report.

To make shrimp meal for feeds, shrimp byproducts are cooked in an oven at 90-95 C and then dried (Autin 1997). Feed manufacturing companies then process shrimp meal under different temperature-time regimes, depending on the final product being made. According to one US feed manufacturer, 99.9% of shrimp feeds manufactured in the United States are processed at temperatures of 76.6-137.7 C, with most feeds subjected to 87.7-110 C (T. Ziegler, Minutes of the stakeholder meetings) – although he does not mention the length of time that high temperatures are maintained.

Flegel (1995) reports that YHV was inactivated by exposure to 60 C for 15 minutes, concluding that YHV is not transmitted by shrimp feeds. IHHNV is inactivated at 80 C (Al-Mazrooei 1995, cited in Lotz 1997). There appear to be no data concerning time-temperature inactivation of TSV and WSSV. However, potentially relevant to WSSV, another shrimp baculovirus , *Baculovirus pennaei*, is inactivated in 10 minutes at temperatures of 60-90 C (LeBlanc and Overstreet 1991, cited in Lotz 1997).

In short, US shrimp feeds are unlikely to transmit YHV or IHHNV. Data about temperature-time regimes to inactivate TSV and WSSV are clearly needed. Compared to many of the other data needed to assess the risks of shrimp viruses, collection of data concerning inactivation of TSV and WSSV should be relatively quick and straightforward – and should be a high priority.

Question:

17. How can changes in wild shrimp populations be used to interpret the effect (or lack of effect) of introduced shrimp viruses? How could shrimp population models be used in the future?

Answer/question:

Shrimp populations fluctuate considerably from year to year – a 25% change is not uncommon in the Gulf of Mexico. Shrimp population models based on physical factors such as temperature and on recruitment strength and used to forecast shrimp harvests have historically been fairly accurate in predicting population fluctuations (J. Nance, pers. comm to P. Baker).

A large disparity between the harvest predicted by a forecasting model and an actual shrimp harvest – as there was this past season in the western Gulf of Mexico – could indicate shrimp mortality from a virus. However, viral disease would be only one of a number of possible explanations for an unexpected reduction in shrimp harvests.

Low levels of mortality from shrimp viruses would likely not be detected by comparing the results of predicted and actual shrimp harvests.

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Howard Harder

PRE-MEETING COMMENTS

Management goals, assessment endpoints, and the conceptual model

1. The management goal is adequate for what is going to be the initial phase of a ongoing investigation.

2. The stated assessment endpoints are sufficient. Shrimp viruses presumably will affect the target animal to a greater extent than they will affect related organisms. From the evidence to-date, determining whether or not shrimp viruses have an ecologically significant effect on wild shrimp populations will be challenge enough for the workgroup.

3. This is overly ambitious for the initial phase.

Viral stressors and factors regulating shrimp populations

4. In terms of the management goal, there is both relevant and irrelevant laboratory information on the infectivity of viral agents to our native shrimp species. Much of the public's concern originates from reports that native species are affected by various viruses. The vast majority of these reports comes from studies that cause infection by direct syringe injection of the viral agents and, therefore, the public's reaction is based on non-applicable information. As risk assessment is based on probabilities, it seems reasonable to base decision making on the most probable vectors of per os or water borne exposure, not the least probable vector of transmission through hypodermic use. (Even per os studies may not mimic the real world if the fed material is heavily loaded with viral particles and/or if infected material is the sole food source for the test animals, but these types of studies currently allow the best assessment of actual risk.)

5. Inoculations seem to work for a wide range of animals; it could be assumed that they would be effective for shrimp. The wild population of _Penaeus vannamei_ in Central America apparently has been inoculated with Taura Syndrome Virus (TSV) and the only lasting effect on the population level seems to be an increased resistance to the disease. A much less studied situation in South Carolina suggests the same conclusion as the wild population of _Penaeus setiferus_ contains a "White Spot Virus" that has been in the population for a number of years with no noticeable affect on population numbers (see question 10). (This virus will be labelled WSV in this paper to distinguish it from the Asian White Spot Syndrome Virus (WSSV)). This WSV only expressed itself when the shrimp were confined and stressed. This virus has now been found in shrimp from Georgia, and (anecdotally) from Texas and Washington state.

6. With the exception of catastrophic viral outbreaks (die-offs) in a wild population, it may be impossible to separate natural or man-made effects on population levels from subtle effects of diseases. For example, in South Carolina, it has been documented that winter temperatures are a major factor in determining the magnitude of the following fall harvest of white shrimp which can vary by a factor of 2; a disease outbreak with a mortality rate of 20 or 30 percent may not be detectable.

7. With no evidence to the contrary, it can be assumed that there is no effect on humans by the viruses of concern in this report. With the millions of pounds of virus-laden shrimp that is imported and consumed yearly with no reports of health related problems, even among individuals with depressed immune systems, this seems reasonable certain. This conclusion should be bolstered by the amount of shrimp eaten raw as sashimi.

8. This should be considered a two part question, asking both if the current identification techniques adequately reliable, and are there enough identification centers available. The first part is better left to the disease experts, but the second question is easily answered shrimp aquaculturists. There are not enough facilities and experts to allow for all phases of disease screening that is desirable for the culture industry in the United States. With so few centers available and the volume of "routine" analyses (from both within and without the U.S.) they are asked to perform, it is inevitable that backlogs develop. Rapid identification <u>AND</u> confirmation of diseases is all-important, yet is currently not possible as even with priority given to samples from outbreaks, definitive results can take weeks. Culture facilities face a two-edged sword as it is desirable to hold animals until their disease-free status is assured, yet the longer they are held at high densities, the more stressed they become and the more susceptible they are to infections, both from outside vectors and from forcing the expression of latent diseases.

Viral pathways and sources

Aquaculture

9& 10. Aquaculture operations can be a source of viral introduction but existing evidence indicates that the introduction is confined to the culture facility. With the exception of the Gulf of California study discussed elsewhere, are there any instances where outbreaks originated on a facility and significant mortality subsequently occurred outside the facility?

In South Carolina, three viruses have been identified over a number of years. In the late 1980's and early 1990's, IHHNV was a problem on several farms. It was known that this virus was imported with *P. vannamei* post-larvae (pl's) but was considered a risk associated with the culture of this species. During these years there was no restriction on water discharge and there were unintentional releases of infected animals from the farms. Over the last 10 years, no native shrimp have been diagnosed with IHHNV.

In 1996, a number of farms experienced a massive TSV outbreak. Again, there was water exchange prior to recognition of the disease and there was significant bird predation on dead and dying animals, with likely aerial transport of tissue and feces to the surrounding environment. Despite intensive monitoring of the wild population subsequent to this outbreak, no noticeable effects were observed; indeed, the following year was a "bumper crop" of native shrimp.

WSV was discovered in South Carolina during the winter of 1996-97. Two separate and discreet collections of adult *P. setiferus* (to be overwintered as broodstock) were taken to a state agency and a private farm, respectively. Despite the two collected populations being captured, transported, and held separately, both groups exhibited low-grade, but significant, mortailities that was diagnosed as some form of a White Spot Virus. These circumstances indicated the virus was present in the wild and a subsequent survey of areas along the South Atlantic coast confirmed the presence of the virus in shrimp and other crustaceans. The historical presence of the disease was confirmed in archival samples from previous years. In 1997, at least one farm experienced an outbreak of this WSV in ponds stocked with *Penaeus stylirostris* (that had previously tested negative for WSV), illustrating that the movement of the disease was from the wild to the farm.

Shrimp processing

11. & 12. It is a certainty that processing plants have processed virus-infected product for years, and retailers have sold virus-infected product for years. Without question, discharges from plants that processed infected shrimp have reached the environment, and retailers have sold infected shrimp that ended up as bait. Whether this processing or selling constitutes a significant threat to wild populations is unknown.

Other potential sources and pathways

13. The WSV in South Carolina apparently was not introduced by aquaculture as it was never identified in any of the farmed species. If aquaculture is ruled out as an introducing vector,

then this virus was introduced by another vector or was a naturally occurring disease in the native population. This would seem to argue for the tiered approach with the first assessment being to determine the naturally occurring diseases in the areas and species of interest.

14. Discount manufactured feed as a potential vector. Shrimp farms should be discouraged from using, or supplementing with, natural feeds such as baitfish, trawler by-catch, etc.

Stressor effects

15. In the best possible light, there is no evidence of detrimental effects of introduced viruses on wild shrimp populations. In addition to a possible viral cause, there seem to be indications that non-viral causative agents may be responsible for the temporary decline in the wild populations of shrimp in the Gulf of California in the 1980's, including the poor water quality often attributed to the upper reaches of the Gulf. (Mexican officials have said that a combination of weather conditions and overfishing may be determining factors in the population decline.) Shrimp population numbers are characterized by cyclical fluctuations over time in the absence of viruses, and it is questionable as to whether potential viral impacts on these numbers can be separated from all of the other impacts. In the worst light, IHHNV caused a reduced harvest of shrimp in the Gulf of California for several years before the population rebounded.

The TSV situation in South America underscores the importance of addressing the concerns presented in question 16. TSV was identified and largely confirmed as the causative agent in massive pond mortalities in cultured shrimp. Subsequently, TSV was identified in wild shrimp from surrounding areas. Without background data, is it not possible, even likely, that TSV was endemic to the wild population and unnoticed until it entered the culture environment and amplified? This would be similar to what is suspected with the WSV that appears to have been endemic in South Carolina for some time.

16. It is critical that background information be available for any type of risk assessment. In some cases it is possible to track a viral outbreak to a particular source, such as a farm experiencing a disease event and the problem being traced back, through infected pl's, to a hatchery. In other cases it is not known where the disease originated and without background information, the possibility of a culture pond being infected from the wild cannot be discounted.

17. Given the current lack of scientific information concerning all aspects of viruses in the wild, and the natural fluctuations in populations over time, it appears unlikely that trends in

population numbers, or population models, can be of significant use in interpreting effects of viruses on wild populations. (See questions 15 & 16)

18. Perhaps if a conclusion is reached that viruses do pose significant risks to native shrimp populations, then further investigations of risks to other organisms are warranted, but not at this time. (See question 2)

Comprehensive risk assessment and research needs

19. One significant contribution of the risk assessment approach should be to present a concise and factual report of what is, and is not, known about the shrimp virus situation. It appears that interest groups only present information favorable to their point of view and the media only reports items that may appear sensational; which often leaves the general public misled.

Similarly, this review and workshop should allow all participants to be more fully informed of the current state of viral affairs; experts in one field rarely get the opportunity to see the "big picture" in "real time".

20. Some immediate needs: Background assessment of current state of the wild population with respect to the incidence of viral occurrence; compilation and dissemination of available pertinent literature on viruses and their effects. Unsure of timeframe and cost.

21. & 22. Should be considered after workshop.

Fritz Jaenike

WRITTEN COMMENTS FOR "CHARGE TO PANEL MEMBERS"

Fritz Jaenike

1. The management goal reflects the dimensions of the shrimp virus problem , but needs to be qualified a great deal when utilizing it as the corner stone of the whole process. Some of the qualifiers which should be considered include:

   A. What is a "new" virus versus an "established" one? How do we know that background levels of virus are not naturally occurring or already present? i. e. /in South Carolina WSV (or WSV like) is widespread and detectable in a number of marine and estuarine species. Is this considered a "new" virus?

   B. When considering "disease-causing" viruses can you accurately lump all viruses into the same category or not? The disease causing abilities of IHHNV is certainly much different than WSV with regards to Gulf of Mexico and S. Atlantic shrimp species, which have been tested in the laboratories. Should imported shrimp with WSV be considered differently than imported shrimp with IHHNV? In aquaculture we would consider them quite differently when evaluating risks.

2. The first assessment endpoint in the JSA report " Survival , growth and reproduction of wild penaeid shrimp populations in the Gulf of Mexico and southeastern U. S. Atlantic coastal waters." is already so broad that it will be hard to measure. To expand the endpoint further to the entire marine ecosystem seems completely burdensome. The second endpoint of "Ecological structure and function of coastal and near-shore marine communities as they affect wild penaeid shrimp populations." is a multiyear undertaking that will probably lead the assessment to remain unresolved for years. If the risk assessment

is tiered and the policy makers decide "better safe than sorry" until we know the answers to all the questions, then we may end up with unrealistic recommendations and fail to determine a clear, realistic course of action. The determination of more specific answers related to virus policies may not be accomplished with goals that are so broad. Of course I would like to have an additional assessment endpoint. Protection of Shrimp mariculture industry from imported shrimp viruses.

3. A broader assessment considering alternate seafood production methods or other land usages as stressors to the health of the natural shrimp populations would be a huge undertaking. Deciding which of these stressors would be likely to antagonize or be synergistic to backround viral levels in wild shrimp would be even more difficult. By broadening the assessment we may lose focus of the intended outcome of the questions at hand. The main concern seems to be focused on shrimp viruses as it relates to risks to native shrimp populations. What should be the policy of the government on the imports of viral containing shrimp or with regards to outbreaks on aquaculture farms?

4. We need to evaluate the trials conducted in Texas on TSV and native shrimp as an example. Lab trials demonstrated problems with pL P. setiferus, while field trials failed to show similar effects. Lab trials are too intensive to be widely utilized to predict wild population effects. It does, however seem relevant to assume that if you can't kill shrimp in the lab it should be considered very low risk for a problem to occur in wild populations.

5. Very likely. The use of wild P. vannamei in ponds in Central and S. America over several years has generally demonstrated a decreased susceptibility to TSV with time. Gulf of California P. stylirostris utilized in aquaculture are

demonstrating less susceptibility to IHHNV than in previous years. Some strains of P. stylirostris have even been selected in aquaculture and are now resistant to IHHNV.

6. It's tough to say since past information on natural swings in shrimp populations are not associated with analyses which substantiate presence or absence of viruses. If you take historical information on shrimp population variations, the determination of which environmental or human activity was the major or most likely cause has seemed very subjective. A quantitative basis for determining variation is lacking.

7. From the bulk of historical information I would think the risk factor of shrimp viruses harming humans could be reduced to next to zero if not zero.

8. Some are some are not. Dr. Don Lightner could answer this question best. The need for holding in shrimp in stressful conditions followed by bioassays on known susceptible species is probably the most reliable indicator vs. some diagnostic tool by itself. This would particularly be the case with environmental media.

9. Information on the wild populations is so sketchy and incomplete it's hard to base any conclusions. Texas and South Carolina facilities operated with IHHNV present in pond raised shrimp for several years. I have not yet heard of a positive IHHNV occurrence in the wild populations. South Carolina may now be the leading information source on virus in wild populations utilizing the newest diagnostic tools. It appeared that WSV was present in the wild population prior to its detection in any aquaculture facility. More examples on how the wild

populations are a source of virus to aquaculture exist in other countries where wild seed are used in ponds.

10. This observation is very prevalent for shrimp viruses in South America, Mexico and Asia as evidenced by information from Roland Laramore on TSV in wild P. vannamei which is published in the JSA report.

11. There is not a great deal of information on the viral status of local wild shrimp utilizing the most recent diagnostic tools to base any opinions on. I am not aware of any survey on the viral status of wild shrimp from areas adjacent to major processing areas located in Alabama, Mississippi or Louisiana. The most data points on viral status of local shrimp that I am aware of is in South Carolina and I have been told there is not a great deal of processing going on there.

12. Yes, retailers, restaurants and food service.

13. Importers besides processors, bait and ship ballast water. Importers.

14. Not a source according to Tim O'Keefe with Rangen Feeds.

15. With caution. The role of IHHNV in the decline of shrimp populations in the 1980's needs to be considered along with the other stressors to the populations. How can we be sure that the viruses were introduced versus being at some baseline concentration within the wild population then expressing themselves in aquaculture and or environmental stress situations?

16. There should be a database established on background levels of viruses in wild shrimp populations utilizing the most sensitive diagnostic tools. Concentration

and holding of shrimp populations may need to be done to obtain low level or baseline levels of some viruses. Samples from processing areas which have not been surveyed should be prioritized in addition to aquaculture areas and control areas where neither exist. Use what information is available but rely on sensitive diagnostic techniques or those utilizing amplifications. This data gap should not be assumed for a risk assessment.

17. With or without analyses its tough to pin a decline on a virus. Shrimp population models are tough to use due to the number of factors such as weather which can cause normal variations.

18. There is need to evaluate what the case was in the Gulf of California with regards to non-shrimp species during the shrimp decline of the late 80's. The non-shrimp invertebrate populations in Asia where WSV and YHV are known occur and to be carried by other invertebrates besides shrimp should be evaluated.

19. Information from a risk assessment can contribute much to management decisions. South Carolina as a case point which is presently occurring should be considered. A lot of data should be evaluated in terms of effects on wild populations to help in determining management decisions. If we can identify the most likely problem causing viruses and the areas in which they are handled we can manage accordingly.

20. Gather more information in South Carolina. I don't know how much it will cost, but a concerted effort should produce some results relatively quickly.

Fritz Jaenike

21. Yes, we should prioritize the most likely inputs of virus to the U. S. (imported shrimp), and decide how best to implement practical , cost effective precautionary measures.

22. First, specific exposure scenarios should be identified and ranked according to most exposure to least. Then pole the stakeholders in those respective areas of measures which could be practically implemented to reduce the risk of exposure.

Donald Lightner

COMMENTS ON THE DOCUMENT:

MINUTES OF THE STAKEHOLDER MEETINGS OF THE JSA SHRIMP VIRUS WORK GROUP

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December 14, 1997

Management goals, assessment endpoints, and the conceptual model

1. How well does the management goal reflect the dimensions of the shrimp virus problem?

On page 14 of Appendix D, Report of the "JSA Shrimp Virus Work Group" the management goal is given as:

"Prevent the establishment of new disease-causing viruses in wild populations of shrimp in the Gulf of Mexico and southeastern U.S. Atlantic coastal waters, while minimizing possible impacts on shrimp importation, processing, and aquaculture operations."

In late 1995 and early 1996, when the shrimp virus issue was emerging, this goal may have been appropriate. The viruses, TSV, IHHNV, WSSV, and YHV (= Taura Syndrome Virus, Infectious Hypodermal and Hematopoietic Necrosis Virus, White Spot Syndrome Virus, and Yellow Head Virus, respectively), were "new" at that time in the sense that none of the three had been previously detected in farm raised or wild shrimp in Texas or elsewhere in North America. The management goal was based on the premise that none of these agents had become established in U.S. coastal or surface waters. There is increasing evidence that at least one of these agents, WSSV, has become established in wild stocks of the white shrimp, *Penaeus setiferus* in the Gulf of Mexico and in the western Atlantic off South Carolina. Hence, this management goal, as written, may no longer be appropriate, at least for this virus, in U.S. coastal waters.

2. Some have suggested modifying the assessment endpoints to emphasize potential risks of shrimp viruses to non-shrimp organisms and the larger estuarine ecological system or alternatively, to the aquaculture industry. Please comment on the assessment endpoints as the focal point for the ecological risk assessment.

Two "endpoints" are given in section 3, page 18 of the JSA report. The first centers around assessment of the threat of the shrimp viruses to "*survival, growth, and reproduction of wild penaeid shrimp populations in the Gulf of Mexico and southeastern U.S. Atlantic coastal waters*", and the second on assessing the effect of the viruses on the "*ecological structure and function of* *coastal and near-shore marine communities as they affect wild penaeid shrimp populations.*"

At first glance these seem to be reasonable "endpoints" of the risk assessment. The first is far more straight forward than the second. Some aspects of the first endpoint can be tested in controlled laboratory studies. Nonetheless, as pointed out by the JSA document and by various other persons in the "Proceedings of the Stakeholder Meetings", there are numerous important data gaps for all four shrimp viruses that will need to be filled before the JSA (or other group) can make an informed assessment on these "endpoints." Although the studies required to fill these data gaps are desirable, the time and resources required to run even a portion of the required studies is substantial if not impossible. For example, how can a study be run to determine the effect of an introduced pathogen on an ecosystem without actually introducing the pathogen? Hence, I have to recommend that the "endpoints" be kept narrowly focused (as the JSA report has generally attempted to do) so that meaningful data can be generated and used in the risk assessment process.

3. It has been suggested that the scope of the proposed risk assessment is too narrow and that it should be broadened to consider the impacts of such stressors as alternative land uses and seafood production methods in coastal areas. Please comment on this suggestion.

It is not at all clear to me what is being suggested here. Is it being suggested that all **anthropogenic** changes (i.e. alternative land uses) to coastal areas be considered in the shrimp virus risk assessment? Hence, without having the suggestion (or question) clarified, I cannot comment.

Viral stressors and factors regulating shrimp populations

4. How relevant to virus effects on wild shrimp populations is information on infectivity and effects that is derived from laboratory or intensive aquaculture operations?

Data of the sort referred to here, which is obtained from laboratory studies or from intensive aquaculture operations, provides an indication of the potential effects of a given "stressor" or "factor" on wild shrimp populations. Correctly run laboratory studies test only one variable. The environmental conditions in aquaculture farms is highly controlled, and thus the number of variables, while more than in a lab settings, is far less than in a "wild setting". Hence, while such data provides only an indication of what might be, it is the best and most reliable data available.

5. How likely is it that exposure of wild shrimp populations to viral diseases could lead to the development of immunity and reduced effects on population survival over time?

The available data on this question suggests that it is very likely that wild shrimp populations will develop "resistance" (the term "immunity" may be an inappropriate term in arthropods) to introduced viral pathogens. Penaeid shrimp have an extremely high fecundity. This high fecundity, paired with natural selection for resistance to a given pathogen (in the continuous presence of the pathogen), translates into a high potential for the relatively rapid development of specific pathogen resistance with each successive generation. Only survivors that are resistant to a particular pathogen live to breed. This phenomenon has occurred in the wild *P. stylirostris* stocks in the Gulf of California in response to the introduction and establishment of IHHNV. It has been used in the development of specific pathogen resistant (SPR) breeding lines (for IHHNV and TSV) by several groups in the shrimp farming industry. Perhaps, the apparently steadily improving resistance of wild postlarvae used in Latin American shrimp farms to TSV has likewise resulted from natural selection of some wild stocks of *P. vannamei* where the virus has become enzootic.

6. How can the strong influence of both natural and non-viral anthropogenic factors on shrimp populations be separated from risks associated with viral stressors?

To obtain the sort of information required here, single (or multiple factors) have to first be identified and defined. Then controlled laboratory studies, in which the effects of varying the values of single (or multiple) factors, can be designed and run to gain some insight as to their potential effect in natural settings. When coupled with controlled virus challenge studies, the effect of some factors such as changing salinity, temperatures, or other natural or non-viral factors, can be estimated.

7. Can human health effects from shrimp viruses be ruled out as a concern? Why or why not?

Nothing in living systems is absolute. However, shrimp viruses can only affect human health indirectly through loss of income: shrimp that die from virus infections cannot be harvested (from farms or the wild) and sold. Despite the opportunity for infection presented over the past 30 to 50 years by the millions of tons of shrimp that have been harvested from all over the world from wild fisheries and farms, have been processed, packed, and cooked by human hands, and finally consumed by humans, no case of a shrimp virus infecting a human (or any other mammal) has ever been reported.

8. Are the available identification techniques for shrimp viruses reliable enough to allow definite conclusions to be drawn about the occurrence of viruses in shrimp and environmental media?

This question can best be addressed with the following table. The table lists most of the methods available for the detection of infections by the viruses TSV, IHHNV, WSSV and YHV. Good methods for detection of infection are readily available for all but YHV. Application of these methods to "environmental media" may be more problematic, and is largely untested.

Table 4. Summary of diagnostic and detection methods for the major viruses of concern to the shrimp culture industries of the Americas (modified from Lightner 1996a).				
Method*	**IHHNV**	**TSV**	**YHV**	**WSSV**
Direct bright field light microscopy (LM)	-	++	++	++
Phase Contrast LM	-	-	-	+
Dark-field LM	-	-	-	++
Histopathology (of acute infections)	++	+++	+++	+++
Enhancement/Histology	++	+	-	++
Bioassay/Histology	++	+++	+++	++
Transmission electron microscopy (EM)	+	+	+	+
Scanning EM	-	+	-	+
Fluorescent antibody with PABs or MAbs	r&d	r&d	r&d	r&d
ELISA with PABs	-	r&d	r&d	-
ELISA with MABs	+/r&d	++/kit	r&d	-
DNA Probes	+++/K	+++/K	+/r&d	+++/K
PCR	+++	++/r&d	+/r&d	+++

* Definitions:

- \- = no known or published application of technique.
- \+ = application of technique known or published.
- ++ = application of technique considered by author to provide sufficient diagnostic accuracy or pathogen detection sensitivity for some applications.
- +++ = technique provides a high degree of sensitivity in pathogen detection.
- K = diagnostic kit or product available from DiagXotics, Inc. (Wilton, CT, U.S.A.).

Methods: BF = bright field LM of tissue impression smears, wet-mounts, stained whole mounts;

LM = light microscopy;

EM = electron microscopy of sections or of purified or semi-purified virus;

ELISA = enzyme-linked immunosorbent assay;

PAbs = polyclonal antibodies;

MAbs = monoclonal antibodies;

r&d = techniques in research and development phase.

Viral pathways and sources

Aquaculture

9. U.S. aquaculture operations have had problems with viral diseases for several years. How does information from local wild shrimp populations support or refute the importance of aquaculture operations as a source for the virus?

While shrimp farms in the United States have had a history of disease episodes caused by IHHNV, TSV, WSSV, and possibly YHV, only strains of WSSV have been detected in populations of wild shrimp. Because only specific pathogen-free (SPF; shown to be free of IHHNV, TSV, WSSV, YHV, and other major shrimp pathogens by routine testing over multiple generations in captivity) *P. vannamei* or indigenous *P. setiferus* had been cultured at the affected farm in 1995 (and in 1993 and 1994), the probability is extremely low that the *P. vannamei* stocks were the source of TSV, WSSV, and YHV that appeared in Texas in 1995. Contamination of the affected farm (TSV in May, and WSSV and YHV in October, 1995) came from some other source. Likewise, monitoring of the stocks used at the farms in Texas and South Carolina in 1996 and 1997, clearly demonstrated that TSV entered some farms that year through a breech in the SPF program. However, WSSV and the YHV agent were not detected in the *P. vannamei* stocks used in 1995-1997, **unless wild *P. setiferus* was also present.** These data implicate shrimp farming only in the re-occurrence of TSV the U.S. in 1996, but not in initial appearance of TSV in Texas in 1995, nor of the appearance of WSSV and YHV in 1995 and 1997. Wild *P. setiferus* have been clearly shown to be the source of contamination in these latter cases.

10. It has been widely held that it is highly unusual for domesticated animals to infect wild animal populations; usually it is the other way around. How well does this observation apply to the relationship between shrimp in aquaculture and wild shrimp populations, with regard to shrimp viruses?

First of all, the basic premise of this question is wrong! It is not difficult to find examples in the literature (in mammals, birds, fish, mollusks, and crayfish) where serious pathogens (viral, bacterial, protozoan, and fungal) have been transferred from domesticated (or captive non-indigenous) stocks to wild stocks. Reducing the risk of accidental introduction of non-indigenous pathogens to wild stocks with introduced domesticated or captive-wild stocks are among the expressed purposes of the ICES Guidelines and of the USMSFC SPF program.

Shrimp processing

11. Some believe it likely that shrimp processing operations have processed virus-infected shrimp from foreign sources for several years. How does information from local wild shrimp populations support or refute the importance of shrimp processing as a potential source for the virus?

The answer depends on the virus. While apparently not enzootic in the U.S., IHHNV and TSV are enzootic in cultured and wild shrimp stocks in most shrimp farming areas of North America. WSSV and YHV are not. Other than in Asia and the Indo-Pacific, WSSV and YHV have only been found in wild or cultured shrimp in the U.S. If we look at what is different between the U.S. and other major penaeid shrimp farming or fishing countries in the Americas, it is apparent that one difference is that the U.S. imports and processes vast quantities of Asian shrimp, while the other countries, who have not yet had cases of WSSV or YHV, do not import and/or process shrimp from areas where WSSV and YHV are prevalent.

12. Should the retailers who distribute (rather than process) shrimp products receive additional evaluation as potential sources of exposure?

Yes. Sport fishermen commonly purchase penaeid shrimp from retail outlets (grocery stores, as well as from specialized bait dealers) and introduce these potentially contaminated shrimp where they fish. Imported shrimp are commonly used as bait in marine, estuarine, and freshwater sport fisheries in the U.S.

Other potential sources and pathways

13. After considering the sources addressed in the shrimp virus report, what sources other than aquaculture and shrimp processing are most critical for evaluation in a risk assessment of shrimp viruses? Given time constraints, which of these should be the focus of discussion at the workshop?

Bait shrimp should be considered. Ship ballast water, visitors, birds, feeds and feed ingredients, and other vehicles of transport are far less likely to provide an effective means of virus transport than are the live or frozen hosts of these pathogens. Therefore, all live and frozen shrimp products should be the focus of discussion at the workshop.

14. Is manufactured shrimp feed a potential virus source, or is the processing temperature sufficient to rule this source out?

As I answered to one of the questions earlier in this discussion, nothing is absolute. However, the relative risk posed by shrimp feed (that contains shrimp or crab meals) is extremely low. Were this not the case and shrimp feeds were the source of these viruses in the U.S., other countries using far more shrimp feed from the same sources, should have been even more severely impacted by the pathogens in question than has the U.S. industry.

Stressor effects

15. How should the available evidence concerning the effects of introduced viruses on wild shrimp populations be interpreted?

Volumes could be written on this question. The effect of an introduced virus on a wild population is affected by several factors. Among the most important of these are: 1) the relative naivety (susceptibility) of the host population to the virus; 2) the virus' mode(s) of transmission; 3) efficiency of transmission by horizontal or vertical routes; 4) life stages when acute disease typically occurs; 5) environmental factors that could influence disease expression at the susceptible life history stages; and 6) other factors. With this in mind, the available evidence should be considered individually for each virus in each host system. For example, the prognosis for an IHHNV infection in naive *P. stylirostris* in the Gulf of California in 1988-1992 is not the same as the prognosis for TSV infection in wild Ecuadorian *P. vannamei*. We know from controlled laboratory studies that the latter resulted in more survivors than the did former.

16. There is presently a lack of basic data on background levels of pathogenic viruses in wild shrimp populations in U.S. waters. How should this gap be evaluated in a risk assessment?

There have been a number of pathogen and parasite surveys carried out on shrimp from U.S. waters. Some of these date back to the 1960's; some have been thorough multi-year studies in which samples of shrimp in various life stages were taken and examined for viral or other pathogens. Likewise, the academic and commercial aquaculture industries in the U.S. have collected, cultured and studied wild shrimp on and off since the late 1960's. From all of these studies, BP is the only viral pathogen documented in wild shrimp in U.S. waters prior to 1995. While not explicitly tested for, signs of infection by WSSV, YHV, IHHNV, and TSV were not noted in these studies. Had pathogens like WSSV been present before 1995, it would have made its presence known, especially in captive live animals in laboratories or bait camps. The "gap" in the data is not as large as the question implies.

17. How can changes in wild shrimp populations be used to interpret the effect(or lack of effect) of introduced shrimp viruses? How could shrimp population models be used in the future?

Population models are only as good as the data fed into them. In order to have any validity, studies done on shrimp viruses in wild populations will require that the populations of interest are appropriately sampled and tested for the pathogens of concern. The resulting incidence and prevalence data can then be used to make predictions and draw conclusions from population models.

18. How important are potential viral effects on non-shrimp species?

This question may only apply to WSSV. For IHHNV, TSV, and probably YHV, penaeids (or very closely related shrimps) seem to be susceptible to infection and prone to disease if infected. In marked contrast, WSSV can infect, and kill in some cases, a wide variety of crustaceans. Among the hosts killed by WSSV are some species of freshwater crayfish. The wide host range of WSSV makes it an important potential pathogen of North American crustaceans, both freshwater and marine.

Comprehensive risk assessment and research needs

19. How will a comprehensive risk assessment contribute to management of the shrimp virus problem, i.e., will it add significantly to the information presently available?

A comprehensive risk assessment has the potential of gathering virtually all of the available information on this topic in one place and extracting from it the facts necessary to make informed management decisions. The key to the appropriateness of the decisions made, may depend in large part, on how well the available data is acquired and evaluated.

20. What type of assessment should be conducted next (e.g., quantitative risk estimates using shrimp population models), and what would be the likely time frame and cost?

This question might best be deferred to the NMFS where I presume the latest models are available.

21. Should a future risk assessment consider the risk reduction potential of a range of treatment options associated with specific exposure scenarios?

What treatment options?

22. Summarize the critical research needs for completing such a risk assessment?

I cannot comment here because it is not at all clear to me what is being asked in question #21.

Jeffrey Lotz

MEMORANDUM

From: **J. M. Lotz**
Date: **18 December 1997**
To: EPA/ERG Shrimp Virus Peer Review and Workshop
Subject: Comments on questions

Management Goals, Assessment Endpoints, and the Conceptual Model

1. *How well does the management goal reflect the dimensions of the shrimp virus problem?*

> "*Prevent the establishment of new disease-causing viruses in wild populations of shrimp in the Gulf of Mexico and southeastern U.S. Atlantic coastal waters, while minimizing possible impacts on shrimp importation, processing, and aquaculture operations.*"

The genesis of this workshop appears to be the possible introduction and establishment of one of four viral agents of shrimp aquaculture in the U.S. Gulf of Mexico and the Atlantic Ocean. The agents are WSV, TV, YHV, and IHHNV. However, as is perhaps common to these kinds of activities the management goal appears to lack precision.

(a) The viruses are not specifically identified. The phrase "new disease-causing viruses" implies management of as yet unknown and undiscovered viruses. If this breadth is to be applied to viruses generally why not include other categories of pathogens and potential pathogens?
(b) What is meant by establishment? Would the finding of a positive animal in a wild populations meet the report's definition of establishment, should it be found over some period of time, should it be a self maintaining population of virus.
(c) The word "shrimp" implies more than *P. aztecus*, *P. duorarum*, and *P. setiferus*.

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(d) The word disease-causing is a very slippery word. If infected animals are not seen to be diseased are they not to be considered for management or does disease causing imply "potentially an agent of disease". In this case any parasite could be a pathogen in some species of host.

The second concern is that if one or more of the agents under consideration have already been introduced then the management goal can not be met and the exercise seems irrelevant to the management goal. There is some evidence that at least one of the viruses have already been established in both bodies of water.

The goal as stated ranks the endpoints. Highest priority is prevention of establishment of the viruses. Taking second position is the minimization of impact on business. Otherwise the wording would be something like "minimize the probability" or "reduce the probability" of establishment. If the goal is to guarantee that new viruses are not established (the phrase says "to prevent" not "to reduce the chance s") from aquaculture then there can be no aquaculture if the goal is to guarantee no establishment from imported shrimp then there can be no imported shrimp. My guess is that the goal is really to balance the risks of establishment with the risks of guaranteeing that establishment will not occur.

2. *Comment on the scope of the risk assessment to be limited to effects of viral establishment on populations of "shrimp".*

Shrimp is in fact a rather wide category and the risk assessment is broader than our knowledge base. Broadening the assessment more will put a greater distance between our knowledge and the decisions. Nonetheless the unforseen consequences are usually the ones that come back to haunt any decision. Although I think that the overall assessment should be clearly placed in the context of the ecosystem, the effect on the ecosystem can not be the focus of the risk assessment. This is way beyond our ability to estimate.

3. *Comment on increasing the scope to include not only viral stressors that might affect shrimp populations but also other kinds of stressors that might affect shrimp populations.*

The farther afield from the problem at hand the process gets the less valuable the process will be. Although I can understand why the risk assessment should consider the effects of viral establishment on the ecosystem, I can't fathom why this risk assessment should be expanded to include the effect of global warming or alternative land uses on shrimp populations.

Viral Stressors and Factors Regulating Shrimp Populations

4. *How relevant to virus effects on wild populations is information on infectivity and effects that is derived from laboratory or intensive aquaculture operations?*

In general information derived from laboratory studies is quite relevant to natural settings. However, one has to look at the conditions in the particular laboratory experiment or the aquaculture setting. It is often assumed that laboratory or aquacultured animals are at much higher densities than natural populations but that is not always the case. If one assumes that the Gulf of Mexico is a large aquaculture pond or a large aquarium then the conclusions based on experiments will not translate to the Gulf of Mexico. However, if one views the Gulf of Mexico as composed of a large number of aquaculture ponds or aquaria, then the results of laboratory experiments are likely to translate more realistically. If wild animals get the same dose and have access to consumption of dead animals as they do when they are taken into the laboratory or into an aquaculture setting then they will act in the wild like they do in the artificial settings.

The adjective "intensive" changes the flavor of the question? Does the question assume that the relevance of information derived from semi-intensive or extensive aquaculture is unassailable?

5. *How likely is it that exposure of wild shrimp populations to viral diseases could lead to the development of immunity and reduced effects on population survival over time?*

Assuming all else is equal and that some members of the shrimp population possess genes that would impart resistance then it is quite likely that over several generations there would be changes in the genetic composition of both the shrimp population and the viral population that might reduce the effects of the pathogen on the dynamics of the shrimp populations. However, the ability to predict such changes assumes that the genetic traits that code for resistance to a virus are not linked to some other fitness lowering traits such as ability to avoid predators. It is often assumed that less virulent viruses are more fit than the virulent viruses but that is not always the case. If more than one pathogen was established and resistance to one did not provide resistance to the other but actually increased the virulence of the second then no net change would be observed; some members of the shrimp population would be resistant to one pathogen and not the other. If the virus was actually maintained in one species that acted as an unaffected "carrier" the resistant carrier might actually use the virus to displace less resistance species. The virulence of the virus might be unaffected by this situation. This is the case with crayfish plague in Europe where introduce resistant crayfish are displacing wild susceptible crayfish by carrying crayfish plague.

6. *How can the strong influence of both natural and non-viral anthropogenic factors on shrimp populations be separated from risks associated with viral stressors?*

It is always difficult if not impossible to separate the effect of two factors that operate at the same time particularly if they co-vary. What is needed is a series of natural experiments, that is, several populations of host, some with the virus some without the virus, some subject to the anthropogenic stressor some not, and some with combinations of the various factors. The populations can be separated by either time or space. In time one could look at a population prior to the introduction of a virus but with the second factor present then compare the population after the establishment of the virus. Occasionally one can use data from an unrelated host and parasite that mimics the situation of interest to determine what might in an analogous situation.

7. *Can human health effects from shrimp viruses be ruled out as a concern? Why or why not?*

I am not concerned with the human health effects of shrimp viruses. However, one can never be absolutely certain that a virus of a non-human host will not become infectious to humans. Influenza viruses jump from pigs, chickens, etc. to humans regularly. In addition viruses of insects are transmitted to humans all of the time. The arboviruses multiply in both human and arthropod hosts. Nearly anything is possible.

8. *Are the available identification techniques for shrimp viruses reliable enough to allow definitive conclusions to be drawn about the occurrence of viruses in shrimp and environmental media?*

In general yes; however some are more reliable than others. The question should not be asked outside of an understanding that the reliability of any single diagnostic test can only be determined after lengthy evaluation and clinical trials. Clinical trials have not occurred for the shrimp diagnostic procedures to the extent that they have for pathogens of poultry or cattle or humans. Further the trials that have been done have not been done for surveys of wild shrimp. For the most part the viruses are new, the diagnostic procedures are new, and even the aquaculture of shrimp is new. Most of the molecular diagnostic tools have not been adequately tested to be used on wild shrimp without a second backup benchmark. The typical benchmark diagnostic test is a histological exam; however, in critical cases, particularly for surveys of wild shrimp, follow-up bioassays are required. In some cases the histological evaluation is not completely reliable. The histological pathology associated with some of the viruses may look like pathology caused by another virus.

Viral Pathways and Sources

9. *U.S. aquaculture operations have had problems with viral diseases for several years. How does information from local wild shrimp populations support or refute the importance of aquaculture operations as a source for the virus?*

In the U.S. there is evidence that a shrimp virus may be present in in wild populations but the source is not known. There have been small surveys of penaeid shrimp in the U.S. for evidence of the viruses but those surveys have not turned up conclusive evidence that any of the four viruses are present in U.S. waters. The introduction of IHHNV into the Gulf of California is the best documented case of an the introduction of a viral pathogen into wild shrimp populations from aquaculture. It also may be that Taura virus has been introduced into wild shrimp in parts of Central and South America and that introduction was from aquaculture. The difference between the likelihood of aquaculture as a source for the introduction of viruses into Mexican, Central and South American wild shrimp probably lies in the much higher aquaculture levels that occur in those regions.

10. *It has been widely held that it is highly unusual for domesticated animals to infect wild animal populations; usually it is the other way around. How well does this observation apply to the relationship between shrimp in aquaculture and wild shrimp population, in regard to shrimp viruses?*

The situation in terrestrial livestock agriculture may appear to be different because of differences between the states of development of terrestrial agriculture and aquaculture. The vast majority of livestock used in terrestrial agriculture have no wild stocks of the same species that are exploited for commercial purposes, therefore few are concerned that say an outbreak of hoof and mouth disease in cattle will spread into wild populations of cattle. There are no wild cattle.

There are a number of examples of aquaculture as the cause for an outbreak or introduction of a disease agent into wild populations, e.g., crayfish plague, whirling disease, Anguillicola sp., several salmon bacteria and viruses. The movement of pathogens into wild species has the consequence that the wild animals then become a future source of infections once farmers eliminate the pathogen from their farmed stocks by replacement of animals imported from other farmers or regions. The wild animals are then of concern to farmers and their livestock eventhough the original introduction of the pathogen into the wild population was from aquaculture. I would not be surprised if terrestrial livestock agriculture had followed a similar scenario during its early history of domestication of stocks. Therefore the "unusual" situation in aquaculture is not unusual at all it is just that aquaculture and terrestrial livestock agriculture are at simply different phases in their development.

Shrimp Processing

11. *Some believe it likely that shrimp processing operations have processed virus-infected shrimp from foreign sources for several years. How does information from local wild shrimp populations support or refute the importance of shrimp processing as a potential source for the virus?*

The information from local wild shrimp populations is very meager. However, there is evidence that at least one of the viruses is present in wild shrimp in the Gulf and the Atlantic. The source of it is unknown and by itself doesn't suggest processing rather than some other source. There is clear evidence that infectious virus is present in at least some frozen shrimp destined for domestic processing. Another piece of evidence that might point to processing as an indirect source is that the

U.S. aquaculture industry is the only industry in the western hemisphere that has reported WSV. WSV has not been reported from aquaculture of shrimp in Mexico, Central America, nor South America. There is much less processing of shrimp imported from Asia (where WSV is common) in Mexico, Central America and South America. This may suggest that processing of shrimp from Asia resulted in contamination of U.S. aquaculture. This of course assumes that the U.S. WSV is Asian in origin.

12. *Should the retailers who distribute (rather than process) shrimp products receive additional evaluation as potential sources of exposure?*

The evaluations that have been done are minimal; however, infectious virus has been found in shrimp in supermarkets. If these shrimp are purchased and "processed" at home the disposal of the home waste could be a source of contamination. There should be further evaluation of shrimp that may carry infectious virus regardless of whether they are to be processed or not. The focus should be on the viruses, the infectiousness of the virus, and how those viruses might contact susceptible hosts.

Other Potential Sources and Pathways

13. *After considering the sources addressed in the shrimp virus report, what sources other than aquaculture and shrimp processing are the most critical for evaluation in a risk assessment of shrimp viruses? Given time constraints, which of these should be the focus of discussion at the workshop?*

One source for the establishment of a virus into U.S. waters, especially into the Gulf of Mexico, might be the natural spread of virus from a point of establishment outside of U.S. waters into shrimp of U.S. waters. In particular, it might be that an establishment in the Gulf of Mexico or the Caribbean Sea might spread into the U.S. by migration and contact among susceptible species. It would be important to know whether any of the viruses of interest are already present in areas of shrimp aquaculture along the coasts of nations bordering the Gulf of Mexico.

14. *Is manufactured feed a potential virus source, or is the processing temperature sufficient to rule this source out?*

The temperature of processing of manufactured feeds depends upon the method of preparation. IHHNV would need to be heated to 80°C. It may be necessary to treat Taura Virus to an even great temperature to prevent infectiousness. There are however a number of fresh uncooked feeds that are associated with shrimp aquaculture, algae, brine shrimp, squid, and blood worms among others. A live organism could conceivably carry one or more of the viruses (WSV has been shown to have a wide host range among crustaceans). Any fresh feed could act as a mechanical vector any of the viruses. This would be particularly likely if a processing plant processes shrimp and one of the fresh feeds in particular squid is likely to be processed by the same processors as shrimp since both are used as food for people.

Stressor effects

15. *How should the available evidence concerning the effects of introduced viruses on wild shrimp populations be interpreted? (For example, what was the role of IHHNV in the decline of shrimp populations in the 1980's in the Gulf of California? What about TSV release from aquaculture into the wild in South America?)*

There should be no question that viruses have been introduced into wild shrimp populations from aquaculture. Since the stated management goal of the risk assessment is to "prevent the establishment" of viruses then the pertinent data is that that can happen. When the question is, "can one predict what will happen if a virus is introduced into a wild shrimp population?" one has to again look at the available data. The data from the Gulf of California clearly show that IHHNV was introduced from aquaculture and that *P. stylirostris* were found with IHHN disease. What is less clear is how was IHHNV introduction related to the decline in catch. The catch data that I have seen (reported in a Tucson newspaper) is that the catch was already in decline prior to the introduction of IHHNV. I am not familiar with the data for catch of shrimp in areas where TV or WSV have been introduced. There are however, examples of the introduction of pathogens into other kinds of aquatic systems. For example the outbreak of a virus in hard head catfish (*Arius felis*) in the Gulf of Mexico during 1996 caused a definite short term (same year) decline in the numbers of catfish that were caught in sampling gear by state agencies in Mississippi. However, there does not seem to be any shortage of hardhead catfish in 1997. Of course hard head catfish are not an economically important species so the numbers are not well known.

From a theoretical perspective we can consider the consequences of introducing an additional risk factor (virus) into a shrimp population. For example if the survival of shrimp in the absence of the additional factor is 1%, that is 99% of them die from some other cause and a shrimp subjected to mortality from the additional factor alone has a 75% chance of dying (about the mortality rate for *P. vannamei* infected with Taura Virus) then a shrimp subjected to both the additional factor (virus) and the general mortality factors has a 99.75% chance of dying. (The chances of surviving both TV and general mortality is (1-.75)*(1-.99)=0.0025 the composite mortality rate is .9975.) The net increase due to the additional factor is only 0.75%, that is out of 10,000 shrimp 9900 would die in the absence of the additional factor and 9975 would die in its presence. There are certainly other considerations that need to be taken into account but the general result is that the increase in the mortality rate from the addition of another mortality factor is actually quite small when the initial mortality rate is quite high.

16. *There is presently a lack of basic data on background levels of pathogenic shrimp viruses in wild shrimp populations in U.S. waters. How should this data gap be evaluated in a risk assessment?*

The data gap can only be evaluated as lacking. I guess the reason for a risk assessment is to deal with data gaps. There may be more data than one thinks. There is at least one unpublished data set on the seasonal dynamics of *Baculovirus penaei* (BP) in *P. aztecus*. BP is a fairly pathogenic virus of shrimp that is native to the Gulf of Mexico.

17. *How can changes in wild populations be used to interpret the effect (or lack of effect) of introduced shrimp viruses? How could shrimp populations models be used in the future?*

I think that changes in wild populations are an extremely valuable source of information. However, one needs good data on variation over several years prior to the introduction of a virus. The data need to be appropriately collected. There are real problems with landings as indicators of shrimp numbers. If fishery independent data on abundances of shrimp are available prior to an introduction and the dynamics can be followed subsequently then good conclusion can be made. Another approach is to look at natural experiments as alluded to in my comments to number 1.

Population models of shrimp are important. More important are models of shrimp and their pathogens. These models can be very helpful in identifying what rates need to be determined and what parameters need to be estimated. For example epidemiological models can be built that incorporate the population dynamics of shrimp populations and they can be used to suggest which factors are important to the establishment of a pathogen and the consequences of that establishment on shrimp populations. Not only can population dynamic models be useful but also genetic and evolutionary modes should be considered.

18. *How important are potential viral effects on non-shrimp species?*

Very important. For example, if a virus reduces the numbers of a species that serves as food for an important fishery species then there could be a reduction in the abundance of that fishery species. In addition, other species may serve as reservoirs for outbreaks in other wild or cultured species. Certainly if the goal is to prevent establishment then the role of non-shrimp species needs evaluation.

Comprehensive Risk Assessment and Research Needs

19. *How will a comprehensive risk assessment contribute to management of the shrimp virus problem, i.e., will it add significantly to the information presently available?*

A comprehensive risk assessment should contribute to understanding and defining what the problem is and what might be done to prevent establishment. In addition the assessment will probably point out areas for future research and information that is needed to answer specific questions related to introduction if the viruses. The process seems to be rather lengthy. Pathways are now open that appear to have a considerable amount of virus already. Establishment might actually occur before the assessment is done.

20. *What type of assessment should be conducted next (e.g., quantitative risk estimates using shrimp population models), and what would be the likely time frame and cost?*

I think that it is important to get really good estimates of how much infectious virus is coming into the U.S. and where the virus might be contacting wild populations. I think that the most important factor in determining whether a virus will be established in a susceptible wild population is how many times introduction is tried. I think that determining whether a particular virus will become established will require detailed knowledge of the doses that wild populations are actually exposed to, the distribution of shrimp in the wild, the virulence of the virus to the species of interest, and the transmission potential of the viruses in water or by contacting infected shrimp. These kinds of parameters can be put into epidemiological models that will help understand whether a virus is likely to become established at various values of dose, susceptibility and transmissiblility.

21. *Should future risk assessment consider the risk reduction potential of a range of treatment options associated with specific exposure scenarios?*

Yes.

22. *Summarize the critical research needs for completing such a risk assessment.*

We need to know how much virus is contacting wild shrimp populations and what the infectiousness of the contacting virus is. We need information on the transmission rate within and among wild populations of the species of wild shrimp. We need evaluation of the virulence of the viruses in the species of wild shrimp of interest. It is also critical to determine what the temporal and spatial distribution of wild shrimp populations are in the Gulf and Atlantic. This kind as well as other similar kinds of information will be needed for epidemic models that will allow good guesses for the likelihood of establishment through various pathways. Another piece of information that is needed is to know whether or not the pathogens of interest have are already established in the Gulf and Atlantic.

Roy Martin

Premeeting comments are not available at this time.

Larry McKinney

Larry D. McKinney

RESPONSE TO QUESTIONS FROM - LARRY McKINNEY

MANAGEMENT GOALS, ASSESSMENT ENDPOINTS, AND THE CONCEPTUAL MODEL

1. **How well does the management goal reflect the dimensions of the shrimp virus problem?**

   The management goal: *Prevent the establishment of new disease-causing viruses in wild populations of shrimp in the Gulf of Mexico and southeastern U.S. Atlantic coastal waters, while minimizing possible impacts on shrimp importation, processing, and aquaculture operations* is on target and appropriate for a risk assessment exercise.

2. **Some have suggested modifying the assessment endpoints to emphasize potential risks of shrimp viruses to non-shrimp organisms and the larger estuarine ecological system or, alternatively, to the aquaculture industry. Please comment on the assessment endpoints as the focal point for the ecological risk assessment.**

   The assessment endpoints as proposed seem appropriate, although the second assessment endpoint: *The ecological structure and function of coastal and near shore marine communities as they affect wild penaeid shrimp populations* - may be too broad even in the context of a risk assessment. It is my understanding that this endpoint represents the "valued ecological entity" and that *Survival, growth and reproduction of wild penaeid shrimp populations in the Gulf of Mexico and southeaster U.S. Atlantic coastal waters* - is intended to represent an attribute of that entity, in the context of risk assessment process, that are important to protect and are potentially at risk. I would not recommend expanding these endpoints to include additional risks.

3. **It has been suggested that the scope of the proposed risk assessment is too narrow and that it should be broadened to consider the impacts of such stressors as alternative land uses and seafood production methods in coastal areas. Please comment on this suggestion.**

   I do believe that the impact of additional stressors should be assessed. Some that were included in testimony were: Operational methods, especially associated with wastewater discharges, bait production for recreational use, shrimp feed production, human waste, direct importations to retailers. Intuitively, some would seem of low probability, but I would think they need some level of consideration.

<u>VIRAL STRESSORS AND FACTORS REGULATING SHRIMP POPULATIONS</u>

This topic includes basic information about shrimp viruses as well as the full range of natural and anthropogenic factors that regulate shrimp populations. Questions for consideration:

4. **How relevant to virus effects on wild populations is information on infectivity and effects that is derived from laboratory or intensive aquaculture operations?**

   It is very relevant because is establishes one endpoint in assessing the probability that wild populations could be infected.

5. **How likely is it that exposure of wild shrimp populations to viral diseases could lead to the development of immunity and reduced effects on population survival over time?**

   I cannot answer that, I lack the expertise. At least one of the studies presented as testimony asserts such an effect.

6. **How can the strong influence of both natural and non-viral anthropogenic factors on shrimp populations be separated from risks associated with viral stressors?**

   Unless the effect of the viral stressor is significant (overwhelming), I am not sure that we have adequate data to separate out natural and non-viral anthropogenic factors.

7. **Can human health effects from shrimp viruses be ruled out as a concern? Why or why not?**

   I cannot answer that, I lack the expertise.

8. **Are the available identification techniques for shrimp viruses reliable enough to allow definitive conclusions to be drawn about the occurrence of viruses in shrimp and environmental media?**

While I lack the direct expertise, my review available techniques indicates that they are inadequate.

VIRAL PATHWAYS AND SOURCES

The shrimp virus work group considered aquaculture and shrimp processing to be the primary pathways of concern leading to exposure to pathogenic shrimp viruses, but is also identified a number of other potential pathways. Some related questions are listed below.

AQUACULTURE

9. U.S. aquaculture operations have had problems with viral diseases for several years. How does information from local wild shrimp populations support or refute the importance of aquaculture operation as a source for the virus?

Data is inadequate to reach a conclusion

10. It has been widely held that it is highly unusual for domesticated animals to infect wild animal populations; usually it is the other way around. How well does this observation apply to the relationship between shrimp in aquaculture and wild shrimp populations, with regard to shrimp viruses?

I think that it is unsound to use such an analogy in regards to aquaculture. The experiences upon which that conclusion is based comes from land based agriculture. Water, the universal solvent, provides a significantly enhanced transmittal medium and very different circumstances.

SHRIMP PROCESSING

11. Some believe it likely that shrimp processing operations have processed virus-infected shrimp from foreign sources for several years. How does information from local wild shrimp populations support or refute the importance of shrimp processing as a potential source for the virus?

Data is inadequate to reach a conclusion.

12. Should the retailers who distribute (rather than process) shrimp products receive additional evaluation as potential sources of exposure?

Yes

<u>OTHER POTENTIAL SOURCES AND PATHWAYS</u>

13. **After considering the sources addressed in the shrimp virus report, what sources other than aquaculture and shrimp processing are most critical for evaluation in a risk assessment of shrimp viruses? Given time constraints, which of these should be the focus of discussion at the workshop?**
    Bait shrimp and Non-Shrimp Translocated Animals (example: the growing culture of Australian red claw crayfish).

14. **Is manufactured shrimp feed a potential virus source, or is the processing temperature sufficient to rule this source out?**
    The testimony provided at the hearings appears conflicting on this issue. Until that can be resolved shrimp feed cannot be ruled out as a source.

<u>STRESSOR EFFECTS</u>

These next questions concern the possible consequences to wild shrimp populations and marine communities from exposure to pathogenic shrimp viruses.

15. **How should the available evidence concerning the effects of introduced viruses on wild shrimp populations be interpreted? (For example, what was the role of IHHNV in the decline of shrimp populations in the 1980's in the Gulf of California? What about TSV release from aquaculture into the wild in South America?)**
    There is no substantive evidence (which I have reviewed) that introduced viruses have had an effect on wild shrimp populations. Available information does provide evidence of transmittal of viral disease between wild populations and cultured shrimp. The evidence establishes a pathway, but does not contribute greatly to the assessment of risk.
16. **There is presently a lack of basic data on background levels of pathogenic shrimp viruses in wild shrimp populations in U.S. waters. How should this data gap be evaluated in a risk assessment?**
    As a significant data gap that must be addressed.

17. **How can changes in wild shrimp populations be used to interpret the effect**

(or lack of effect) of introduced shrimp viruses? How could shrimp population models be used in the future?

Clearly, any population change (decline) outside norms would indicate an effect, although not necessarily from disease (hypoxia, el Niño effects, etc) would have to be accounted for and some empirical evidence would need exist for linking a decline to disease. Shrimp population models that adequately explain observed variability do not currently exist and until they do (even if possible) they will not be useful in this context.

18. How important are potential viral effects on non-shrimp species?

They can be very important, especially on susceptible species with low populations (ie listed endangered/threatened species) or with restricted distributions

COMPREHENSIVE RISK ASSESSMENT AND RESEARCH NEEDS

19. How will a comprehensive risk assessment contribute to management of the shrimp virus problem, i.e., will it add significantly to the information presently available?

I am sorry, but "comprehensive" risk assessment is not defined in any of the supplied documents so I cannot ascertain what is contemplated. If you mean by comprehensive - taking a tiered approach and extending it beyond the qualitative levels into quantitative levels as new information is developed according to identified needs, then yes, that approach will make a positive contribution.

20. What type of assessment should be conducted next (e.g., quantitative risk estimates using shrimp populations models), and what would be the likely time frame and cost?

A quantitative assessment using shrimp population models would be useful if it were sensitive enough, but likely will not be timely or inexpensive. The taskforce report (page 53) estimates one year and $200-300K. That is optimistic at best and a case can be made that such a model would lack the sensitivity to meet the need. I lack the expertise to make such a judgement, but have some concern about it.

21. Should a future risk assessment consider the risk reduction potential of a range of treatment options associated with specific exposure scenarios?

Yes, if I understand the question correctly this approach would likely give risk managers some better options to work with that they now have.

22. Summarize the critical research needs for completing such a risk assessment.

Three important research needs are: 1) *Assessing the presence and distribution of pathogenic viruses in wild stocks* - One insufficiency in assessing the efficacy of disease management strategies is a lack of baseline information on the presence and distribution of pathogenic viruses in our native stocks. The recent occurrence of a "whitespot" type virus in native species held in the Texas Agriculture Research Center in Corpus Christi illustrates that need; 2) *better information on infectivity, transmissibility and virulence of viruses* - one of the most immediate risk management needs is how can we minimize risk until some of the critical research needs are met. A more clear understanding of what is known about this topic and how that knowledge can be used to isolate cultured from wild shrimp is a critical management need; 3) *Assessing the relationship between stress and disease susceptibility in shrimp and evaluating the interaction among multiple stressors* - aquaculture conditions typically initiate stress sufficient to increase disease susceptibility and this is primarily due to over crowded conditions. If such conditions are not likely in wild populations can other stressors have a similar effect? 4) *Assessing the potential of shrimp processing activities in disease transmittal* - the risk we know the least about is that associated with the processing of imported shrimp. Based on sheer volume, it could overwhelms all others. Adequately assessing that risk will likely form the basis of future management strategies.

Wayne Munns

Shrimp Virus Workshop
Pre-Workshop Response to Questions
Wayne R. Munns, Jr.

Management goals, assessment endpoints, and conceptual models

1. The draft management goal (p. 14 of the JSA Shrimp Virus Report) adequately captures two primary management concerns: 1) prevention of establishment of a potentially disruptive suite of viral agents in wild shrimp populations, and 2) minimization of the potential negative impacts on the sector of commerce involved with distribution of shrimp products to the North American market. A third management concern not addressed by the draft management goal might be stated as "minimization of potential negative impacts on resource populations and ecological systems other than wild shrimp". The focus of the draft management goal currently is limited to shrimp and the shrimp industry. Because the degree to which the viral agents can affect other species is not know with high certainty, some reflection of this concern may be warranted.

2. The first assessment endpoint (p. 18) clearly reflects the first aspect of the draft management goal, and summarizes nicely the environmental value (and its attributes) of primary interest. A minor word smithing change may be warranted, however. Strictly speaking, "populations" do not "survive, grow, and reproduce"; rather, these are attributes associated with individuals. Replacing the first occurrence of "of" with "in" would correct this.

   The second assessment endpoint, however, is less well crafted. It again focuses primarily upon shrimp populations, reflecting a focus on other ecology components only as support systems for the shrimp populations themselves. Effects on these support systems should be adequately reflected in the shrimp "survival, growth, and reproduction" attributes expressed in the first assessment endpoint. As a corollary, it does not address potential effects on components of ecological systems which are more-or-less independent of shrimp populations, but which might represent high risk to these components. Inclusion of a third assessment endpoint addressing risks to non-shrimp components of ecological systems would be warranted given sufficient management concern (see Response 1 above).

3. My belief is that, with the possible exception of the inclusion of a third assessment endpoint (see Response 2), the assessment should not be broadened to include stressors other than shrimp viruses, unless these other stressors interact with virus establishment, transport, and consequence pathways and processes. As communicated in the conceptual models described in the JSA report, pathways that to some degree reflect land use and production methods are considered, but only within the context of shrimp viruses. To broaden the scope to include other aspects of the shrimp industry would risk diffusion of the assessment effort.

Viral stressors and factors regulating shrimp populations

4. This question is difficult to answer. We know from other situations that predictions based upon exposure to stressors of naive laboratory test subjects often fail in validations against actual field situations. Pre-exposure to the stress can lead to compensatory responses (immunologic, homeostatic, and evolutionary responses) which reduce susceptibility to subsequent exposure. Recognition of this phenomenon (as well as the opposing situation of pre-exposure leading to enhanced susceptibility) will be important when identifying assessment uncertainties.

5. This is an area of obvious great uncertainty, and the answer to this question is critical to understanding the potential long-term consequences of virus establishment. That wild

shrimp populations occur in areas of the world in which shrimp viruses are indigenous suggests that some degree of immunity can be developed. The characteristics of these "compensated" populations, with respect to attributes such as productivity, stability, resilience, and susceptibility to other stressors, also is unknown. Also cogent is the time course of development of immunity. Although the potential development of immunity may minimize the long-term consequences of virus establishment in North America, the severity and extent of short-term ecological effects on shrimp populations may be unacceptable from a risk management standpoint.

6. This will be difficult within the context of the risk assessment itself. As a data need, however, it is important to be able to separate the influences and risks associated with viral infection from other potential causes and stressors. Information regarding natural variability in the dynamics of wild shrimp populations, and the responses of those populations to anthropogenic stress, should be evaluated to provide expectations against which to overlay the effects predicted to result from viral infection. Further, the potential synergistic or antagonistic interactions between viral infections and other stressors represent a significant uncertainty for the assessment.

7. Statements to this effect are made in the JSA Report, but the data (as communicated) appear circumstantial at best, and precedents of "trans-species jumping" by viral agents exist (ebola comes to mind). Although this likely is of minor management concern at the moment, further investigation of shrimp virus epidemiology as it affects humans may be warranted.

8. No.

Viral pathways and sources

9. As with potential risks to humans, little information exists regarding the epidemiology of shrimp virus transmission to wild shrimp populations. Although the lack of confirmed infection of wild U.S. populations would suggest a low probability of establishment from aquaculture operations, the data are too scant to evaluate aquaculture operations as a source of viral release. This represents a critical data gap in the aquaculture exposure pathway.

10. The potential transmission of viruses from domesticated animals to wild population likely is controlled in large part by three factors: 1) exposure of wild animals to domesticated animals and their by-products; 2) differences in the immunities of the two groups to pathogens; and 3) the frequency of infection in domesticated animals. The first factor is an explicit component of the conceptual model, and therefore will be evaluated as part of the risk assessment; the second represents an important data gap; and we have data addressing the third. These factors will be explored as part of a risk assessment.

11. Little information exists regarding the epidemiology of shrimp virus transmission to wild shrimp populations. Although the lack of confirmed infection of wild U.S. populations would suggest a low probability of establishment from shrimp processing operations, the data are too scant to evaluate shrimp processing operations as a source of viral release. This represents a critical data gap in the shrimp processing exposure pathway.

12. The probability of release of viral agents as part of the distribution process likely is lower than that of the other pathways to be evaluated, but retail distribution as a potential source should be evaluated in the qualitative risk assessment.

13. An evaluation of existing data with respect to probabilities of transmission and establishment should be evaluated for all other sources (at least as identified in the JSA Report). Insufficient information is available to prioritize among these other sources.

14. Information provided in the JSA Report suggests that processing temperatures often are insufficient to kill viruses. Manufacture of shrimp feed should therefore be included along the pathways of shrimp processing and aquaculture.

Stressor effects

15. Such evidence provides direct information concerning the potential consequences of virus release and establishment in U.S. waters. Examination of shrimp populations in South America and Asia should provide useful data with which to bound the potential long-term consequences of viral infection. Cursory examination of that information suggests that because wild populations continue to exist, compensatory responses may occur that mitigate total devastation of those populations. Given the data at hand, however, it is impossible to determine the time course of such responses, and further to determine whether those populations are "impacted" relative to an uninfected condition.

16. This data gap is directly relevant to the issue of immunity and susceptibility of wild shrimp populations. As referenced in Responses 5 and 22, it is critical to understand whether immunity is a viable compensatory mechanism to mitigate the negative impacts of infection. As such, this will be an important source of uncertainty in the risk assessment.

17. Assuming that pathways can be established that link the release of viral agents with subsequent exposure to wild shrimp populations, and that infection of those shrimp can be documented, the responses of such populations can be used to predict (at least empirically) the responses of naive populations which might be exposed in the future. The time course of population change would provide information regarding the potential short-term consequences of infection, as well as provide indication of potential compensatory responses (e.g., development of immunity). Population modeling could assist in this evaluation in a number of ways, including: 1) supporting development of expectations of population dynamics (incorporating natural temporal and spatial variability) against which to evaluate short-term responses; and 2) providing predictive tools relating the biological effects of infection to ultimate population response. The former application might require empirical evaluation of long-term data sets, whereas the latter would require mechanistic understanding of both direct viral influences on shrimp demographic characteristics (survival, growth, and reproduction) and potential compensatory mechanisms (e.g., immunity).

18. Unknown. This represents a critical data gap, particularly with respect to the third assessment endpoint suggested in Response 2.

Comprehensive risk assessment and research needs

19. The answer to this question will be determined in large part by the uncertainties recognized in the qualitative assessment we are about to conduct.

20. The answer to this question will be determined in large part by the uncertainties recognized in the qualitative assessment we are about to conduct. A more comprehensive risk assessment could incorporate quantitative estimates of the probability of virus transmission, as well as quantitative models of both viral and shrimp population dynamics.

21. Should the initial qualitative, or subsequent more quantitative assessments suggest that the risks of establishment and the consequences of establishment be unacceptably high, then an assessment comparing various mitigation options (including treatment options) may be warranted.

22. Assuming the question to refer to a comprehensive risk assessment, the critical research needs from my perspective include concrete information concerning:

    1. potential compensatory responses (e.g., development of immunity) of wild shrimp populations exposed to the viral agents, including insight into the time course(s) of such responses

    2. susceptibility of non-shrimp native species to viral infection and the consequences of such infection

    3. the basic epidemiology of shrimp virus disease transmission, including identification of potential intermediate vectors, natural attenuation rates, etc.

    Additionally, diagnostic methods for surveillance of shrimp viruses in wild populations are needed to establish current and future levels of infection. Such data would help to address the three research needs identified above.

Gary Pruder

Dr. Gary D. Pruder, VP
The Oceanic Institute
U.S. Shrimp Farming Program

Premeeting Comments
Shrimp Virus Peer Review

1. Management Goal: *Prevent the establishment of new disease-causing viruses in wild populations of shrimp in the Gulf of Mexico and southeastern U.S. Atlantic coastal waters, while minimizing possible impacts on shrimp importation, processing and aquaculture operations.*

The introduction of disease causing viruses to shrimp farming operations has been shown to have immediate and drastic impact. Suggest that the management goal be expanded to exclude the introduction of disease causing viruses to shrimp farms..

2a. Assessment Endpoint (1): *Survival, growth and reproduction of wild shrimp populations in the Gulf of Mexico and southeastern U.S. Atlantic coastal waters.*

In keeping with #1 above, suggest that survival and growth of shrimp in farms be added as assessment endpoints.

2b. Assessment Endpoint (2): *Ecological structure and function of near shore marine communities as they effect wild shrimp populations.*

Perhaps such an effort is out of reach.

3. *It has been suggested that the scope of the proposed assessment be broadened to consider the impacts of alternative land use and seafood production methods in coastal areas.*

Seafood production methods will likely be included in preventing the introduction of viruses. Recommend against expanding the scope to include other environmental impacts at this time.

4. *How relevant to virus effects on wild populations is information on infectivity and effects that are derived from laboratory or intensive aquaculture operations?*

Likely that information from laboratory and shrimp farming operations will represent worst case scenarios in individual mortality and survival percentages.

5. *How likely is it that exposure of wild shrimp populations to viral diseases could lead to development of immunity and reduced effects on population survival over time?*

It appears to be a reasonable course of events. It would be valuable to know what genetic changes if any, accompany increased resistance, if any, to the disease agents.

6. *How can strong influence on both natural and non-viral anthropogenic factors on shrimp populations be separated from risks associated with viral stressors?*

Sometimes but not often. The systems and the interactions are complex and do not lend themselves to controlled experiments.

7. *Can human health effects from shrimp viruses be ruled out as a concern?*

Not sure.

8. *Are the available identification techniques for shrimp viruses reliable enough to allow definitive conclusions to be drawn about the occurrence of viruses in shrimp and environmental media?*

Probably yes for some viruses and unsure for others.

9. *U.S. aquaculture operations have had problems with viral diseases for several years. How does information from local wild shrimp populations support of refute the importance of aquaculture operations as a source for the virus?*

Aquaculture operations do not create viruses. However, if a farm become infected it is likely that the virus will be multiplied and subsequently be transferred with shrimp product, shrimp waste and/or discharge waters. Presently, high health shrimp farms are subject to infection transfer from wild animals. It is critical that steps are taken to exclude viral diseases from shrimp farms.

10. *It has been widely held that it is highly unusual for domesticated animals to infect wild animals; usually it is the other way around. How well does this observation apply to the relationship between shrimp in aquaculture and wild shrimp populations, with regard to shrimp viruses?*

Perhaps not too well. The differences between domesticated shrimp and wild shrimp and not yet substantial. Our experience to date in breeding shrimp, has indicated that wild shrimp are more resistant to many stresses including disease.

11. *Some believe it likely that shrimp processing operations have processed virus-infected shrimp from foreign countries for several years. How does information from local wild shrimp populations support or refute the importance of shrimp processing as a potential source for the virus?*

It is only recently that virus related problems were recognized as serious problems by foreign shrimp producers. It is unlikely that shrimp processed over the past twenty years carried significant viral infections. However, those processed over the last three or four years are known to carry high levels of virus

12. *Should retailers who distribute (rather than process) shrimp products receive additional evaluation as potential sources of exposure?*

The practice of selling older shrimp products as bait should be discouraged.

13. *After considering the sources addressed in the shrimp report, what sources other than aquaculture and shrimp processing are most critical for evaluation in risk assessment of shrimp viruses? Given time constraints, which of these should be the focus of discussion at the workshop?*

Live shrimp and bait shrimp are likely carries of shrimp viruses and potential transfer products.

14. *Is manufactured feed a potential virus source, or is the processing temperature sufficient to rule this source out?*

It is unlikely that feeds are involved in the current problem. I do not know about temperature.

15. *How should the available evidence concerning the effects of introduced viruses on wild shrimp populations be interpreted.*

No comment

16. *There is presently a lack of basic data on background levels of pathogenic viruses on wild shrimp populations in U.S. waters. How should this data gap be evaluated in risk assessment?*

Recent findings confirm the presence of exotic viruses in wild populations. The real issue goes back to #15.

17. *How can changes in wild shrimp populations be used to interpret the effect (or lack of effect) of introduced shrimp viruses? How could shrimp models be used in the future?*

No comment

18. *How important are potential viral effects on non-shrimp species?*

Direct economic impact would be much less. Do not know about long term indirect impacts.

19. *How will a comprehensive risk assessment contribute to management of shrimp virus problems?*

The assessment will organize existing information. Hopefully it will also support the need for research to fill information gaps.

20. *What type of assessment should be conducted next and what would be the likely time frame and cost?*

Suggest a combined modeling and multiple case study be undertaken to set some reference points. I suggest $15 MILLION over the next three years. In the meantime, aquaculture and processing operations should be assisted in developing economic methods to disinfect both incoming and effluents.

21. *Should a future risk assessment consider the risk reduction potential of a range of treatment options associated with specific exposure scenarios?*

Development of treatment options should be undertaken immediately.

22. *Summarize the critical research needs for completing such a risk assessment?*

Suggest we follow the data gap and research need recommendations page 49-51 of the Evaluation Report by the JSA shrimp Virus Work Group

Paul Sandifer

Responses from Paul A. Sandifer, SC Department of Natural Resources

Management goals, assessment endpoints, and the conceptual model

1. How well does the management goal reflect the dimensions of the shrimp virus problem?

The goal is very clear and does a good job of incorporating most of the elements of the problem. However, I recommend the following minor modification suggested changes noted in bold):

> "Prevent the establishment of new disease-causing viruses in wild populations of **penaeid** shrimp in the Gulf of Mexico and southeastern U.S. Atlantic coastal waters, while minimizing possible impacts on shrimp importation, processing, aquaculture operations **and the ecosystems upon which wild penaeid shrimp stocks depend**."

2. Some have suggested modifying the assessment endpoints to emphasize potential risks of shrimp viruses to non-shrimp organisms and the larger ecological system or, alternatively, to the aquaculture industry. Please comment on the assessment endpoints as the focal point for the ecological risk assessment.

I think that the emphasis of the risk assessment should remain on penaeid shrimp, but other information should be included where it is available and pertinent. However, the available information on the occurrence and impacts of various viruses in penaeid shrimp populations is very sketchy at best, and that for other organisms appears to be extremely limited. Nevertheless, a minor modification of the second assessment endpoint as noted below (suggested change in bold) might be helpful, since it would not limit the assessment of ecological effects to just those dealing with marine shrimp populations:

> "Ecological structure and function of coastal and near-shore marine communities, **especially** as they affect wild penaeid shrimp populations."

3. It has been suggested that the scope of the proposed risk assessment is too narrow and that it should be broadened to consider the impacts of such stressors as alternative land uses and seafood production methods in coastal areas. Please comment on this suggestion.

I am adamantly opposed to much broadening of the risk assessment, because I believe such would result in the EPA's inability to draw any useful conclusions within a reasonable time frame. Broadening the scope of the assessment to include other areas with very limited data pertinent to the occurrence and impacts of shrimp viruses would needlessly complicate the process and, in my view, likely ensure its failure.

Viral stressors and factors regulating shrimp populations

4. How relevant to virus effects on wild populations is information on infectivity and effects that is derived form laboratory or intensive aquaculture operations?

Very relevant, since in most cases this is the primary information we have about potential pathological effects. However, this question could probably be better addressed by epidemiologists with experience with viral diseases of arthropods (e.g., insects). Information from other better known situations, such as some virus diseases of insects or domesticated animals or plants might prove very enlightening.

5. How likely is it that exposure of wild shrimp populations to viral diseases could lead to the development of immunity and reduces effects on population survival over time?

It is quite possible that effects in wild populations (and probably cultured populations as well) might diminish over time with repeated exposures. Whether or not such diminution would be the result of an acquired "immunity" or some sort of accommodation (see Flegel and Pasharawipas, viracom 23 June 97) is unknown. Also unknown is how long it might take for wild populations to develop such protection, if at all, and the possible effects on survival of the wild stocks until such accommodation occurred.

6. How can the strong influence of both natural and non-viral anthropogenic factors on shrimp populations be separated from risks associated with viral stressors?

One would have to look very carefully at long-term data series on shrimp populations and then attempt to correlate population level effects (if any) that were greater than those associated with "normal" environmental variation and persistent.

7. Can human health effects from shrimp viruses be ruled out as a concern? Why or why not?

I would leave this to those with expertise in human health in relation to virus diseases.

8. Are the available identification techniques for shrimp viruses reliable enough to allow definitive conclusions to be drawn about the occurrence of viruses in shrimp and environmental media?

NO.

Viral pathways and sources

9. U.S. aquaculture operations have had problems with viral diseases for several years. How does information from local wild shrimp populations support or refute the importance of aquaculture operations as a source for the virus?

In most situations it does neither, since there are few if any baseline (before aquaculture) data on the incidence (if any) of viral infections in wild shrimp populations for comparison, and little if any work has been done to determine if archived samples such as in museum collections could be analyzed in any way to provide such "before" data.

10. It has been widely held that it is highly unusual for domesticated animals to infect wild populations; usually it is the other way around. How well does this observation apply to the relationship between shrimp in aquaculture and wild shrimp populations, with regard to shrimp viruses?

I am not sure. It is clear that aquaculture operations have spread viral diseases from one facility to another, and they may well have spread viruses to wild shrimp populations, but documentation of this latter appears to be lacking. Again, the lack of baseline data on the occurrence of viruses in wild shrimp populations, and indeed the distribution of viruses in wild crustaceans worldwide, makes it difficult to draw many conclusion. Further, at least in the US, it is my impression that relatively little sampling has been done of wild shrimp populations, even around aquaculture operations, for viral analysis, and what analyses have been done have generally followed disease outbreaks in the aquaculture operations. Thus, it is difficult to determine in many situations whether the disease came to the aquaculture operation from the wild or whether the aquaculture operation introduced the disease to the wild.

11. Some believe it likely that shrimp processing operations have processed virus-infected shrimp from foreign sources for several years. How does information from local wild shrimp populations support or refute the importance of shrimp processing as a potential source for the virus?

I have not seen enough data from analyses of virus incidence in local wild shrimp populations to draw any conclusions in this matter.

12. Should the retailers who distribute (rather than process) shrimp products receive additional evaluation as potential sources of exposure?

Yes. It is my understanding that some shrimp are harvested from apparently diseased ponds in South America at very small size and then packaged whole in bags for direct sale in the US for fish bait. The only processing these shrimp undergo is external washing, packaging in small plastic bags, and freezing. Many other shrimp products come into the US with the potential to be carrying viral diseases and go directly into wholesale and retail distribution networks, with little or no additional processing and certainly none that would affect the viability of any viruses they may carry.

13. After considering the sources addressed in the shrimp virus report, what sources other than aquaculture and shrimp processing are most critical for evaluation in a risk assessment of shrimp viruses? Given time constraints, which of these should be the focus of discussion at the workshop?

The most important other potential source of some virus infections that deserves considerable discussion at the workshop, in addition to aquaculture, shrimp processing, shrimp importation and retail sales, is the local wild stocks themselves. Evidence is mounting that there is widespread occurrence of a "white spot complex virus" in crustaceans, including penaeid shrimp, in US South Atlantic waters, and that this virus has moved from the wild into culture facilities. Whether the virus has been in the wild for a long period of time or was introduced only relatively recently needs much further study. It may well be that there are a number of viruses naturally occurring in native wild shrimp populations, and that these could affect aquaculture operations and/or wild populations.

14. Is manufactured shrimp feed a potential virus source, or is the processing temperature sufficient to rule this out?

I believe that feed should be considered a potential virus source until ruled out by testing for viable virus particles. Not all feeds provided to aquaculture operations in this hemisphere are likely to be processed at high temperatures, and it is quite possible that some lots fail to get cooked as much as they should. Experimentation should be undertaken to resolve this question. For example, one might incorporate some shrimp tissue known to be infected with virus into the shrimp feed preparation and then process it as normal. The final product tested would then be tested for the presence of viable virions.

Stressor effects

15. How should the available evidence concerning the effects of introduced viruses on wild shrimp populations be interpreted? (For example, what was the role of IHHNV in the decline of shrimp populations in the 1980's in the Gulf of California? What about TSV release from aquaculture into the wild in South America?)

I have seen no evidence that conclusively links an outbreak of virus disease in aquaculture operations with failures of a local wild stock, although the potential for such effects certainly appears to be present. The problem with the correlation of IHHNV with the decline of the *Penaeus stylirostris* fishery in the upper Gulf of California is that it was a single factor correlation, and other potential contributing factors apparently were not taken into consideration. At this time, it seems impossible to determine just how much, if any, of the problem in that fishery was the result of IHHNV. The situation with regard to TSV in wild stocks in South America is even more confusing. It appears likely that the virus was spread by shrimp farms, but it originated from the wild somewhere, perhaps in South America, perhaps elsewhere. Clearly the virus is widespread now in wild stocks in much of the region, but I do not know if there is sufficient evidence to determine whether it existed in these same stocks prior to being observed on shrimp farms or not. Also, I am not aware of whether there are data on the wild stocks, either from the fisheries themselves or from fishery-independent surveys, that suggest any collapses of local populations in association with observations of the virus in the wild.

16. There is presently a lack of basic data on background levels of pathogenic shrimp viruses in wild shrimp populations in U.S. waters. How should this data gap be evaluated in a risk assessment?

I believe that an immediate effort must be made to at least partially fill this data gap before any realistic assessment of risk can be completed. This is probably the most pressing need.

17. How can changes in wild shrimp populations be used to interpret the effect (or lack of effect) of introduced shrimp viruses? How could shrimp population models be used in the future?

This will be very difficult over the short term. Wild shrimp populations are notoriously variable, primarily in response to environmental factors. Unless one sees something like a catastrophic decline in population abundance at the same time that environmental factors are considered "good" for shrimp — and one has reliable data on incidence of one or more viruses in the wild population, with associated and evident pathology — it will be very difficult to draw firm cause-and-effect conclusions. It may be possible to use one or more of the existing empirical shrimp population models to estimate an effect of a virus outbreak in a wild population, if the model has a good track record of predicting effects of environmental factors and then something occurs in the population that makes the predicted value considerably different from the observed. At best, however, this would be an indicator, not a clear signal of cause.

18. How important are potential viral effects on non-shrimp species?

Very, but they may be difficult to evaluate in the short term.

Comprehensive risk assessment and research needs

19. How will a comprehensive risk assessment contribute to management of the shrimp virus problem, i.e., will it add significantly to the information presently available?

I do not know if it will add to the information available, but it will certainly result in a synthesis and assessment of the currently available information that will be of great use to many involved with the shrimp virus problem. Agencies such as the one I work for (the SC Department of Natural Resources) will undoubtedly use the risk assessment in formulating regulatory policy and setting priorities for research, development and management activities.

20. What type of assessment should be conducted next (e.g., quantitative risk estimates using shrimp population models), and what would be the likely time frame and cost?

Quantitative risk assessment is clearly needed, but much more data than is currently available will be needed before beginning such. A badly flawed quantitative assessment based on poor data would likely do more harm than good. I have little experience in this area, but would guess that a minimum time frame would be 5 years, with a cost on the order of $5-10 million over that period.

21. Should a future risk assessment consider the risk reduction potential of a range of treatment options associated with specific exposure scenarios?

Yes. As much as possible, note of such treatment options should be included in the present qualitative risk assessment.

22. Summarize the critical research needs for completing such a risk assessment?
A comprehensive evaluation would take much more time than I have at present, but the following are some of the most pressing needs.

a) Further refinement, testing and validation of diagnostic techniques for the viruses in question, coupled with development of more user-friendly techniques that could be used on a broad range of kinds and numbers of samples.

b) Development of a reliable and detailed data base on the incidence and effects of viruses in wild shrimp populations and populations of other near-shore and coastal crustaceans. This should include identification and examination of archived samples from as many years ago as practical.

c) Development, testing and demonstration of reliable and cost-effective methods for treating infected aquaculture facilities, including large outdoor ponds, to eradicate shrimp viruses and prevent escape to the environment.

d) Based on studies from other fields (e.g., insect population studies), as well as direct observation and carefully crafted experiments, determine the likely effects of shrimp viruses in wild populations.

e) Experimentally evaluate the potential for acquired "immunity" or accommodation to the viruses in question by captive shrimp.

f) While not a research issue per se, one of the most pressing needs is for a standardized process and bureaucratic mechanism for inspection and certification of brood and seed stock shrimp for distribution around the country to aquaculture facilities.

Max Summers

Premeeting comments are not available at this time.

Max D. Summers
December 18, 1997

Preliminary response to "Charge to Panel Members"

A. *Diagnostic techniques for shrimp pathogens*

1). How good are the diagnostic techniques relative to specificity and sensitivity?
2) Can these detection and identification tests be equally applied for all pathogens of concern?
3) With the diagnostic tests available, what is the level of detection and identification suitable for reliable risk assessment (endpoint) analyses?

B. *With highly sensitive, specific and reproducible diagnostic techniques, one can more quantitatively and qualitatively develop feasible risk assessment data for:*

A. *Management goals, conceptual risk models:*

2. Potential to spread to U.S. shrimp populations.
3. Potential to spread to "non-host" populations.

B. *Viral stressors and factors regulating shrimp populations:*

4. Correlation of empirical laboratory data with virus infection and spread in wild populations.
5. The development of disease resistance.
6. The effects of natural and non-viral anthropogenic influences for virus introduction and spread.
7. Potential effects on human health.
8. The credibility and reliability of shrimp virus diagnostics - this area needs a constructively critical and comprehensive assessment by epidemiologists/epizoologists who are expert in these applications for monitoring experimental and natural populations of animals and man. I would suggest a team of individuals working with shrimp pathogens and those who are expert and knowledgeable of predicting potential for pathogen introduction and spread in human populations.

C. *Viral pathways and sources:*

10. Diagnostic tools are key to evaluating the potential for virus spread in exposed shrimp populations.

D. *Comprehensive risk assessment and research needs:*

22. A critical assessment of diagnostic techniques; and the program of how such is to be used and implemented to detect/identify the target pathogen in any potential source of introduction and spread within populations.

Suzanne Thiem

Comments on the Shrimp Virus Report

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Management goals, assessment endpoints, and the conceptual model

1. The stated management goal is "Prevent the establishment of new disease-causing viruses in wild populations of shrimp in the Gulf of Mexico and southeastern U.S. Atlantic coastal waters, while minimizing the possible impacts on shrimp importation, processing and aquaculture operations." From the material presented in the report, this goal seems to be too narrow. It appears that the presence of diseased shrimp in aquaculture ponds and importation and processing of diseased shrimp, in particular, could negatively impact native shrimp populations in many ways. A broader statement, such as "Maintain the health and ecology of wild penaeid shrimp populations in the Gulf of Mexico and southeastern U.S. coastal waters, ..." would encompass non-viral shrimp diseases as well as other stressors.

2. The assessment endpoints established for this report are:
Primary: "Survival growth and reproduction of wild penaeid shrimp populations in the Gulf of Mexico and southeastern U.S. coastal waters."
Secondary: Maintain? Preserve?."Ecological structure and function of coastal and near-shore marine communities as they affect wild penaeid shrimp populations." (this is not a sentence)
The primary assessment endpoints seems appropriate at this time since it should be a reasonable indicator of the impact of viruses as well as other stressors on natural shrimp populations and provide at least minimal feedback on the health of the ecosystem. However if shrimp population declines are observed, this endpoint can not distinguish if virus infection is the reason for the decline. As for the second endpoint, I'm not sure how it could be measured.

3. I would agree that the scope is probably too narrow, even if the primary concern is the health of the native shrimp populations and/or other fauna. In addition to the issues of other shrimp diseases and exotic shrimp species- other factors impacting coastal waters such as development and seafood production certainly should be considered since they can effect nutrient and oxygen levels in the water, temperature, etc. If the shrimp or other organisms

are stressed they also may become more susceptible to diseases including introduced or naturally occurring pathogens.

Viral stressors and factors regulating shrimp populations

4. Studies were cited about the transmission of several of these viruses to different shrimp species and to other crustaceans as well as other arthropods, yet without further knowledge of how these transmissions were evaluated, it is impossible to judge the value of these results for risk assessment. Specifically, it is often possible to transmit a disease in laboratory situations but not in a natural situation. Thus, in the natural environment, it is not clear how susceptible native shrimp species are to the viruses infecting non-native shrimp species. Also with a few exceptions, viruses tend to be specialized, generally having relatively narrow host ranges. However, since these shrimp species are related they may well be susceptible and possibly even more sensitive to viruses from other locales. Without evaluating the methods used to obtain the data, in particularly how the virus input and virus from the resulting infections were validated, I am suspect of reports of transmission to other organisms such as crabs. Laboratory results can certainly give baseline data and in particular demonstrate if transmission is possible- but can not accurately predict outcomes in natural situations. Likewise an intensive aquaculture operation is quite different from a natural situation. For example, to become infected a shrimp would have to encounter the virus, yet we don't know the distribution of viruses in the natural habitat or how likely it would be for the host to come in contact. In an intensive aquaculture system, the spread of viral disease is greatly enhanced.

5. I don't know if shrimp can or will develop "immunity" to virus diseases- little is known about immune responses of invertebrates to viruses and they lack the immunological memory of vertebrates. However it is possible that resistant populations will develop.

6. Other stressors surely have an impact on shrimp populations and I believe it will be difficult to separate the impact of these factors from the risks of viral stressors.

7. It is highly unlikely that these viruses can effect human health. Viruses co-evolve with their hosts and become highly adapted to particular hosts. Given the tremendous evolutionary distance between vertebrates and invertebrates (approx. 540 million years) it is improbable that these viruses could infect humans or other vertebrates even by mutating.

8. I am not familiar enough with the identification techniques used for identifying these viruses to make a judgment on their reliability.

Additional comments on viral stressors: Are these viruses really new? These viruses are described as new or exotic throughout the report. However, from the material presented I'm not convinced that similar viruses are not already present in native shrimp populations, but data to support or refute this idea are lacking. If some of these viral diseases are detected in native populations how will we know if we are detected a domestic cousin- or an exotic variety? Viral disease outbreaks can be expected to occur when populations are crowded since virus levels can be amplified and spread, as they have in aquaculture operations in Asia and South America. If native species were grown in high density aquaculture, disease outbreaks from native pathogens would be expected, particularly if appropriate sanitary/hygienic procedures were not routinely applied.

Viral pathways and sources
Aquaculture

9. There is not sufficient information on virus infections in wild shrimp populations to support or refute the importance of the aquaculture operations as source of virus infection in wild populations. However, aquaculture is one of the most likely potential source for virus inoculum because large amounts of virus can be produced during disease outbreaks. In addition, other diseases such as bacterial, fungal, or rickettsial diseases, have the potential for adversely affecting native shrimp populations as much as viral diseases. Again high density aquaculture could provide a means of amplifying these diseases as well and increasing the risk of their spread to native populations.

10. There is not enough information to determine if shrimp in aquaculture can infect wild populations. The two most important factors for the infection of wild animals by diseases of domestic animals are the probability of exposure and susceptibility to the disease agent. In the case of shrimp neither of these parameters are well characterized.

Shrimp processing

11. There is insufficient information to support or refute the claim that processing virus-infected shrimp is a source for viruses infecting native populations. However the practice

of some shrimp producers to harvest and ship diseased shrimp makes this one of the more likely sources for virus contamination of native shrimp populations.

12. It seems less likely that shrimp in the retail distribution system would be a substantial source for virus exposure of native species than aquaculture or processing since it would be less likely that viruses from this source would enter the coastal waters.

Other potential sources and pathways

13. Of the other sources mentioned in the report, bait shrimp and ballast water are the most likely virus sources that could impact native populations. However unlike aquaculture and shrimp processing operations that process imported shrimp that may be diseased, virus levels from these sources are unlikely to be as high.

14. Not enough information on the manufacturing of shrimp feed was given to evaluate its potential as a virus source. The report stated that shrimp meal was not heated enough to kill viruses and it was added to feed. But it is not clear how extensive the use of this shrimp meal is for feed stock for shrimp aquaculture. In any case, it would impact aquaculture primarily. Thus, its impact would be secondary- increasing infection rates in aquaculture leading to greater risk of exposure of native species from this source (see #9).

Stressor effects

15. What is the impact of shrimp viruses amplified in aquaculture on natural populations in Asia and South America? Since these viruses are pathogens of the native species, I would expect that if there was significant transmission of disease from aquaculture (or other sources) to native populations it would be observed in these situations resulting in greater mortality from virus than would normally be observed in the absence of aquaculture opperations. The one cited example of shrimp decline from IHHNV in the Gulf of California was disputed by Dr. Alvarez, Instituto Nacional de la Pesca, Mexico, who suggested other causes for the decline. Another report by C. R. Laramore on viruses in native shrimp populations in Honduras following TSV outbreaks in aquaculture showed no noticeable effects on the native populations. These data are not sufficient to make any conclusions on the effects of introduced viruses on native populations. Both are correlative but not conclusive.

16. Due to a lack of knowledge about native pathogenic viruses, I would approach the risk assessment conservatively by assuming a minimal impact of native viruses until more data is available. Thus until it can be shown otherwise assume virus infection observed is from introduced viruses. That way risks from introduced viruses would be less likely to be underestimated.

17. Shrimp population data could be used in monitoring the overall health of the shrimp populations, but additional data on virus loads within the population is needed to make any correlations with virus impact To get a good handle on the effects of virus vs. a multitude of other stressors a database should be developed over time that includes populations, pathogen loads from sampled specimens, and physical data such as temperatures, dissolved gases, etc. This type of data may be currently available sans the virus loads. This would help determine the impact of various factors on shrimp populations and make it possible to develop shrimp population models that could be used to more accurately evaluate the effects of different stressors including viruses.

18. Shrimp viruses could impact non-shrimp species in two major ways. First, shrimp are an important link in the food web, severe losses of shrimp from virus infection (unless other species fill their niche) would impact shrimp predators. Secondly, if these viruses do indeed infect other species they could have a direct impact on these species. It is difficult to judge how big or important these impact would be since it would depend on the extent of the viral disease and the magnitude of the loss. Again there is a major data gap on how these viruses are transmitted in natural conditions as well as their persistence in the environment..

Comprehensive risk assessment and research needs

19. A comprehensive risk assessment is a good idea. Clearly shrimp viruses, previously identified in foreign aquaculture operations, are present in both domestic aquaculture operations and in imported shrimp indicating that they are a potential risk to native shrimp in our coastal waters. A significant problem in assessing the magnitude of the risk is the lack of good data on a number key issues. A comprehensive risk assessment will serve to identify and prioritize these gaps. As I see it the greatest uncertainties are biological, particularly as it relates to exposure of native populations and possible establishment of these viruses in the wild.

20. I'm not sure what retrospective data is available on native shrimp populations, but it would appear to be necessary to draw any conclusions about the impact of viruses vs. other stressors on shrimp populations. I'm also not sure what type of model could be developed with so little knowledge of the virus distribution and life cycle. Cost? I don't have the experience to begin to estimate the cost of such an assessment. A tiered assessment might be a more reasonable approach. That way qualitative estimates of risk could be used until sufficient data were available to get a better quantitative risk assessment.

21. It would be prudent to consider treatment options to reduce the risk of exposure.

22. In my estimation the most critical research needs for a risk assessment for exposure to shrimp viruses are their likely impact on shrimp populations: 1) Determining the likely chance for exposure in the wild from any exogenous virus source. This would include the fates of viruses that are released into environments, such as likely location in the water column with relationship to locations of susceptible shrimp populations, the length of virus viability in natural habitats, and the viruses mode of entry into hosts. 2) Determining the susceptibility of native shrimp species under natural conditions, including which developmental stages are most susceptible. 3) The nature and extent of viruses in wild shrimp populations in coastal waters, including "native" and putative introduced viruses needs to be assessed. This may require development of new diagnostic and survey techniques.

<u>Other comments:</u> If viral pathogens in insects are used as a model for shrimp viruses, disease outbreaks (epizootics) are generally cyclic are correlated with high insect population densities. Because viruses are obligate parasites their levels can only increase when they infect a susceptible host. Viruses in the environment are gradually inactivated so that only low levels remain. Therefore the probability of an insect encountering an infectious virus is low and if an insect does become sick and die, the probability of another susceptible insect encountering the diseased insect or amplified virus is also low. However, when host densities are high the insect that is infected by chance encounter and becomes sick will be in close contact with additional susceptible insects which allows the virus to be amplified and spread extensively within that population leading to a population crash and the deposition of large quantities of virus in the environment. Release of high amounts of virus (naturally or artificially) into the environment increases the chance that a susceptible host will come in contact with the virus and become infected even at low host densities.

Gerardo Vasta

SHRIMP VIRUS REVIEW WORKSHOP

(A) MANAGEMENT GOALS, ASSESSMENT ENDPOINTS AND THE CONCEPTUAL MODEL

(1) How does the management goal reflects the dimensions of the shrimp problem? Overall, the management goal reflects quite adequately the dimensions of the shrimp problem to be addressed in the short term. The proposed ecological risk assessment concerning shrimp viruses is appropriate because the potential threats to the natural ecosystems and the shrimp industry are both serious and urgent. These potential threats to US native wild shrimp populations from nonindigenous shrimp viruses arises from possible escapes of imported shrimp and insufficiently treated effluent from aquaculture facilities, shrimp processing solid waste and effluents, scavenger sea birds, bait for recreational fishing, human waste/sewage, ballast water and others. It is also well documented that under both experimental and natural conditions, shrimp viruses can infect various shrimp species and other crustaceans. Therefore, the potential for transmission of these viruses, principally from aquaculture and processing operations to native wild crustacean populations, although not yet well documented, should be a serious concern.

(2) Some have suggested modifying the assessment endpoints to emphasize potential risks of shrimp viruses to non-shrimp organisms and the larger estuarine ecological system, or, alternatively, the aquaculture industry. Please, comment on the assessment endpoints as the focal point for the ecological risk assessment: The potential negative effects of viruses on shrimp wild populations, organisms other than shrimp, and the ecosystem as a whole, that may result from the aquaculture and processing industries and other factors, are relevant and worth addressing with urgency. In fact, the consensus among the environmentalists seems to be that protection of wild shrimp must take precedence over shrimp aquaculture, and clearly, a substantial industry in the Gulf coast is based on domestic shrimp fisheries. However, the success of imported shrimp processing and mariculture operations in satisfying the consumer demand for shrimp (70-80% of the shrimp market), may alleviate the pressure on wild shrimp populations, food webs and the ecosystem as a whole. Furthermore, it should be considered that many marine ecosystems have been transiently or permanently damaged by commercial fishing practices, and current shrimp fishing methods may have similar environmental effects. Because of greater efficiency and potential to control its environmental effects, food farming is now preferred to food capture. Thus, the risks associated with shrimp viruses on wild shrimp populations, shrimp mariculture and the ecosystem as a whole, should eventually be assessed as an integrated initiative. What is badly needed are (a) the resources to conduct monitoring at the three assessment points (b) the tools to carry out the monitoring (c) to interpret the data as a coordinated effort in order to truly understand the sources and pathways of the disease agents. In the long term, US native species may be selectively bred and genetically improved to become useful mariculture species, avoiding the need of farming nonindigenous species. In fact, there is a precedent of this possibility in the attempts to farm *P. setiferus* in Texas.

(3) It has been suggested that the scope of the proposed risk assessment is too narrow and that it should be broadened to consider the impacts of such stressors as alternative land uses and seafood production methods in coastal areas. Please comment on this suggestion. If the risk assessment does not address the need to preserve and improve coastal current mariculture operations, we should be prepared to accept the risk of increasing alternative food production methods, such as shrimp trawling with the associated fish and turtle kills and high pressure on the wild shrimp populations and, ultimately, on the food webs. If coastal shrimp farming is to be stopped, alternative agricultural land uses that would produce runoffs with fertilizers or chicken/pig feces could have serious environmental impacts such as the algal blooms, including the *Pfiesteria piscicida* outbreaks, observed on the Atlantic coast. Any use of coastal land will have an impact on the coastal marine ecosystem and appropriate land use policies, such as the establishment of buffer zones, and rational management practices should be developed in order to minimize the impact.

(B) VIRAL STRESSORS AND FACTORS REGULATING SHRIMP POPULATIONS

(4) How relevant to virus effects on wild populations is information on infectivity and effects that is derived from laboratory or intensive aquaculture operations? The contributions of scientific research to several of the issues under consideration, represent the only body of evidence on which a solid base for a risk assessment initiative, and clearly indicate that this information is not only is very relevant, but much more of it is needed to elaborate a useful risk assessment. Needless to say that like in both the laboratory or pond setting, experiments have to be correctly designed, adequately controlled, and the data interpreted with caution. In the absence of reliable field data on wild shrimp populations, the aforementioned experimental approach sometimes constitutes the only source of knowledge we can rely upon.

Although the laboratory conditions may not exactly replicate the changing environmental conditions, most variables can be manipulated and controlled in a way that even those environmental conditions that are not very frequently observed can be simulated. The resulting data can then be used to gain insight in problems of infectivity of nonindigenous viruses for native shrimp species in the environment. Native species such as *P. setiferus*, *P. aztecus* and *P. duorarum* can be infected experimentally with IHHNV under laboratory conditions, by injection or by offering virus-infected tissues as sole food source. Experimental studies demonstrated that *P. setiferus*, but not *P. aztecus* or *P. duorarum*, could be killed by TSV. Furthermore, it was concluded that the three US. native species can serve as carriers or reservoir hosts of TSV without necessarily exhibiting disease (Overstreet et al, 1997). Although disease or mortalities did not necessarily occur in all the experimental animals and, therefore, it cannot be concluded that infection, disease or mortalities will happen in open waters, the potential risk of this event taking place cannot be ignored. Infection or a carrier status, should be considered a determinant factor that underscores the possibility that these viruses may have detrimental effects in native shrimp species and the environment overall. Stressful environmental conditions affecting infected, although not diseased, shrimp may determine different outcomes. Additionally, mutation of the established virus may lead to more virulent strains in an unpredictable manner. The genetic susceptibility of cultured *P. vannamei* to infectious HIINV and *Baculovirus penaei* has been recently examined and the possible relationship with growth status and metabolic gene expression characterized (Alcivar-Warren et al, 1997). The transmission of viruses in the wild shrimp populations is a documented fact and experiments can be designed to determine the viral doses that may lead to infection in open waters. Therefore, the laboratory experimentation has revealed the potential threat of exposure of native species to nonindigenous viruses, and it should be considered as the first step of a process that generates the scientific knowledge necessary to develop risk assessment and management strategies.

Results obtained from intensive aquaculture operations are very relevant, particularly in the absence of detailed field information on the wild populations. Although the aquaculture setting, particularly under high density rearing, is stressful in nature, it is important to understand the potential risks for the native species under those stressful conditions. For example, pond trials have yielded controversial results concerning the risk of TSV infectivity for native species, such as *P. setiferus*, as compared to *P. vannamei*.. In some studies, *P. setiferus* was not affected by the presence of TSV-surviving *P. vannamei* or by the presence of TSV-infected *P. vannamei* in adjacent ponds. Studies on the influence of salinity on the susceptibility of farmed *P. vannamei* to TSV, and the impact of aquaculture on wild shrimp populations in Honduras, illustrate how intensive aquaculture operations may be used to gain insight in viral infection and disease. However, additional experimentation under controlled conditions in the laboratory and intensive aquaculture operations will be necessary to establish the risk involved in cross-species infectivity of nonindigenous viruses and disease.

(5) How likely is that exposure of wild shrimp populations to viral diseases could lead to the development of immunity and reduced effects on population survival overtime? It has been shown that some short term immunity in arthropod species can be induced by challenge of with non-self materials, but overall, invertebrates are not endowed with immune memory and neither permanent nor long term immunity has been demonstrated so far. Invertebrates lack a B cell/T cell/immunoglobulin-mediated adaptive immune system, but are able to recognize and respond to non-self substances at least as efficiently as vertebrates do. Invertebrates rely on non-specific innate mechanisms that although may be inducible, only result in short-lived responses that in most cases do not discriminate between individual pathogens. Therefore, responses mounted by invertebrates to potentially infectious agents are mediated by immune systems only in the sense that they resemble qualitatively the "innate" or, "natural" immune responses of vertebrate myeloid cells and non-immunoglobulin, humoral components. Passive immunization with rabbit antibodies against a luminescent *Vibrio harveyi* strain 820514 originally isolated from diseased *P. monodon*, has been recently studied and results suggest and enhanced disease resistance in the treated animals for the first two weeks (Lee et al 1997).

Invertebrate defense responses exhibit common themes such as phagocytosis and encapsulation, but the underlying molecular recognition and effector mechanisms can be considerably diverse. The best characterized components of immunity in the crustacea are the glucan-binding proteins and lectins as recognition molecules, and the prophenoloxidase system and antibacterial peptides as effector factors. However, is not yet clear how the various components interact in the internal defense system against viruses. Some of the factors involved, such as a-2-macroglobulins, C-reactive proteins, antibacterial peptides, serine proteinases and proteinase inhibitors have been substantially conserved through the evolutionary lineages leading to the chordates, whereas others, such as C-type lectins and complement-related factors, only retained those regions of the molecule or single amino acid residues that are relevant to recognition/effector functions. Finally, for other factors such as glucan-binding proteins and some antibacterial peptides from crustacea, no homologues have been identified in vertebrates so far, and appear to be exclusive of invertebrate species. Penaeidins, a new family of antimicrobial peptides isolated from the hemolymph of *P. vannamei*, has been recently described (Destoumieux et al, 1997)

In addition to phagocytosis, encapsulation and nodule formation can be observed in the crustacea. Pathogens often elicit encapsulation, with consequent inactivation or death of the invader through toxic intermediates from an enzymatic cascade pathway that results in melanization. The recognition/effector mechanism responsible, is the prophenoloxidase activating system, that is present in most invertebrates and contains factors that are directly involved in communication between invertebrate hemocytes. A plasma recognition protein binds the polysaccharides or glycoproteins on the pathogen surface and induces activation of a prophenoloxidase-activating enzyme that will cleave the proenzyme prophenoloxidase to yield phenoloxidase. This active enzyme will catalyze the oxidation of phenols to quinones that will polymerize and form melanin, all exhibiting anti-microbial properties. In the shrimp *P. paulensis*, the great majority of the prophenoloxidase activity is found in shrimp hemocytes, is cation (Ca, Mg)-dependent, and is enhanced by microbial cell wall components such as LPS and β1-3 glucans suggesting a role in non-self recognition. Associated factors involved in cell adhesion and degranulation are also present (Perazzolo and Barraco, 1997). The interaction of hemocytes with foreign materials can further trigger clotting of body fluid (i.e. plasma) that would aid in internal defense by blocking or slowing the spread of microbes in the body cavity. Among the non-self recognition molecules, members of the immunoglobulin superfamily have been clearly identified in arthropods. However, only hemolin, a protein isolated from insects, can be induced upon pathogen challenge. Lectins (carbohydrate-binding proteins) are widespread, usually constitutive or inducible, components of invertebrate body fluids and tissues. Commonly multivalent, these molecules can aggregate microbes with the appropriate saccharide moieties on their surfaces. Simple aggregation of microbes can aid internal defense by restricting the distribution of potentially pathogenic agents and promote their phagocytosis. Such opsonization may be the result of conformational changes on the lectin upon binding to ligand that are recognized by the phagocytes.

Because it is unlikely that true immunity will be induced by exposure to the viral pathogen, reduced effects on population survival cannot be expected. At best, the continued impact of a viral pathogen on the shrimp populations could lead to selective survival of disease-resistant individuals, strains or races. In the Gulf of California the wild *P. stylirostris* shrimp populations rebounded, from the presumed IHHNV-caused mass mortalities, to harvestable levels after six years. It would be very interesting to examine if the current shrimp populations in the Gulf of California are equally or less susceptible to IHHNV than other populations from locations that have not been exposed to the disease.

(6) How can the strong influence of both natural and non-viral anthropogenic factors on shrimp populations be separated from risks associated with viral stressors?: It is possible that changes in salinity, and temperature, heavy metals or other pollutants, such as fertilizers in run offs that cause eutrophication of the environment, could stress coastal or estuarine shrimp populations and increase their susceptibility to viral disease. In the case of the Mexico's Gulf of California some evidence points to an association between detection of IHHNV in wild *P. stylirostris* shrimp and a decline in those populations, but other environmental factors may have compounded the problem. Further, it has been proposed that overfishing may have significantly contributed to the decline. Basic laboratory studies on effects of environmental factors such as temperature, salinity, heavy metals on the immune capabilities of shrimp are urgently needed in order to gain insight in the risks of climatic changes, such as El Niño, or anthropogenic factors on shrimp viral disease. Similarly, the recovery of the populations may have been due to either the return to "normal" environmental conditions, or the selection of shrimp races or strains with enhanced disease resistance. Therefore, although experimental research can provide valuable information on the effect of each environmental variable on shrimp susceptibility to disease, it may be difficult to separate these factors from the risks associated with viral stressors, without oversimplifying the problem.

(7) Can human health effects from shrimp viruses be ruled out as a concern? Why or why not?: In general virus that infect invertebrates do not infect mammals and, although viruses can change substantially over time in host-specificity and virulence, shrimp virus infections in humans are unlikely to take place. However, factual scientific evidence that would completely rule out this possibility is lacking. Some estuarine invertebrates, such as mussels and oysters, can transmit human viral diseases such as hepatitis and bacterial diseases such as those caused by *Vibrio* spp. Accordingly, another possibility to consider when addressing human health issues, is that virus-infected shrimp may be less able to control the proliferation of certain components of their associated bacterial flora, such as *Vibrio* spp., and thus become vectors of microbes that are pathogenic to man.

(8) Are the available identification techniques for shrimp viruses reliable enough to allow definitive conclusions to be drawn about the occurrence of virus in shrimp and the environmental media?: In the past, tests for the detection of shrimp viruses have yielded mixed results with regards to reliability. Bioassays, histological examination and serological methodologies have been applied alone or in combination but their specificity and sensitivity have been difficult to assess. Substantial progress, however, has recently been made in the development of fast, specific, and sensitive molecular identification and quantification methods for the diagnosis of viral diseases in shrimp. Particularly, PCR-based and DNA hybridization technologies have proven extremely useful in this regard (Chang et al 1996; Lo et al, 1996a,b; Loy et al 1996; Wang et al, 1996; Nunan and Lightner, 1997; Hasson et al, 1997)

(C) VIRAL PATHWAYS AND SOURCES

AQUACULTURE

(9) US aquaculture operations have had problems with viral diseases for several years. How does information from local wild shrimp populations support or refute the importance of aquaculture operations as a source for the virus? It is clear that aquaculture operations suffer from catastrophic outbreaks of viral disease, but unquestionable data on the transmission and establishment of nonindigenous viruses in the environment are not readily available. Therefore, the hypothesis that aquaculture of nonindigenous shrimp constitutes a source for virus spreading to the wild shrimp populations, lacks the necessary factual evidence at present time. A small number of cases of viral and bacterial disease in wild shrimp populations have been proposed to originate in coastal aquaculture or processing operations. In the case of the Mexico's Gulf of California, based on the available evidence, it has been proposed that IHHNV transmitted from animals farmed in coastal ponds and hatcheries, may have caused a decline in wild *P. stylirostris* shrimp populations. Interestingly, Mexico does not allow the aquaculture of nonindigenous shrimp species, and in this example this policy may have aided in the transmission of viral disease from the aquaculture setting to the environment, if this was the case. Accidental releases to the environment of nonindigenous shrimp species have been documented in the US aquaculture operations. Furthermore, under shrimp aquaculture systems in which ponds for high density rearing and waste disposal sites are open to the environment, with wastewater routinely discharged directly into coastal waters, it is likely that potentially pathogenic viruses will spread into the environment. Under those conditions, the improbable event of a nonindigenous virus becoming established in the environment, may become possible if repeated effluent discharge takes place over time. In this context, it is questionable whether shrimp aquaculture can operate in coastal areas without posing a threat to native shrimp, fish and wildlife stocks in surrounding bay and estuarine ecosystems. However, with proper management practices that include biosecurity and containment measures, continued disease-monitoring and careful treatment of the waste, the risk can be minimized. Finally, there is insufficient scientific knowledge concerning species-specificity of the viruses and the dynamics of their transmission in the environment, to make any accurate predictions of the potential hazard of coastal pond shrimp farming.

(10) It has been widely held that it is highly unusual for domesticated animals to infect wild animal populations: usually it is the other way around. How well does this observation apply to the relationship between shrimp in aquaculture and wild shrimp populations, with regard to shrimp viruses? It has been documented that viruses that infect "domesticated" shrimp species such as *P. monodon* , can cross-infect wild US shrimp species under experimental conditions or in intensive rearing ponds. It is not clear that this can happen in the environment, but the potential for this happening can not be ruled out.

SHRIMP PROCESSING

(11) Some believe it likely that shrimp processing operations have processed virus-infected shrimp from foreign sources for several years. How does information from local wild shrimp populations support or refute the importance of shrimp processing as a potential source for the virus? About 80% of the shrimp processed in the US is imported. Because some foreign aquaculture operations will harvest a pond at the first sign of disease and commercialize the product, the likelihood of infected shrimp being processed in US seafood factories is relatively high. Some processing operations consist of "unloading/shipping" plants and their potential as virus sources are small. In some others, the shrimp is thawed, peeled, deveined and repackaged. In the latter processing scenario, potentially infectious waste is produced and, if not adequately treated, may represent a significant source of virus. In some facilities, wastewaters are routed through sewage treatment plants, that include chlorination and hydrogen peroxide injection, before the effluent is discharged in the environment. Untreated solid waste may be used in landfills and, if infected, the potential of transmission to aquaculture facilities by scavenger birds cannot be ignored. Anecdotal evidence indicates that in the Gulf coast, a *Vibrio* sp. outbreak in wild shrimp was associated with areas where presumable infected shrimp harvested in Texas was processed.

OTHER POTENTIAL SOURCES AND PATHWAYS

(12) Should the retailers who distribute (rather than process) shrimp products receive additional evaluation as potential sources of exposure? It has been proposed that because imports of raw frozen seafood are commercialized independently from processing plants, and their waste may eventually reach landfills, dumpsites and waterways, they may represent a potential source of exposure that is not subject to adequate monitoring for virus infection. It should be considered during the decision-making process that if surveillance of the imported products resulted in labeling of the packed seafood as virus-infected, a serious consumer perception problem may be established, and the impact on this sector may be considerable.

(13) What sources other than aquaculture and shrimp processing are most critical for evaluation in a risk assessment of shrimp viruses? Given time constraints, which of these should be the focus of discussion at the workshop? A number of additional sources and vectors have been proposed, including infected shrimp as bait in recreational fishing, scavenger bird feces, human feces and ship ballast water, although their relative importance in virus transmission remains to be determined. Possibly, bird feces should be the priority topic for discussion because there is documented evidence about the presence of virus and it could represent a viral pathway from cultured shrimp to the wild shrimp populations and vice-versa.

(14) Is manufactured shrimp feed a potential viral source, or is the processing temperature sufficient to rule this source out? Because most shrimp farms in the US use exclusively pelleted shrimp feed, this represents a potential viral source. However, the manufacturing process subjects the feed to temperatures between 170 ° F and 230° F, which are sufficient to destroy most viruses. This should be determined experimentally and the issue resolved timely.

STRESSOR EFFECTS

(15) How should the available evidence concerning the effects of introduced viruses on wild shrimp populations be interpreted? The factual documented evidence concerning the presence of introduced viruses on wild shrimp populations is certainly not overwhelming, and it remains unclear that any effects have taken place as a result of these, if in fact have occurred. In the Gulf of California, a decline in wild *P. stylirostris* populations, has been associated with the detection of IHHNV, but it remains unclear that the virus may have been the cause, and other environmental factors and overfishing may have compounded the problem. In fact, wild shrimp populations in areas in South Carolina and Texas where outbreaks of viral disease such as TSV and IHHNV have taken place in coastal aquaculture operations, have not shown any signs of decline in following years. However, this evidence does not demonstrate that viral transmission or disease have not occurred in the wild shrimp populations. Therefore, the evidence has to be interpreted with caution and extensive research is needed to determine (a) the presence, virulence and load of "native" and nonindigenous viruses in wild shrimp populations and (b) the environmental conditions under which these may produce disease in the aforementioned wild populations.

(16) There is presently a lack of basic data on background levels of pathogenic shrimp viruses in wild shrimp populations in US waters. How should this data gap be evaluated in a risk assessment? Unfortunately, this is one of the critical aspects of a risk assessment and that would require considerable investment of resources and research efforts. Most of the "new" viral diseases recently described, have become patent in aquaculture settings and in many cases with catastrophic consequences. However, it remains unclear if these viruses can be present in the wild shrimp populations or in other species, with insignificant or unnoticed effects. Therefore, sensitive and specific, quantitative molecular tools should be applied to the assessment of the presence and levels of native and introduced viruses in the wild shrimp populations and other sympatric crustacean species. Similarly, a similar monitoring initiative should be developed in low and high density rearing ponds in aquaculture operations. At present time, however, this data gap should be evaluated with caution, and it should be assumed that the potential for the establishment of pathogenic shrimp viruses in wild shrimp populations in US waters is substantial.

(17) How can changes in wild shrimp populations be used to interpret the effect (or lack of effect) of introduced shrimp viruses? How could shrimp population models be used in the future? Fluctuations in coastal wild shrimp populations not exposed to aquaculture operations should be determined, and the baseline data compared with those obtained with wild shrimp populations from areas where nonindigenous shrimp farming takes place, and particularly where viral disease outbreaks have occurred. Differences in the population profiles during or after disease outbreaks may provide insight in the effects of introduced viruses in the wild shrimp populations. This has to be accompanied by careful sampling and monitoring of actual presence of the specific virus in the wild shrimp population in order to make the comparisons meaningful.

(18) How important are potential viral effects on non-shrimp species? It is well documented that some viruses can infect other crustacean species. For example, white spot syndrome baculovirus (WSBV) has been detected by PCR techniques in cultured and wild shrimp [*P. monodon*, *P. japonicus*, *P. penicillatus* and *Metapenaeus ensis* (sand shrimp)], prawns (*Macrobrachium rosenbergii*), crabs (*Charybdis feriatus*, *Portunus pelagicus* and *P. sanguinolentus*) and other arthropods, in different Asian countries (Lo et al, 1996). Therefore, the potential threat of shrimp viruses for non-shrimp species in the US and the ecosystem overall, cannot be ruled out.

COMPREHENSIVE RISK ASSESSMENT AND RESEARCH NEEDS

(19) How will a comprehensive risk assessment contribute to management of the shrimp virus problem, i.e., will add significantly to the information presently available? There is no doubt that a comprehensive risk assessment would contribute to a more useful management of the shrimp virus problem. The limitations to conduct such type of initiative reside in the quantity and quality of the available data, resources, and particularly, time. Therefore, in the present situation it may be important to focus on a more limited set of goals and assessment points in order to conduct a risk assessment that will permit limited but immediate management decision making.

(20) What type of assessment should be conducted next (e.g., quantitative risk estimates using shrimp population models), and what

would be the likely frame and cost? To conduct a quantitative risk assessment as the second step of the process would be logical. However, the scientific tools would have to be developed, applied and a large amount of data collected before this initiative could be carried out in a meaningful manner.

(21) Should a future risk assessment consider the risk reduction potential of a range of treatment options associated with specific exposure scenarios? Yes. But again, this type of risk assessment can only be conducted with data that is only partially available.

(22) Summarize the critical research needs for completing such a risk assessment.

1. Continue the development of sensitive and specific molecular probes for the known viruses that affect crustaceans, particularly nonindigenous and native shrimp species. Develop quantitative diagnostic methodology, such as competitive PCR.

2. Identify markers for stress and acute phase response in shrimp species, such as inducible peptides, protease inhibitors and lectins. Develop the molecular tools to detect and quantitate these markers in cultured and wild shrimp.

3. Apply those molecular tools to determine baseline occurrence and levels of viruses and stress indicators in wild shrimp and other crustacean species. Compare the information with that obtained from cultured shrimp, in healthy ponds and during viral disease outbreaks.

4. Develop and apply population models that will explain and aid in predicting natural variability of US wild shrimp populations.

5. Continue and expand experimental work on the species-specificity, infective doses and virulence of the viruses of interest, together with viability outside the host and dynamics of disease transmission. Correlate this information with molecular data on stress markers.

6. Expand efforts to gain insight in the inducible recognition and effector factors that mediate shrimp immune mechanisms and their failure to clear/inactivate their specific pathogens.

7. Apply the molecular qualitative and quantitative tools and bioassays for virus viability to examine possible sources and pathways such as imported processed shrimp, farm pond water, sediments, scavenger bird and human feces, shrimp feeds, ballast water, and others.

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Shiao Wang

1. How well does the management goal reflect the dimensions of the shrimp virus problem?

2. Some have suggested modifying the assessment endpoints to emphasize potential risks of shrimp viruses to non-shrimp organisms and the larger estuarine ecological system or, alternatively, to the aquaculture industry. Please comment on the assessment endpoints as the focal point for the ecological risk assessment.

   There is good evidence now that WSSV infects several non-shrimp arthropods. The effects of WSSV on these non-shrimp hosts are unknown. If the effects of WSSV infection on all arthropods are similar, then there is real cause for concern with regard to estuarine ecosystems. However, our knowledge and fear of shrimp-infecting viruses are based almostly entirely on either laboratory or intensive aquaculture observations. Such observations should not be used to predict what would occur in natural ecosystems.

3. It has been suggested that the scope of the proposed risk assessment is too narrow and that it should be broadened to consider the impacts of such stressors as alternative land uses and seafood production methods in coastal areas. Please comment on this suggestion.

   I think this suggestion warrants consideration. We should learn from problems many foreign countries are currently experiencing with shrimp aquaculture, both ecological and viral, and broaden the risk assessment process to consider potential impacts on coastal areas.

4. How relevant to virus effects on wild populations is information on infectivity and effects that is derived from laboratory or intensive aquaculture operations?

   Information on infectivity is relevant but information on effects is not, in my personal opinion. I can't imagine these shrimp viruses existing in the wild as naked viruses which means that these viruses are a part of the microbial ecosystem. Each virus-bacterial host complex co-exist with many others, probably sharing and competing for similar resources. Diversity is maintained by trophic interactions and resource limitations, preventing the dominance of any one single species. The effects seen in intensive aquaculture operations, an artificial environment, result from the dominance of one particular virus that is infectious to shrimp. Such dominance, in my opinion, would not take place in nature.

5. How likely is it that exposure of wild shrimp populations to viral diseases could lead to the development of immunity and reduced effects on population survival over time?

Anecdotal observations would suggest that this is quite likely. For example, when BP was first reported by Couch in 1974 in pink shrimp (Penaeus duorarum) from Cedar Key, Florida, 20% of feral shrimp were infected. Approximately two years ago, Dr. Kenneth Stuck of the Gulf Coast Research Laboratory collected pink shrimp from the same location and others along the Gulf Coast of Florida looking for BP. Although many hundreds of shrimp were examined, less than 1% were infected with BP. One possible interpretation is that over 20 years, pink shrimp susceptible to BP infection have been selected against and the current population is composed predominantly of those more resistant to infection.

Furthermore, minutes of the 1997 Stakeholder Meetings on the Report of the JSA Shrimp Virus Work Group reported that a severe decline in P. stylirostris population in the Gulf of California was associated with the occurrence of IHHNV in the wild population. The P. stylirostris population has since recovered and returned to normal. Note that an association was reported; no one said that IHHNV was the cause. Nevertheless, IF the decline was due to IHHNV, the recovery would suggest that selection took place and that the present population is more resistant to IHHNV.

6. How can the strong influence of both natural and non-viral anthropogenic factors on shrimp populations be separated from risks associated with viral stressors?

I don't think it is possible. Although laboratory studies have shown that exposure to anthropogenic stressors (such as toxins and pollutants) does not always increase the susceptibility of shrimp to viral infections, it is extremely difficult to convince someone that stressed shrimp are not more susceptible. Therefore, I don't think it will be possible to partition the effects of non-viral anthropogenic factors from viral stressors on shrimp populations.

7. Can human health effects from shrimp viruses be ruled out as a concern? Why or why not?

Yes, the viruses are quite host specific. In addition, the immune system in humans is much more advanced compared to invertebrates and thus should be able to inactivate the viruses.

8. Are the available identification techniques for shrimp viruses reliable enough to allow definitive conclusions to be drawn about the occurrence of viruses in shrimp and environmental media?

Yes, I would say that the identifications techniques (PCR and antibody-based) we have for TSV, IHHNV and WSV are quite accurate in terms of identification. I am still concerned though about making false negative conclusions that are based on PCR results. Shrimp tissues contain unidentified compounds that inhibit DNA polymerase. These compounds can be difficult to separate from DNA thus a negative PCR reaction does not automatically rule out the presence of the virus. Including an internal positive control helps but the problem is still a concern. I haven't kept up about the diagnosis of YHV.

9. U.S. aquaculture operations have had problems with viral diseases for several years. How does information from local wild shrimp populations support or refute the importance of aquaculture operations as a source for the virus?

I don't think we have enought information and experience to make that determination. In terms of scale, we have not had the type of problems that Asian countries are experiencing. There is little doubt that aquaculture operations provide a more concentrated source of pathogens because of their dense or intense nature. However, once discharged into the natural environment, the effect of dilution and microbial interactions on viral infectivity is unknown.

10. It has been widely held that it is highly unusual for domesticated animals to infect wild animal populations; usually it is the other way around. How well does this observation apply to the relationship between shrimp in aquaculture and wild shrimp populations, with regard to shrimp viruses?

I don't think this observation would apply in the case of shrimp. Pathogens dispersed via water are much more difficult to contain than those on land. Farmed animals such as cows and chicken are monitored much more closely thus pathogens have little chance to spread on the farm, much less to wild populations. This is completely different from the way shrimp is cultured.

11. Some believe it likely that shrimp processing operations have processed virus-infected shrimp from foreign sources for several years. How does information from local wild shrimp populations support or refute the importance of shrimp processing as a potential source for the virus?

I don't know the importance of shrimp processing as a potential source for the virus. I don't doubt that shrimp processing operations have processed virus-infected shrimp from foreign sources. However, there is not enough known about viral persistence in the natural environment to determine whether shrimp processing is a significant source of viruses. Studies on the dynamics of virus abundance in coastal seawater have shown large temporal fluctuations in matters of 10 - 20 minutes. Processing operations can introduce virus to the environment, whether the virus persists long enough to infect natural populations is not known. My feeling is that shrimp aquaculture operations present a more significant source of virus in terms of abundance while processing operations present a more significant source in terms of introducing new viruses from afar.

12. Should the retailers who distribute (rather than process) shrimp products receive additional evaluation as potential sources of exposure?

    No, unless we're talking about bait shrimp.

13. After considering the sources addressed in the shrimp virus report, what sources other than aquaculture and shrimp processing are most critical for evaluation in a risk assessment of shrimp viruses? Given time constraints, which of these should be the focus of discussion at the workshop?

14. Is manufactured shrimp feed a potential virus source, or is the processing temperature sufficient to rule this source out?

    I have no personal experience with feed manufacturing but this source should be ruled out. Not only will high temperature inactivate viruses but, at least in the case of BP, the simple process of dry will also do the same.

15. How should the available evidence concerning the effects of introduced viruses on wild shrimp populations be interpreted? (For example, what was the role of IHHNV in the decline of shrimp populations in the 1980's in the Gulf of California? What about TSV release from aquaculture into the wild in South America?)

    These are associations where no cause and effect can be shown. In my personal opinion, we should not extend our observations on the effects of viruses on shrimp in the laboratory or in aquaculture operations to what might take place in the natural environment. The effect of viruses on shrimp populations in the natural environment lies at the heart of the risk assessment process and more research is needed.

16. There is presently a lack of basic data on background levels of pathogenic shrimp viruses in wild shrimp populations in U.S. waters. How should this data gap be evaluated in a risk assessment?

I would disagree with the statement. Information concerning BP, a naturally occurring baculovirus in U.S. waters, is currently available. The natural infection cycle (when infected shrimp occur along the coast each year and the size distribution of those infected) have been well characterized by Drs. Overstreet, Lotz and Stuck at the Gulf Coast Research Laboratory. However, I do think there is an important data gap that needs attention. Although the occurrence of BP has been well characterized, its effect on wild shrimp population dynamics is unknown. Infected shrimp that die or become more susceptible to predation are quickly eliminated and thus never accounted for. Whether this is important in terms of overall shrimp population dynamics needs research.

17. How can changes in wild shrimp populations be used to interpret the effect (or lack of effect) of introduced shrimp viruses? How could shrimp population models be used in the future?

See responses to questions 15 and 16.

18. How important are potential viral effects on non-shrimp species?

See response to question 2.

19. How will a comprehensive risk assessment contribute to management of the shrimp virus problem, i.e., will it add significantly to the information presently available?

    Risk assessment is out of my area of expertise. I'll go directly to question 22.

20. What type of assessment should be conducted next (e.g., quantitative risk estimates using shrimp populations models), and what would be the likely time frame and cost?

21. Should a future risk assessment consider the risk reduction potential of a range of treatment options associated with specific exposure scenarios?

22. Summarize the critical research needs for completing such a risk assessment.

    I think there are two critical questions that we need to address. First, what is the effect of virus infections on the population dynamics of wild shrimp? Second, what is the natural history of viruses that are pathogenic to shrimp in the natural environment? Although empirical evidence to answer the first question will be difficult to obtain, some information is available to model virus-shrimp dynamics in the natural environment. At least for certain viruses, there is information concerning the following areas: 1) effects of shrimp age and condition on susceptibility to infection; 2) viral persistence in shrimp; 3) sublethal effects of viral infections; 4) the effects of genetics on viral resistance. Such information, along with fisheries statistics on the influence of predation and environmental conditions, should be useful in models to determine whether viruses play a significant role in wild shrimp population dynamics.

    The second question may be of greater importance in risk assessment. We can not assess the risk of viruses introduced either by shrimp processing operations or by aquaculture if we do not understand what happens to viruses that are released into the natural environment. With the availability of current molecular techniques to identify and to quantify viruses, a definitive answer to this question can be obtained. The importance of this question with regard to risk assessment warrants additional research.

The premeeting comments of Max Summers that follow were not received by ERG in time to be included in the assembled premeeting comments distributed to peer review experts prior to the workshop.

APPENDIX D

WORKSHOP AGENDA

United States
Environmental Protection Agency
National Center for Environmental Assessment

Shrimp Virus
Peer Review Workshop

Crystal Gateway Marriott Hotel
Arlington, VA
January 7-8, 1998

Agenda

WEDNESDAY, JANUARY 7, 1998

Workshop Chair: Dr. Charles Menzie

8:00AM	**Registration**	
8:30AM	**Welcome and Introductory Remarks**	*Dr. Charles Menzie, Menzie Cura & Associates Chelmsford, MA*
8:40AM	**Opening Remarks**	*Meryl Broussard Representative from the Joint Subcommittee on Aquaculture (JSA)*
8:45AM	**Logistical Information**	*Beth O'Connor Eastern Research Group, Inc. (ERG) Lexington, MA*
8:50AM	**Introduction of Experts**	*Dr. Charles Menzie*
9:05AM	**Introduction and Background**	*Dr. Kay Austin National Center for Environmental Assessment U.S. Environmental Protection Agency (U.S. EPA) Washington, DC*
9:20AM	**Summary of Modified Aquatic Nuisance Species Task Force Risk Assessment Approach**	*Dr. Richard Orr JSA Shrimp Virus Work Group Representative U.S. Department of Agriculture Riverdale, MD*
9:40AM	**Questions and Comments**	

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WEDNESDAY, JANUARY 7, 1998 (Continued)

9:50AM	**Management Goals, Assessment Endpoints and Conceptual Model**
10:10AM	**Questions and Comments**
10:20AM	BREAK
10:40AM	**Summary Presentations of Reviewers' Premeeting Comments**

- Aquaculture Virus Pathways and Sources .... *Dr. Wayne Munns*
  U.S. EPA, Narragansett, RI
- Shrimp Processing Virus Pathways and Sources .... *Dr. Jack Gentile*
  University of Miami
  Miami, FL
- Other Virus Pathways and Sources .... *Dr. Anne Fairbrother*
  ecological planning and toxicology, inc.
  Corvallis, OR
- Viral Stressors and Cross-Cutting Issues .... *Dr. Anne Fairbrother*
- Stressor Effects and Cross-Cutting Issues .... *Dr. Anne Fairbrother*

11:30AM **Questions and Comments**

12:00PM LUNCH

1:00PM **BREAKOUT DISCUSSIONS CONVENE**
Probability of Establishment (discussion topic 1)
Aquaculture .... *Dr. Wayne Munns, Leader*
Shrimp Processing .... *Dr. Jack Gentile, Leader*
Other Sources .... *Dr. Anne Fairbrother, Leader*

2:30PM BREAK

2:45PM **Breakout Sessions Reconvene to Discuss Consequences of Establishment** (discussion topic 2)
Aquaculture .... *Dr. Wayne Munns, Leader*
Shrimp Processing .... *Dr. Jack Gentile, Leader*
Other Sources .... *Dr. Anne Fairbrother, Leader*

4:15PM **Plenary Session Reconvenes: Progress Reports From Breakout Discussion Leaders**

4:45PM **Observer Comments**

5:15PM ADJOURN

THURSDAY, JANUARY 8, 1998

8:30AM	**General Announcements/Review Day Two Charge**	*Dr. Charles Menzie*
8:45AM	**Breakout Discussions Convene to Develop Risk Assessment** (discussion topic 3)	
	Aquaculture	*Dr. Wayne Munns, Leader*
	Shrimp Processing	*Dr. Jack Gentile, Leader*
	Other Sources	*Dr. Anne Fairbrother, Leader*
10:45AM	BREAK	
11:00AM	**Plenary Session Convenes to Discuss Breakout Discussion Findings**	
12:00PM	LUNCH	
1:00PM	**Plenary Session Reconvenes to Review Premeeting Comments on Comprehensive Risk Assessment and Critical Research Needs**	
3:15PM	BREAK	
3:30PM	**Plenary Session Reconvenes to Discuss Breakout Discussion Findings**	
4:00PM	**Observer Comments**	
4:30PM	**Workshop Wrap-Up**	
4:45PM	ADJOURN	

APPENDIX E

PRESENTATION MATERIALS ON THE RISK ASSESSMENT PROCESS DEVELOPED BY THE AQUATIC NUISANCE SPECIES TASK FORCE

Prepared by:

Richard Orr
USDA-APHIS

Lofty Goals

PATHWAY EVALUATION -- Develop a set of criteria to help prioritize pathways that present a risk for introducing non-indigenous aquatic organisms.

RISK ASSESSMENT -- Develop a process that can be used to:

a) evaluate recently established non-indigenous organisms

b) evaluate non-indigenous organisms proposed for deliberate introduction

c) evaluate the risk associated with individual pathways

RISK MANAGEMENT -- Develop a practical operational approach to maximize a balance between protection and the available resources for:

a) reducing the probability of unintentional introductions

b) reducing the risk associated with intentional introductions

Current and Former Members of the Risk Assessment and Risk Management Committee

Walter Blogoslawski
NOAA, National Marine Fisheries Service
Former Member

Joseph McCraren
National Aquaculture Association
Former Member

Richard Guadiosi
U.S. Coast Guard
Former Member

Fred Kern
NOAA National Marine Fisheries Service
Current Member

Richard Orr
USDA, Animal and Plant Health Inspection Service
Current Member, RAM Chairperson

Edwin Theriot
U.S. Army Corps of Engineers
Current Member

Mike Troyer
U.S. Environmental Protection Agency
Former Member

James D. Williams
USGS Biological Resources Division
Current Member

Richard E. Bohn
National Aquaculture Association
Current Member

Sharon Gross
U.S. Fish and Wildlife Service
Former Member

Lauren Kabler
U.S. Coast Guard
Former Member

Marshall Meyers
Pet Industry Joint Advisory Council
Current Member

Richard Sayers, Jr.
U.S. Fish and Wildlife Service
Former Member

Jay Troxel
U.S. Fish and Wildlife Service
Current Member

Bill van der Schalie
U.S. Environmental Protection Agency
Current Member

Example of Risk Assessments that used the Generic Process

I. COMMODITY ASSESSMENTS:

USDA FOREST SERVICE. 1991. Pest Risk Assessment of the Importation of Larch from Siberia and the Soviet Far East. Miscellaneous Publication No. 1495

USDA FOREST SERVICE. 1992. Pest Risk Assessment of the Importation of *Pinus radiata* and Douglas-fir Logs from New Zealand. Miscellaneous Publication No. 1508

USDA FOREST SERVICE. 1993. Pest Risk Assessment of the Importation of *Pinus radiata, Nothofagus dombeyi* and *Laurelia philippiana* Logs from Chile. Miscellaneous Publication No. 1517

II. SPECIFIC ORGANISM ASSESSMENT:

Huettel, R.L.; Griffin, R.L. and Caplen R.T. 1993. Pest Risk Analysis for Pea Cyst Nematode. USDA APHIS PPQ/PPD risk assessment, 15p.

Lehtonen, P. 1993. Pest Risk Assessment on Chinese Water Spinach. USDA APHIS PPQ risk assessment, 22p.

Orr, R.L. and Cohen, S. 1991b. Pest Risk Assessment on Potato Virus Y-N. APHIS PPD risk assessment, 14p.

Orr, R.L. 1991a. Pest Risk Assessment on Apple Ermine Moth. USDA APHIS PPQ risk assessment, 15p.

Orr, R.L. 1991b. Pest Risk Assessment on Cherry Bark Tortrix. USDA APHIS PPQ risk assessment, 13p.

Schall, R.A. 1991. Pest Risk Assessment on Karnal Bunt. USDA APHIS PO risk assessment, 14p.

Schall, R.A. 1992. Pest Risk Assessment on Larch-Poplar Rust. USDA APHIS PO risk assessment, 17p.

RISK – **IS THE LIKELIHOOD AND MAGNITUDE OF AN ADVERSE EVENT**

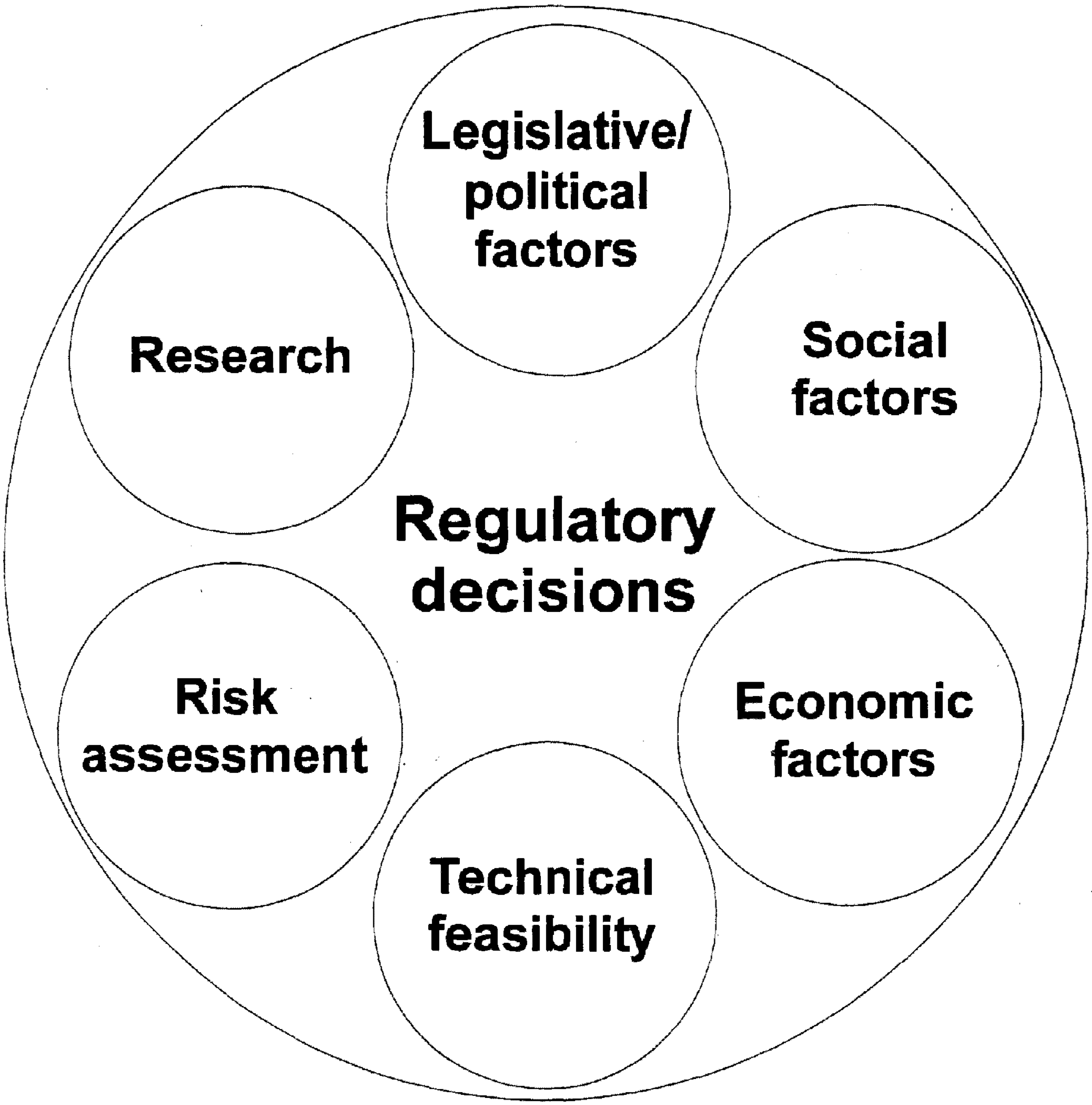
RISK ANALYSIS – **THE PROCESS THAT INCLUDES BOTH RISK ASSESSMENT AND RISK MANAGEMENT**

RISK ASSESSMENT – **THE ESTIMATION OF RISK**

RISK MANAGEMENT – **THE PRAGMATIC DECISION MAKING PROCESS CONCERNED WITH WHAT TO DO ABOUT THE RISK**

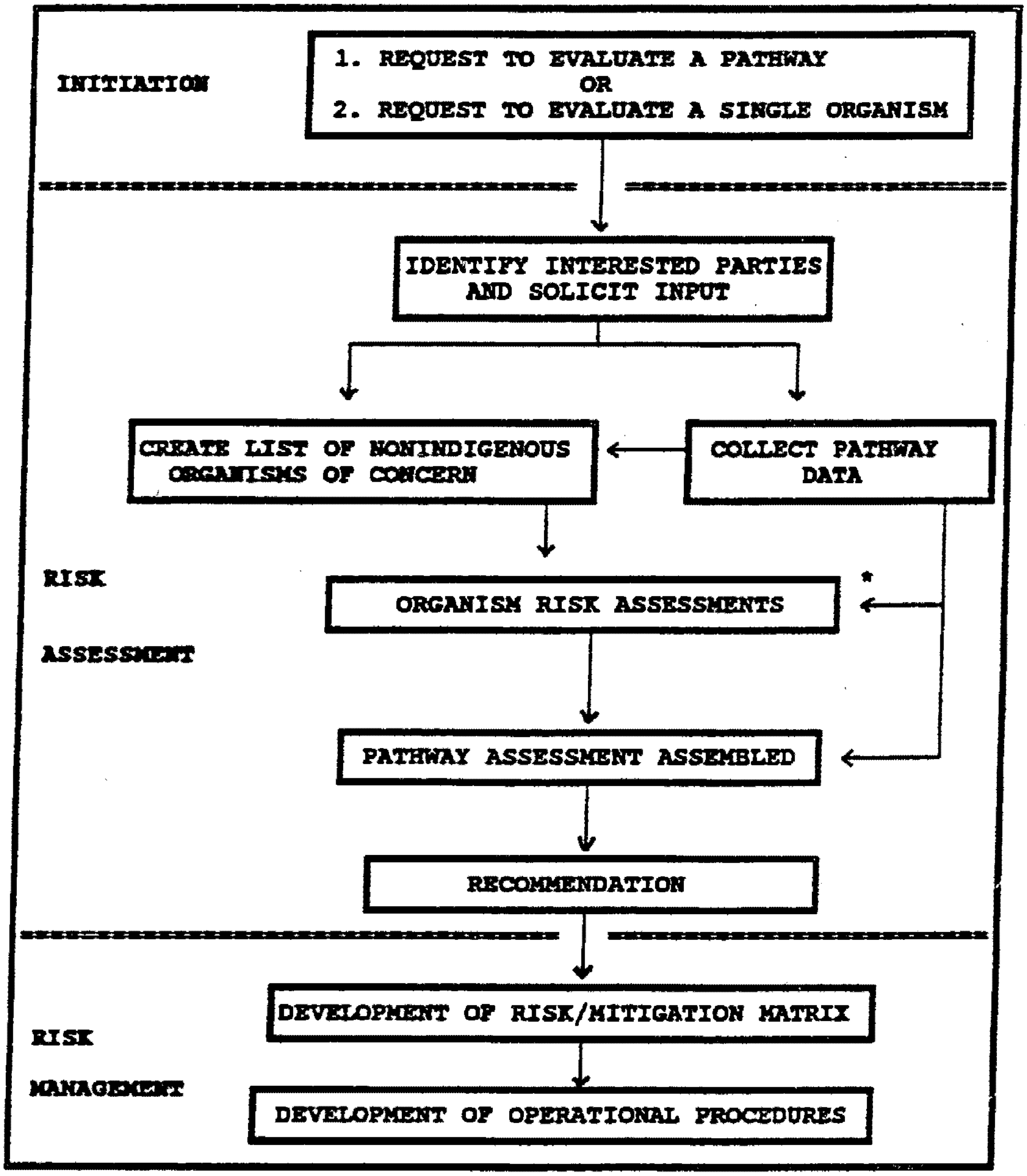
Assessment Criteria

- Comprehensive
- Logically sound
- Practical
- Conducive to learning
- Open to evaluation



Elements in risk management

FIGURE 1. Pathway Analysis: Flow Chart showing the Initiation, Risk Assessment and Risk Management for a pathway.



* = For details on the Organism Risk Assessment see Figure 2 "Risk Assessment Model" page 11. Pathways that show a high potential for introducing nonindigenous aquatic organisms should trigger detailed risk analyses.

Creating a List of Nonindigenous Aquatic Organisms of Concern

The next element in figure 1 (page 8) is "Create List of Nonindigenous Organisms of Concern". The following generalized process is recommended

STEP: 1) Determine what organisms are associated with the pathway.

2) Determine which of these organisms qualify for further evaluation using the table below.

Category	Organism Characteristics	Concern
1a	species nonindigenous not present in country (United States)	yes
1b	species nonindigenous, in country and capable of further expansion	yes
1c	species nonindigenous, in country and reached probable limits of range, but genetically different enough to warrant concern and/or able to harbor another nonindigenous pest	yes
1d	species nonindigenous, in country and reached probable limits of range and not exhibiting any of the other characteristics of 1c	no
2a	species indigenous, but genetically different enough to warrant concern and/or able to harbor another non-indigenous pest, and/or capable of further expansion	yes
2b	species indigenous and not exhibiting any of the characteristics of 2a	no

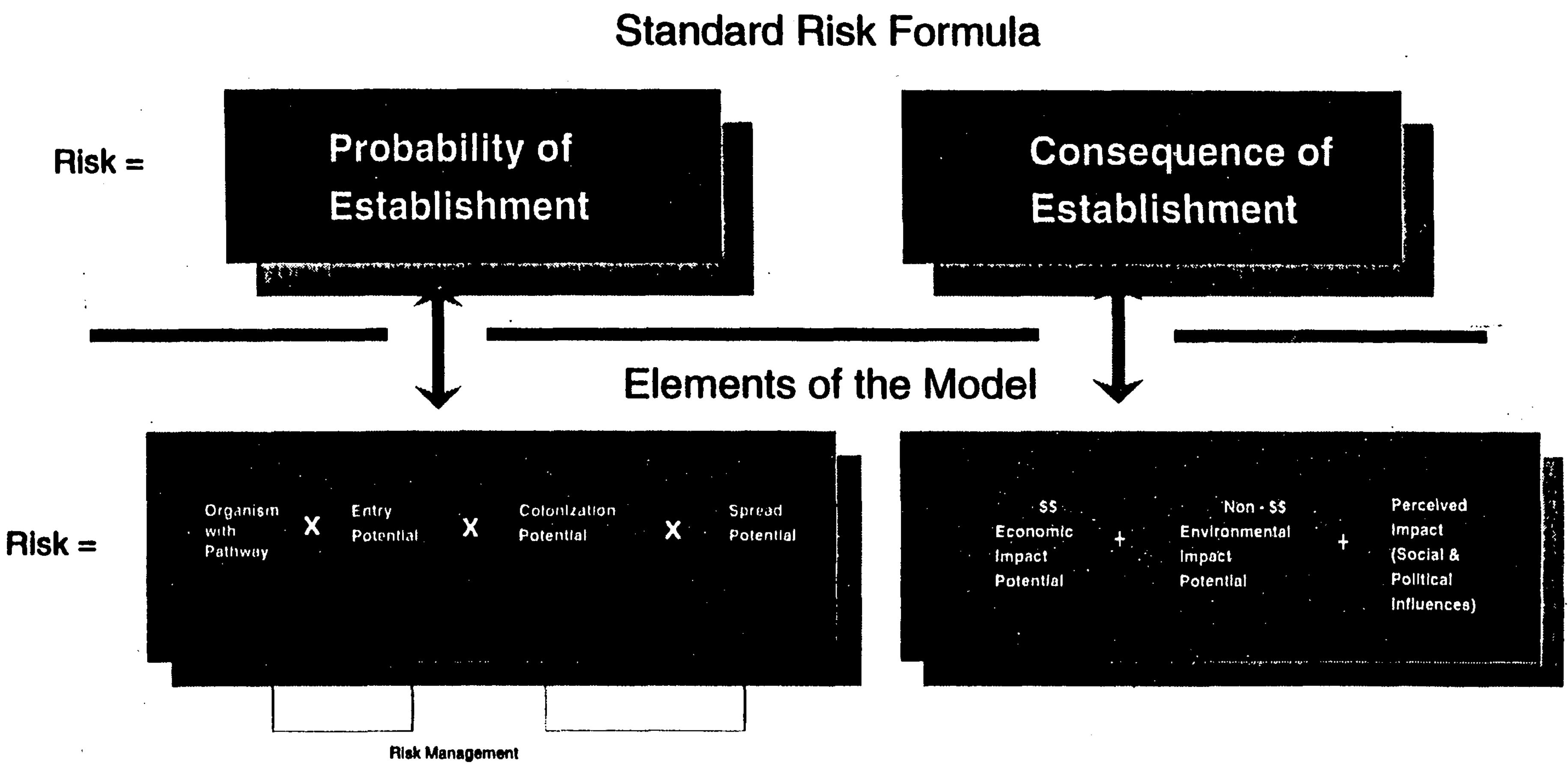
3) Produce a list of the organisms of concern from (step 2) categories 1a, 1b, 1c, and 2a. Taxonomic confusion or uncertainty should also be noted on the list.

4) Conduct Organism Risk Assessments from the list of organisms developed in step 3.

Based on the number of organisms identified and the available resources, it may be necessary to focus on fewer organisms than those identified using the above table. When this is necessary it is desirable that the organisms chosen for complete risk assessments be representative of all the organisms identified. A standard methodology is not available because the risk assessment process is often site or species specific. Therefore, professional judgement by scientists familiar with the aquatic organisms of concern is often the best tool to determine which organisms are necessary for effective screening.

FIGURE 2

Risk Assessment Model



- For model simplification the various elements are depicted as being independent of one another
- The order of the elements in the model does not necessarily reflect the order of calculation

REFERENCE CODES TO ANSWERED QUESTIONS

Reference Code	Reference Type
(G)	General Knowledge, no specific source
(J)	Judgmental Evaluation
(E)	Extrapolation; information specific to pest not available; however information available on similar organisms applied
(Author, Year)	Literature Cited

UNCERTAINTY CODES TO INDIVIDUAL ELEMENTS

Uncertainty Code	Symbol	Description
Very Certain	VC	As certain as I am going to get
Reasonably Certain	RC	Reasonably certain
Moderately Certain	MC	More certain than not
Reasonably Uncertain	RU	Reasonably uncertain
Very Uncertain	VU	A guess

WORKSHEET FOR REVIEW PROCESS

IV. Rating Elements of the Review Risk Model

A. PROBABILITY OF ESTABLISHMENT

- **The probability of the organism being on, with or in the pathway:**
  Ranking = Uncertainty code =

- **The probability of the organism surviving in transit and successfully surviving current regulatory mitigation systems:** Ranking = Uncertainty code =

- **The probability of the organism successfully colonizing:**
  Ranking = Uncertainty code =

- **The probability the organism will be able to spread beyond the colonized area:**
  Ranking = Uncertainty code =

B. CONSEQUENCES OF ESTABLISHMENT

- **The economic impact if established:**
  Ranking = Uncertainty code =

- **The environmental impact if established:**
  Ranking = Uncertainty code =

- **The impact from social and/or political influences:**
  Ranking = Uncertainty code =

C. OVERALL ORGANISM RISK POTENTIAL RATING = Uncertainty code =

V. **Specific Questions:**

VII. **Recommendations:**

APPENDIX F

SUMMARY MATERIALS PRESENTED BY WORKSHOP AND BREAKOUT GROUP CHAIRS

SUMMARY MATERIALS

Prepared by:

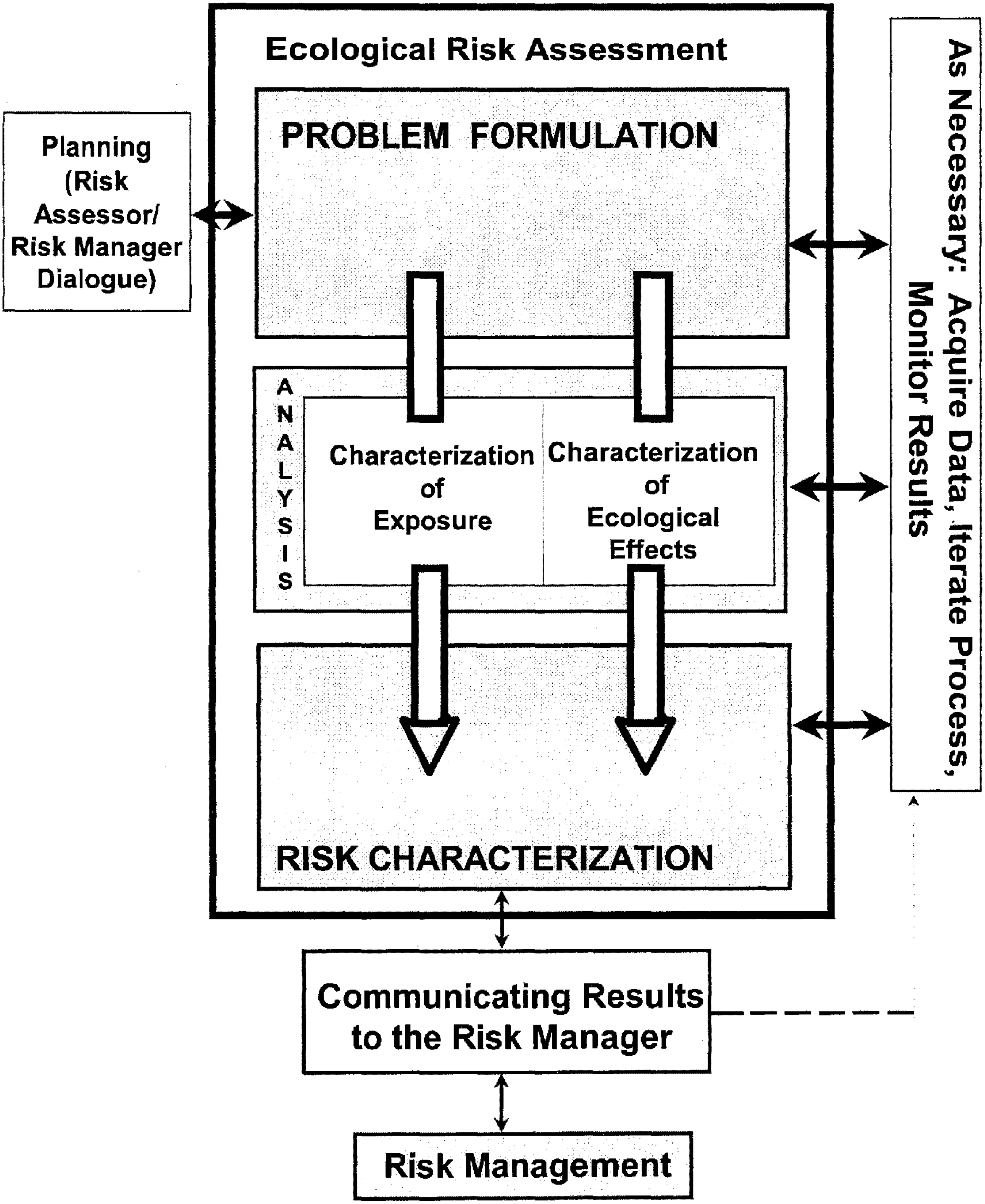
Dr. Charles Menzie
Menzie-Cura & Associates
(Workshop Chair)

WORKSHOP GOALS

- To complete a qualitative assessment of the risks associated with shrimp viruses following the general risk assessment process developed by the Aquatic Nuisance Species Task Force
- To evaluate the need for a future more comprehensive risk assessment
- To identify critical risk-relevant research needs along with possible costs and time implications

PP/1/5/98 - 555b

The Ecological Risk Assessment Process



Ecological Risk Assessment: Problem Formulation

- Define assessment endpoints
  - ✓ Assessment endpoints helps to ensure risk assessment addresses important scientific issues while being responsive to management concerns
- Develop the conceptual model
  - ✓ Models portray the relationships between stressors, their sources, and the ecological effects they may cause.
- Develop an analysis plan
  - ✓ Identify what will be done in an assessment

PP/1/5/98 - 555b

OUR FOCUS AND APPROACH

We will focus on the scientific aspects related to:

1. **likelihood that viruses will become established**
2. **potential consequences of such establishment**

We will rely upon the varied backgrounds and experience represented among the panelists

PP/1/5/98 - 555b

OUR FOCUS AND APPROACH

Three groups will evaluate the following potential viral pathways:

1 Aquaculture

2 Shrimp processing

3 Other

Our work products will be published in a report that will be used, in part, to inform a JSA sponsored workshop on risk management.

SOME GENERAL GUIDELINES

- Remain focused
- Listen well
- Contribute your knowledge and experience
- Be prepared to discuss the issues in an open and thorough manner
- Respect the views of others

OBSERVORS

- You will have an opportunity to comment at the end of each day

- You may also provide oral or written comments/questions to the workshop chair throughout the workshop

PP/1/5/98 - 555b

Management Goals

Prevent the establishment of new disease-causing viruses in wild populations of shrimp in the Gulf of Mexico and southeastern U.S. Atlantic coastal waters, while minimizing possible impacts on shrimp importation, processing, and aquaculture operations.

PP/1/5/98 - 555b

COMMENTS ON THE MANAGEMENT GOAL

40% of us felt it was appropriate (perhaps with clarification or qualification)

Several suggested that it::

- ✓ Should include the aquaculture industry
- ✓ Should consider other pathogenic organisms
- ✓ Should evaluate risk of viruses to other susceptible organisms

Assessment Endpoints

- Survival, growth, and reproduction of wild penaeid shrimp populations in the Gulf of Mexico and southeastern U.S. Atlantic coastal waters.
- Ecological structure and function of coastal and near-shore marine communities as they affect wild penaeid shrimp populations

PP/1/5/98 - 555b

COMMENTS ON ASSESSMENT ENDPOINTS

- Most agreed with assessment endpoint 1. However, a few of you commented on the need to narrow it somewhat to focus on the specific stressors, i.e., introduced viruses.
- Several of you found the second endpoint to be overly broad and perhaps out of reach of assessment.
- Several suggested that the aquaculture industry be incorporated within an endpoint or as an additional endpoint.

PP/1/5/98 - 555b

COMMENTS ON ASSESSMENT ENDPOINTS

A few additional suggestions include:

- ✓ Add an endpoint that relates to possible effects on other species
- ✓ Delete second endpoint and add Maintenance of viable populations and communities of marine organisms, free of virus-induced effects.

PP/1/5/98 - 555b

COMMENTS ON THE MANAGEMENT GOAL

Other suggestions include:

Expand geographic area of interest to include the Pacific coast

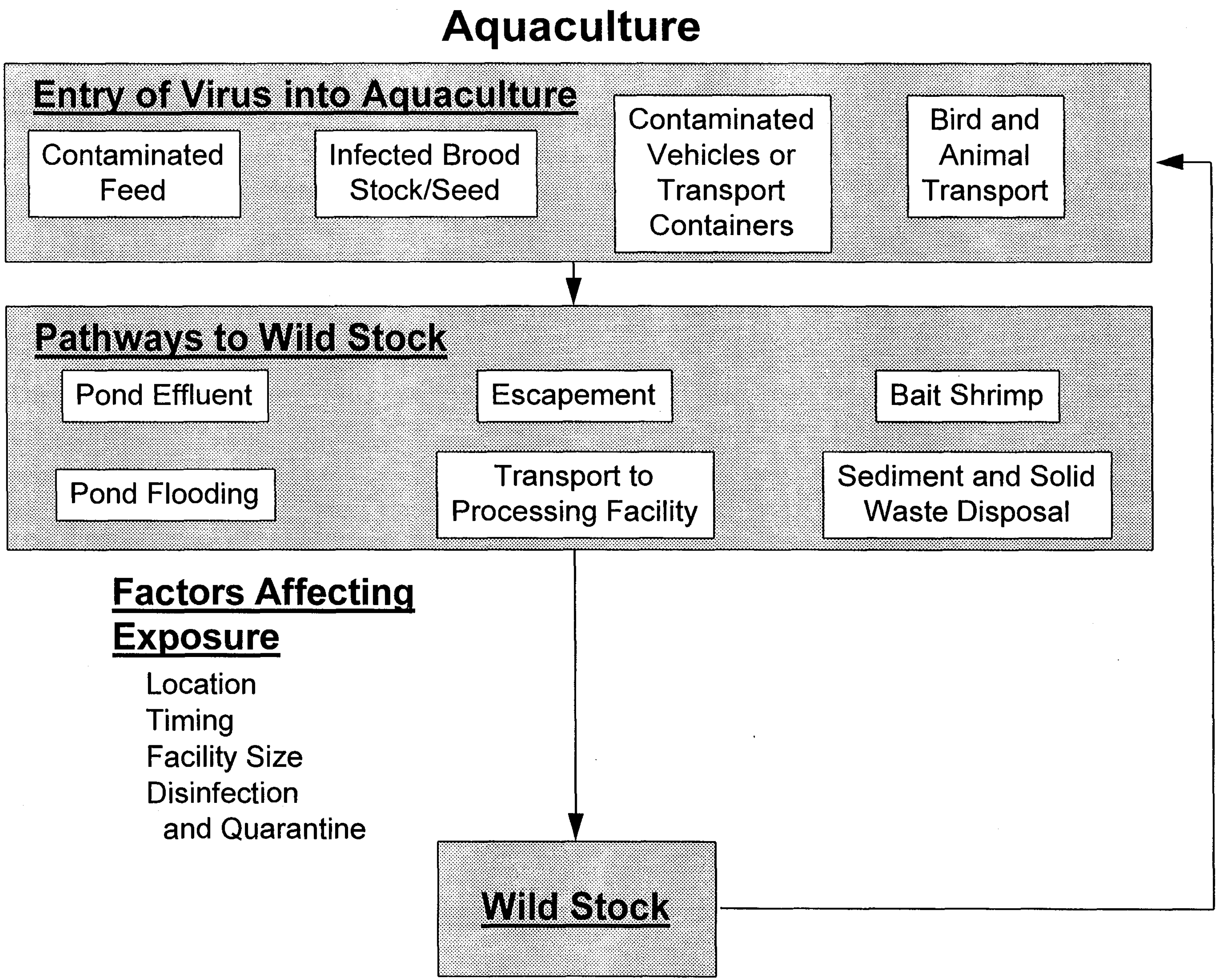
- ✓ Minimize impacts on all industries
- ✓ Specify or confirm that a specific problem exists
- ✓ Prevent recurrent virus epizootic events
- ✓ Emphasize source reduction approaches

SUMMARY PRESENTATION OF
REVIEWERS' PREMEETING COMMENTS

AQUACULTURE VIRUS PATHWAYS AND SOURCES

Prepared by:

Dr. Wayne Munns
U.S. EPA


Aquaculture
Entry of Virus into Aquaculture
Contaminated Feed
Infected Brood Stock/Seed
Contaminated Vehicles or Transport Containers
Bird and Animal Transport
Pathways to Wild Stock
Pond Effluent
Escapement
Bait Shrimp
Pond Flooding
Transport to Processing Facility
Sediment and Solid Waste Disposal
Factors Affecting Exposure
Location
Timing
Facility Size
Disinfection and Quarantine
Wild Stock

Premeeting Comments

Virus Sources and Pathways - Aquaculture

Question 9

How does information from local wild shrimp populations support or refute the importance of aquaculture operations as a source for the virus?

Premeeting Comments

Virus Sources and Pathways - Aquaculture

Question 9

Concensus:

- No direct evidence exists

Issues:

- Simple co-occurrence, or occurrence of mortality, not sufficient
- Examples of escaped cultured shrimp exist

Data Gaps:

- Epidemiology of virus transmission
- Host-specificity of viruses
- Technologies to monitor infection in natural populations and transmission of viruses in discharges (e.g., molecular probes, biomarkers)

Premeeting Comments

Virus Sources and Pathways - Aquaculture

Question 10

It is unusual for domestic animals to infect wild populations. How well does this observation apply to the relationship between shrimp in aquaculture and wild shrimp populations?

Premeeting Comments

Virus Sources and Pathways - Aquaculture

Question 10

Concensus:

- ➢ No direct evidence exists in wild U.S. shrimp; may have occurred elsewhere
- ➢ Numerous examples for other diseases do exist
- ➢ Proposed pathway reasonable

Issues:

- ➢ Evidence of reverse transmission may exist
- ➢ Evidence of facility to facility transmission exists
- ➢ Cultured shrimp not really "domesticated"; analogy may be unsound (transmission by water)

Data Gaps

- ➢ Exposure of wild shrimp to infected cultured shrimp & byproducts
- ➢ Susceptibility and recovery of wild U.S. shrimp

Data Gaps

Virus Sources and Pathways - Aquaculture

- Water exchange with natural waters - protocols for aquaculture operations, water treatment, etc.
- Number, size (and location) of aquaculture operations in relationship to native shrimp populations
- Volume, disposal patterns, and treatment of solid wastes
- Extent of virus contamination of feed, broodstock/seed, vehicles, and birds/animals that could transport virus

- Epidemiology of virus transmission
- Host-specificity of viruses
- Exposure of wild shrimp to infected cultured shrimp and byproducts
- Susceptability and recovery of wild U.S. shrimp
- Technologies to monitor infection and transmission

SUMMARY PRESENTATION OF REVIEWERS' PREMEETING COMMENTS

SHRIMP PROCESSING VIRUS PATHWAY AND SOURCES

Prepared by:

Dr. Jack Gentile
University of Miami

Shrimp Virus Workshop

Shrimp Processing Pathway

John H. Gentile, Facilitator - U. Miami
Ned Alcanthie - NOAA/NMFS
Dwaine Braasch - U Southern Mississippi
Dana Dunkelberger - Palmetto Aquaculture Corp.
Jeffrey Lotz - U. Southern Mississippi
Roy Martin - National Fisheries Institute

Crystal Gateway Mariott Hotel
January 7-8, 1998

Shrimp Processing Pathway

Background

- Sixty countries exporting pond-raised and wild shrimp to the U.S.
- Fifty percent of shrimp processed in U.S. is from Thailand, India, and other countries
- Viral diseases are major problems in these countries
- Foreign shrimp are harvested at early stages of disease
- Increases likelihood of viral contamination of imports
- Virus infected shrimp have been identified in retail stores
- This pathway may pose a significant threat to wild shrimp

Charge to Expert Panel

Shrimp Processing

Question 11a.

Some believe it likely that shrimp processing operations have processed processed virus-infected shrimp from foreign sources for several years.

- What evidence do we have to support this statement?
- What is the magnitude of the problem
- Which foreign sources are shipping infected products
- Do we have accurate, diagnostic screening methods

Charge to Expert Panel

Shrimp Processing

<u>Question 11b.</u>

How does information from wild shrimp populations support or refute the importance of shrimp processing as a potential source for the virus?

- Do we have baseline data on viruses in wild pollutions?
- Do we have the appropriate diagnostic and detection methods?
- What evidence exists to link processing and wild shrimp viruses?
- What do we know of the persistence of viruses in water and sediments

Charge to Expert Panel

Shrimp Processing

<u>Question 12.</u>

Should retailers who distribute(rather than process) shrimp products receive additional evaluation as potential sources of exposure?

- Is there evidence of viruses in retail products?
- What are the potential human health risks from this pathway
- What are the routes from the retail market to the environment?
- Do these routes represent potentially significant sources for the viruses to enter the environment?

Pre-meeting Comments on Shrimp Processing

Question 11a.

Some believe it likely that shrimp processing operations have processed processed virus-infected shrimp from foreign sources for several years.

Agree - 92%

Question 11b.

How does information from wild shrimp populations support or refute the importance of shrimp processing as a potential source for the virus?

Evidence neither supports or refutes - 93%

Question 12.

Should retailers who distribute(rather than process) shrimp products receive additional evaluation as potential sources of exposure?

Should receive additional evaluation - 84%

Shrimp Processing Pathway

Information Needs

- Volume, disposal patterns, and treatment practices for both shrimp processing effluents and solids

- Number, size, and spatial distribution of shrimp processing plants relative to receiving water and habitats for wild shrimp

- Estimates of the extent of virus contamination of shrimp received from foreign sources for processing

- Extent and distribution of contaminated shrimp in retail seafood markets and effluent and solids disposal practices

- Extent of virus contamination of shrimp and fish feeds

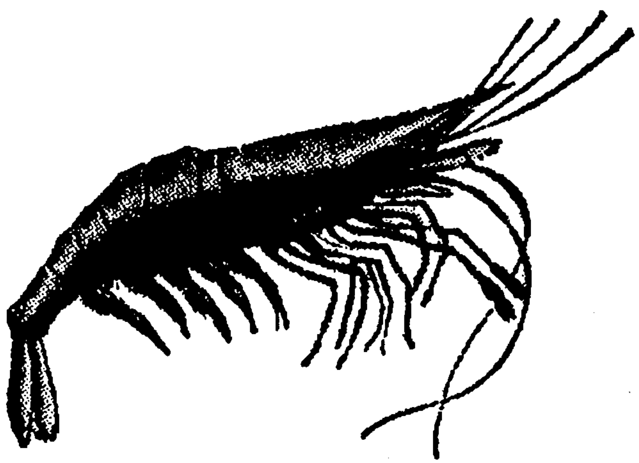
SUMMARY PRESENTATION OF REVIEWERS' PREMEETING COMMENTS

OTHER VIRUS PATHWAY AND SOURCES; VIRAL STRESSORS AND CROSS-CUTTING ISSUES; STRESSOR EFFECTS AND CROSS-CUTTING ISSUES

Prepared by:

Dr. Anne Fairbrother
Ecological Planning and Toxicology, Inc.

Other potential virus sources



- bait shrimp
- ballast water
- other introduced crustaceans
- manufactured shrimp feed
  - high processing temperature (>80 °C/175 °F) would kill viruses
- research and display
- avian vectors
- fishing vessels
- natural spread

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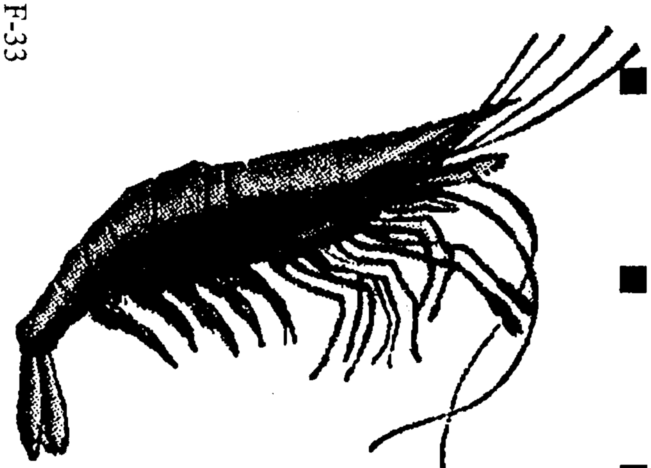
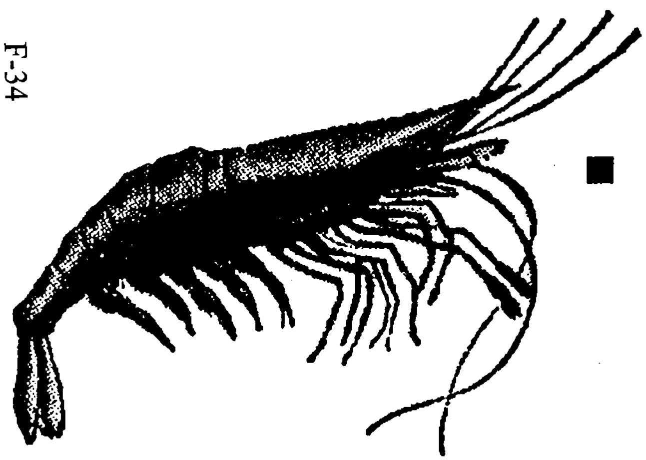
Virus factors



- relevance of laboratory information
- development of immunity and reduction of impact on shrimp
- separating effects of multiple stressors
- human health effects from shrimp viruses
- shrimp virus ID techniques

Relevance of laboratory information



- infectivity information is valuable
- exposures may differ from natural situations
  - injection studies may not be relevant
- stress factors generally are lacking in laboratory studies
  - may make natural populations more or less susceptible
- mode of transmission, viability in the environment, carrier states

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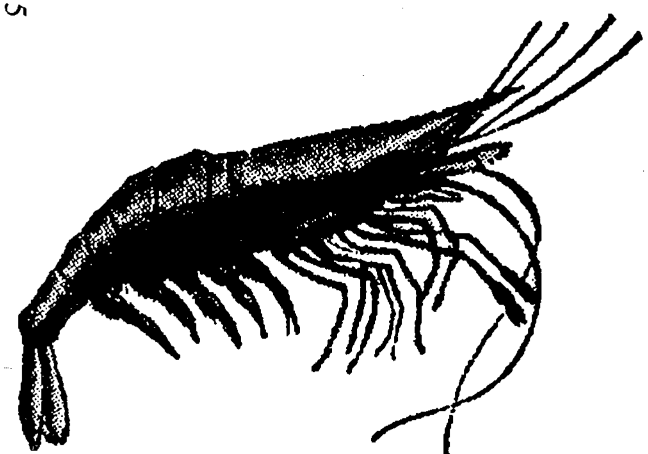
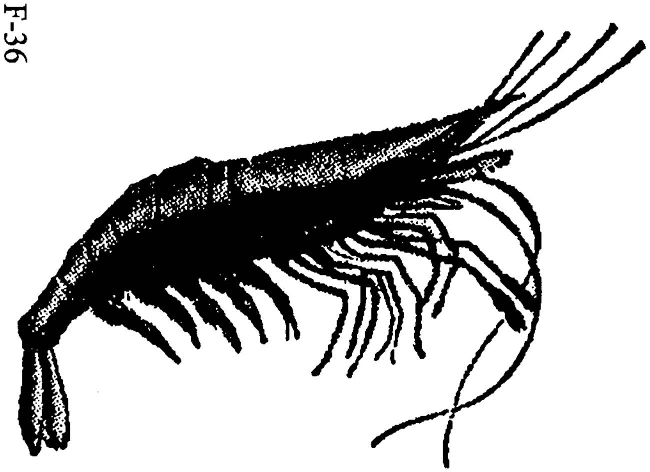
Shrimp immunity



- no immunological memory
- natural selection of disease-resistant individuals more likely
    - historical examples of host / pathogen co-adaptation exists
        - example: Central & South American attempts at inoculating shrimp populations resulted in increased host resistance
    - may / may not be changes in viral virulence

Multiple stressors
pathogens, pollution, salinity, temperature, biota



- it is not possible to separate effects of multiple stressors on shrimp populations
- first do lab studies / controlled experiments
- natural experiments of pops w/ & w/o virus (but all else equal...)
- look for correlations of shrimp pop changes w/ other environ change
- need comprehensive models

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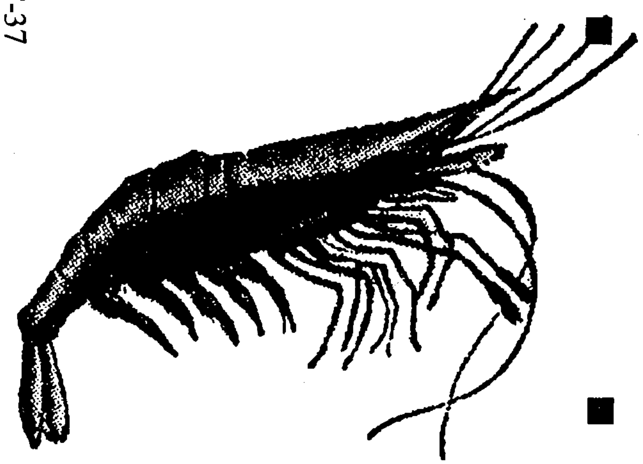
Human health effects



- unknown but presumed low probability
- baculovirues (e.g., WSBV) do not infect vertebrates
- other 3 groups have viruses that are pathogenic to vertebrates
  - ◆ only 1 (rhabdoviruses; YHV) have demonstrated zoonotic potential
- viruses that infect both vertebrates & inverts are in a different virus group (arboviruses)

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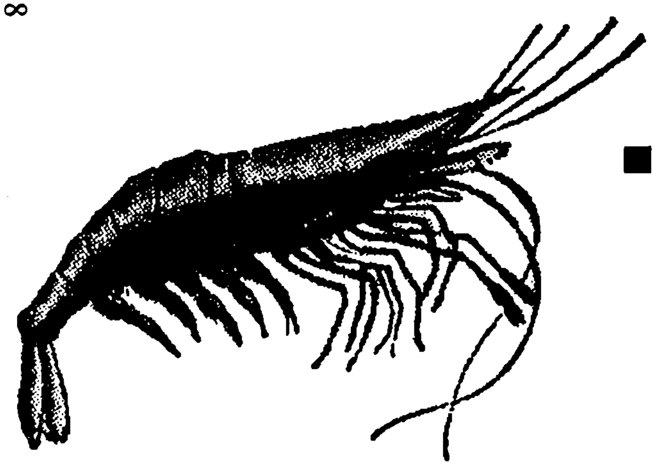
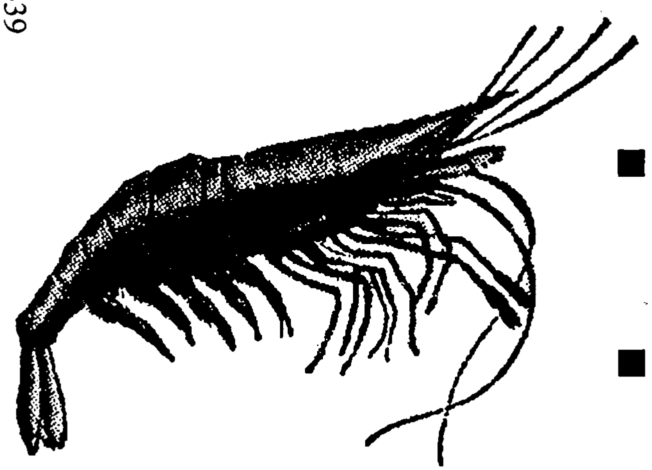
Shrimp virus identification



- some viruses have very reliable techniques (PCR, DNA probes, ELSIAs)
- others till rely on histopathology and electron microscopy

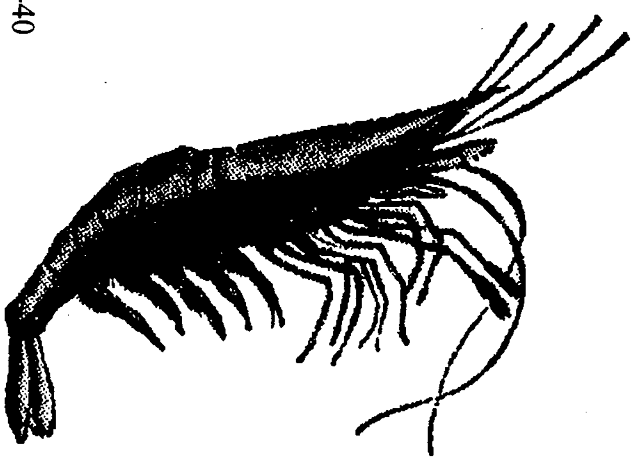
Stressor effects



- interpretation of evidence of prior virus introductions
- evaluation of lack of information on virus prevalence
- use of shrimp models to interpret effects of viruses
- importance of viral effects on non-shrimp species

Prior virus introductions [1]

- Decline of shrimp in Gulf of California
  - IHHNV was not *proven* to be *the* cause (others: pollution and low DO)
  - 25% pop fluctuation not unusual
  - naturally high mortality rate suggests that impact of virus-induced mortality would be minimal

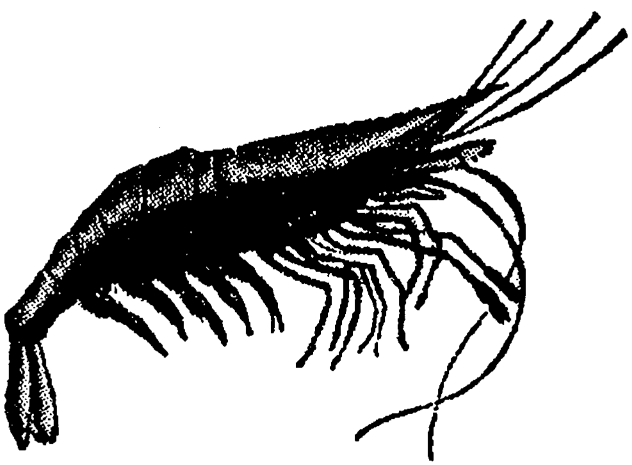


F-40

Prior virus introductions [2]

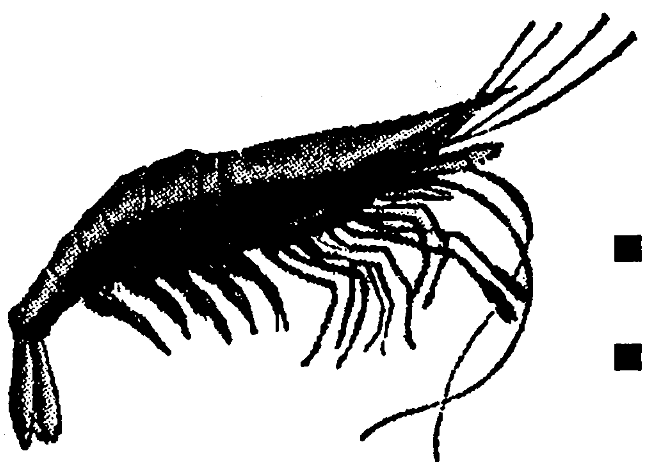
- TSV release from S. American aquaculture
  - unknown if TSV was endemic prior to aquaculture problems
  - other factors: loss of mangroves, antimicrobials, pathogenic bacteria, pollutants

F-41

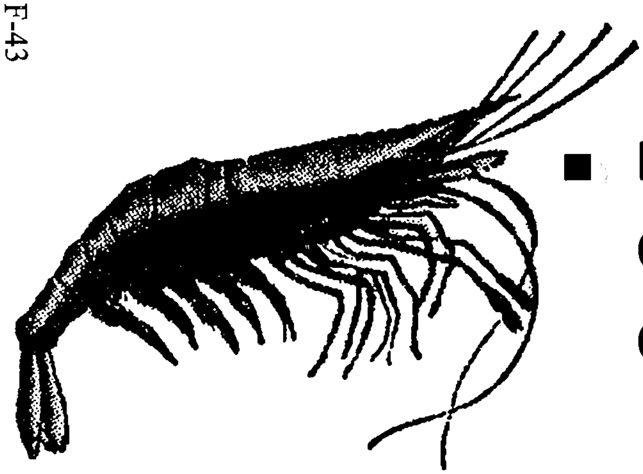


virus prevalence

- need this information for a proper risk assessment
- have some information on baculovirus prevalence, but not about effects
- need good diagnostic methods
- assume naïve population for qualitative estimate of risk of introductions



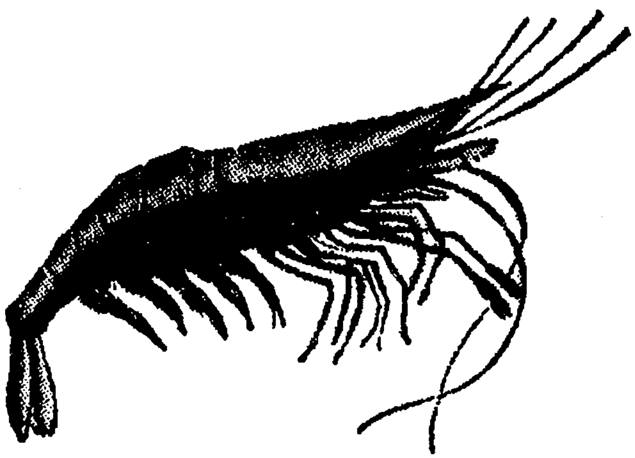
Shrimp models to predict effects [1]



- look for unexplained mass mortality or population declines -- then see if can detect virus pathogenically
  - need info on baseline prevalence
- need to know population controlling factors and what constitutes normal fluctuations
  - 25% change in pop size is normal
  - additional mortality from virus may not be detectable or important

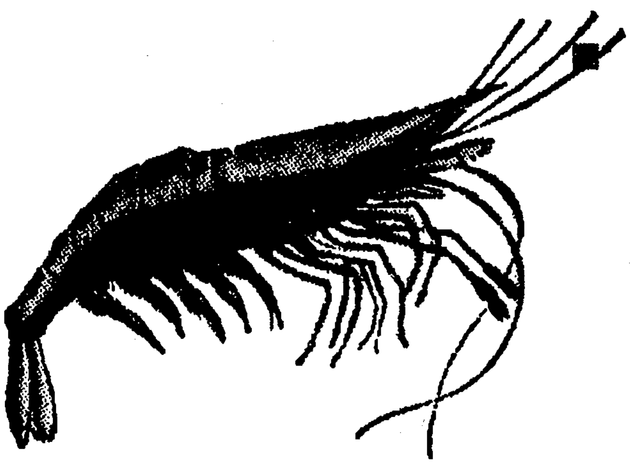
Shrimp models to predict effects [2]

- epidemiological models can provide the parameters of what would be needed for an outbreak to occur
  - genetic structure
  - population demographic factors
  - other stressors and effects
  - virus factors
    - transmission rates, stage-specific mortality, environmental persistence



Importance of viral effects on non-shrimp species

- IHHNV, TSV, and YHV only infect penaeid shrimp. WSSV kills freshwater crayfish, prawns, and other crustaceans
- indirect effects
  - kill what shrimp eat
  - reduce prey base for shrimp predators
  - act as vectors or transport hosts
- look in Asia to see if non-shrimp species carry shrimp viruses



January 7-8, 1998

APPENDIX G

REPORT TO THE AQUATIC NUISANCE SPECIES TASK FORCE: GENERIC NONINDIGENOUS AQUATIC ORGANISMS RISK ANALYSIS REVIEW PROCESS

REPORT TO THE AQUATIC NUISANCE SPECIES TASK FORCE

GENERIC NONINDIGENOUS AQUATIC ORGANISMS RISK ANALYSIS REVIEW PROCESS

(For Estimating Risk Associated with the Introduction of Nonindigenous Aquatic Organisms and how to Manage for that Risk)

Risk Assessment and Management Committee
Aquatic Nuisance Species Task Force
Aquatic Nuisance Prevention and Control Act of 1990

October 21, 1996

Current and Former Members of the Risk Assessment and Risk Management Committee

Walter Blogoslawski
NOAA, National Marine Fisheries Service
Former Member

Joseph McCraren
National Aquaculture Association
Former Member

Richard Guadiosi
U.S. Coast Guard
Former Member

Fred Kern
NOAA National Marine Fisheries Service
Current Member

Richard Orr
USDA, Animal and Plant Health Inspection Service
Current Member, RAM Chairperson

Edwin Theriot
U.S. Army Corps of Engineers
Current Member

Mike Troyer
U.S. Environmental Protection Agency
Former Member

James D. Williams
USGS Biological Resources Division
Current Member

Richard E. Bohn
National Aquaculture Association
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Sharon Gross
U.S. Fish and Wildlife Service
Former Member

Lauren Kabler
U.S. Coast Guard
Former Member

Marshall Meyers
Pet Industry Joint Advisory Council
Current Member

Richard Sayers, Jr.
U.S. Fish and Wildlife Service
Former Member

Jay Troxel
U.S. Fish and Wildlife Service
Current Member

Bill van der Schalie
U.S. Environmental Protection Agency
Current Member

CONTENTS

I. INTRODUCTION

Objective of the Review Process

The Risk Assessment and Management (RAM) Committee was initiated by, and is under the auspices of, the Aquatic Nuisance Species Task Force (Task Force). The Task Force was created for the purpose of developing a strategy in which the appropriate government agencies could meet the goals of the Aquatic Nuisance Prevention and Control Act of 1990. The Task Force was ".. established to coordinate governmental efforts related to nonindigenous aquatic species in the United States with those of the private sector and other North American interests" (ANSP, 1992). The Task Force is co-chaired by the U.S. Fish and Wildlife Service and the National Oceanic and Atmospheric Administration.

The Generic Nonindigenous Aquatic Organisms Risk Analysis Review Process (hereafter referred to as the Review Process) is the risk process developed through the RAM committee to help meet the requirements of the Aquatic Nuisance Prevention and Control Act.

The objective of the Review Process is to provide a standardized process for evaluating the risk of introducing nonindigenous organisms into a new environment and, if needed, determining the correct risk management steps needed to mitigate that risk.

The Review Process provides a framework where scientific, technical, and other relevant information can be organized into a format that is understandable and useful to managers and decision makers. The Review process was developed to function as an open process with early and continuous input from all identified interested parties.

The Review Process was designed to be flexible and dynamic enough to accommodate a variety of approaches to nonindigenous organism risk depending on the available resources, accessibility of the biological information, and the risk assessment methods available at the time of the assessment. The Review Process may be used as a purely subjective evaluation or be quantified to the extent possible or necessary depending on the needs of the analysis. Therefore, the process will accommodate a full range of methodologies from a simple and quick judgmental process to an analysis requiring extensive research and sophisticated technologies.

The specific function of the Review Process is to:

- RISK ASSESSMENT -- Develop a process that can be used to:

  a) evaluate recently established nonindigenous organisms

b) evaluate nonindigenous organisms proposed for deliberate introduction
c) evaluate the risk associated with individual pathways (i.e. ballast water, aquaculture, aquarium trade, fish stocking, etc.)

• RISK MANAGEMENT -- Develop a practical operational approach to maximize a balance between protection and the available resources for:

a) reducing the probability of unintentional introductions
b) reducing the risk associated with intentional introductions

The History and Development of the Review Process

The Review Process was modified from the Generic Non-Indigenous Pest Risk Assessment Process (Orr, et al, 1993) developed by the USDA's Animal and Plant Health Inspection Service (APHIS) for evaluating the introduction of nonindigenous plant pests. The APHIS process has been thoroughly tested both within and outside of the agency with numerous completed individual organism assessments and three high risk pathway studies.

The development of the Review Process has been synchronous and functionally tied to the development of various ecological risk assessment methodologies and nonindigenous organism issues. Foremost was the National Research Council's workshops and meetings for the development of the "Ecological Paradigm" (NRC, 1993). The Review Process's basic approach and philosophy borrows heavily from the NRC's project.

Other major projects and reports which have influenced the direction of the Review Process are: The Environmental Protection Agency's "Ecological Framework" (EPA, 1992a) and associated documents (EPA, 1992b, 1992c, 1994); the United States Congress Office of Technology Assessment's nonindigenous species report (OTA, 1993); and the Forest Service's pest risk assessments on nonindigenous timber pests (USDA, FS, 1991, 1992, 1993).

In addition to the above projects and numerous other pertinent work the following quality criteria (modified from Fischoff et al. 1981) were used in designing the Review Process:

• Comprehensive - The assessment should review the subject in detail and identify sources of uncertainty in data extrapolation and measurement errors. The assessment should evaluate the quality of its own conclusions. The assessment should be flexible to accommodate new information.

• Logically Sound - The risk assessment should be up-to-date and rational, reliable, justifiable, unbiased, and sensitive to different aspects of the problem.

• Practical - A risk assessment should be commensurate with the available resources.

- Conducive to Learning - The risk assessment should have a broad enough scope to have carry-over value for similar assessments. The risk assessment should serve as a model or template for future assessments.

- Open to Evaluation - The risk assessment should be recorded in sufficient detail and be transparent enough in its approach that it can be reviewed and challenged by qualified independent reviewers.

Risk Analysis Philosophy

The risk assessment process allows for analysis of factors for which the dimension, characteristics, and type of risk can be identified and estimated. By applying analytical methodologies, the process allows the assessors to utilize qualitative and quantitative data in a systematic and consistent fashion.

The ultimate goal of the process is to produce quality risk assessments on specific nonindigenous aquatic organisms or with nonindigenous organisms identified as being associated with specific pathways. The assessments should strive for theoretical accuracy while remaining comprehensible and manageable; and the scientific and other data should be collected, organized and recorded in a formal and systematic manner.

The assessment should be able to provide a reasonable estimation of the overall risk. All assessments should communicate effectively the relative amount of uncertainty involved and, if appropriate, provide recommendations for mitigation measures that reduce the risk.

Caution is required to ensure that the process clearly explains the uncertainties inherent in the process and to avoid design and implementation of a process that reflects a predetermined result. Quantitative risk assessments can provide valuable insight and understanding; however, such assessments can never capture all the variables. Quantitative and qualitative risk assessments should always be buffered with careful human judgment Goals that cannot be obtained from a risk assessment are:

1. A risk assessment cannot determine the acceptable risk level. What risk, or how much risk, is acceptable depends on how a person, or agency, perceives that risk. Risk levels are value judgments that are characterized by variables beyond the systematic evaluation of information.

2. It is not possible to determine precisely whether, when, or how a particular introduced organism will become established. It is equally impossible to determine what specific impact an introduced organism will have. The best that can be achieved is to estimate the likelihood that an organism may be

introduced and estimate its potential to do damage under favorable host/environmental conditions.

The ability of an introduced organism to become established involves a mixture of the characteristics of the organism and the environment in which it is being introduced. The level of complexity between the organism and the new environment is such that whether it fails or succeeds can be based on minute idiosyncrasies of the interaction between the organism and environment. These cannot be predicted in advance by general statements based only on the biology of the organism. In addition, even if extensive information exists on a nonindigenous organism, many scientists believe that the ecological dynamics are so turbulent and chaotic that future ecological events cannot be accurately predicted.

If all were certain, there would not be a need for risk assessment. Uncertainty, as it relates to the individual risk assessment, can be divided into three distinct types:

a) uncertainty of the process -- (methodology)
b) uncertainty of the assessor(s) -- (human error)
c) uncertainty about the organism -- (biological and environmental unknowns)

Each one of these presents its own set of problems. All three types of uncertainty will continue to exist regardless of future developments. The goal is to succeed in reducing the uncertainty in each of these groups as much as possible.

The "uncertainty of the process" requires that the risk methodologies involved with the Review Process never become static or routine but continue to be modified when procedural errors are detected and/or new risk methodologies are developed.

"Uncertainty of the assessor(s)" is best handled by having the most qualified and conscientious persons available conduct the assessments. The quality of the risk analysis will, to some extent, always reflect the quality of the individual assessor(s).

The "uncertainty about the organism" is the most difficult to respond to. Indeed, it is the biological uncertainty more than anything else that initiated the need for developing a nonindigenous risk process. Common sense dictates that the caliber of a risk assessment is related to the quality of data available about the organism and the ecosystem that will be invaded. Those organisms for which copious amounts of high quality research have been conducted are the most easily assessed. Conversely, an organism for which very little is known cannot be easily assessed.

A high degree of biological uncertainty, in itself, does not demonstrate a significant degree of risk. However, those organisms which demonstrate a high degree of biological uncertainty do represent a real risk. The risk of importing a damaging nonindigenous organism (for which little information is known) is probably small for any single organism but the risk becomes much higher when one considers the vast number of these organisms that must be considered. It is not possible to identify which of the "unknowns" will create problems -- only assume that some will. Demonstrating that a pathway has a "heavy" concentration of nonindigenous organisms for which little information is present may, in some cases (based on the "type" of pathway and the "type" of organisms), warrant concern. However, great care should be taken by the assessor(s) to explain why a particular nonindigenous organism load poses a significant risk.

This need to balance "demonstrated risks" against "biological uncertainty" can lead assessors to concentrate more on the uncertainty than on known facts. To prohibit or restrict a pathway or specific nonindigenous organism, the reasons or logic should be clearly described.

Risk assessments should concentrate on demonstrated risk. Applying mitigating measures based on well documented individual nonindigenous pests will frequently result in a degree of mitigation against other organisms demonstrating high biological uncertainty that might be using the same pathway.

If we accept that "it is not possible to determine whether a particular introduced organism will become established", and "it is equally impossible to determine what specific impact an introduced organism will have", then we might be asked, "what value is there in doing risk assessments, which consist of assessing the probability of establishment and the consequence of establishment?". The risk assessment process is an effective tool for estimating potential in a systematic fashion.

Some of the information used in performing a risk assessment is scientifically defensible, some of it is anecdotal or based on experience, and all of it is subject to the filter of perception. However, we must provide an estimation based on the best information available and use that estimation in deciding whether to allow the proposed activity involving the nonindigenous organism and, if so, under what conditions.

The assessment should evaluate risk in order to determine management action. Estimations of risk are used in order to restrict or prohibit high risk pathways, with the goal of preventing the introduction of nonindigenous pests.

When conducting risk assessments for government agencies, the most serious obstacles to overcome are the forces of historical precedent and the limitations presented by legal parameters, operational procedures, and political pressure. In order to focus the assessment as much as possible on the biological factors of risk, all assessments need to be completed in an atmosphere as free of regulatory and political influences as possible.

The following quote is taken from the NRC's 1983 Red Book on "Risk Assessment in the Federal Government: Managing the Process":

> "We recommend that regulatory agencies take steps to establish and maintain a clear conceptual distinction between assessment of risks and consideration of risk management alternatives; that is, the scientific findings and policy judgments embodied in risk assessments should be explicitly distinguished from the political, economic, and technical considerations that influence the design and choice of regulatory strategies".

This can be translated to mean that risk assessments should not be *policy-driven*. However, the Red Book then proceeded with a caveat:

> "The importance of distinguishing between risk assessment and risk management does not imply that they should be isolated from each other; in practice they interact, and communication in both directions is desirable and should not be disrupted".

This can be translated to mean that the risk assessment, even though it must not be policy-driven, must be *policy-relevant*. These truths continue to be valid (NRC, 1993).

II. THE REVIEW PROCESS FOR CONDUCTING PATHWAY ANALYSES AND ORGANISM RISK ASSESSMENTS

The need for a risk assessment starts either with the request for opening a new pathway which might harbor nonindigenous aquatic organisms or the identification of an existing pathway which may be of significant risk. All pathways showing a potential for nonindigenous organism introduction should receive some degree of risk screening. Those pathways that show a high potential for introducing nonindigenous organisms should trigger an in-depth risk assessment.

The following details of the Review Process focus on evaluating the risk of nonindigenous organisms associated with an identified pathway. Figure 1, on page 8, outlines the flow of a pathway analysis, dividing the process into initiation, risk assessment, and risk management. Specific organisms needing evaluation which are not tied to a pathway assessment would proceed directly to the "*Organism Risk Assessments*" box in Figure 1 (page 8) and the "*Organism Risk Assessments*" section starting on page 10.

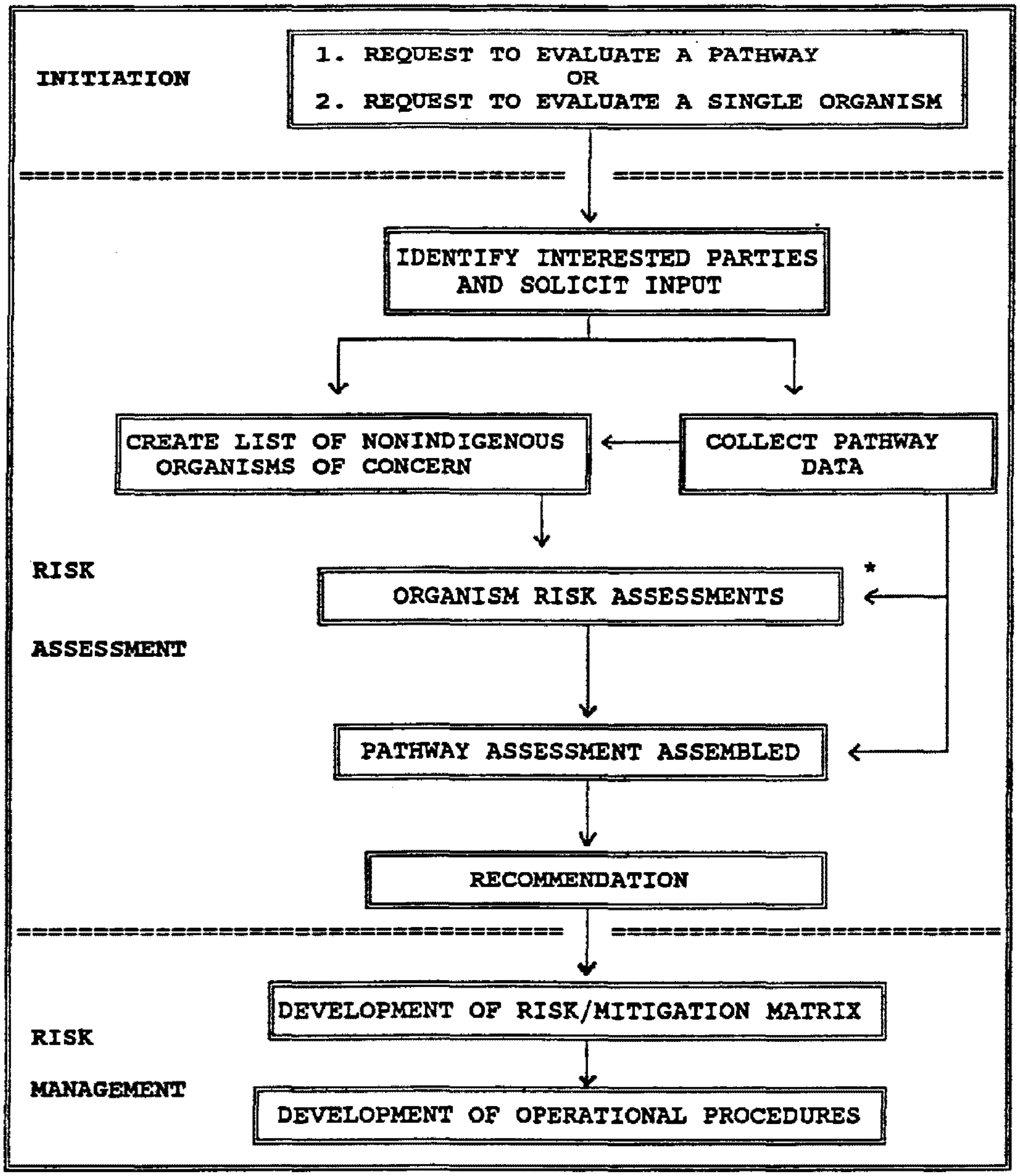
Collecting Pathway Data

Specific information about the pathway must be collected. This information, coupled with additional data (if necessary), would fulfill the "Collect Pathway Data" element in Figure 1, page 8.

Specific information needed about the pathway will vary with the "type" of pathway (i.e. ballast water, aquaculture, aquarium trade, fish stocking, etc.). The following generalized list of information has been useful in other nonindigenous risk assessments.

1) Determine exact origin(s) of organisms associated with the pathway.
2) Determine the numbers of organisms traveling within the pathway.
3) Determine intended use or disposition of pathway.
4) Determine mechanism and history of pathway.
5) Review history of past experiences and previous risk assessments (including foreign countries) on pathway or related pathways.
6) Review past and present mitigating actions related to the pathway.

FIGURE 1. Pathway Analysis: Flow Chart showing the Initiation, Risk Assessment and Risk Management for a pathway.



* = For details on the Organism Risk Assessment see Figure 2 "Risk Assessment Model" page 11. Pathways that show a high potential for introducing nonindigenous aquatic organisms should trigger detailed risk analyses.

Creating a List of Nonindigenous Aquatic Organisms of Concern

The next element in figure 1 (page 8) is "Create List of Nonindigenous Organisms of Concern". The following generalized process is recommended .

STEP:1) Determine what organisms are associated with the pathway.

2) Determine which of these organisms qualify for further evaluation using the table below.

Category	Organism Characteristics	Concern
1a	species nonindigenous not present in country (United States)	yes
1b	species nonindigenous, in country and capable of further expansion	yes
1c	species nonindigenous, in country and reached probable limits of range, but genetically different enough to warrant concern and/or able to harbor another nonindigenous pest	yes
1d	species nonindigenous, in country and reached probable limits of range and not exhibiting any of the other characteristics of 1c	no
2a	species indigenous, but genetically different enough to warrant concern and/or able to harbor another non-indigenous pest, and/or capable of further expansion	yes
2b	species indigenous and not exhibiting any of the characteristics of 2a	no

3) Produce a list of the organisms of concern from (step 2) categories 1a, 1b, 1c, and 2a. Taxonomic confusion or uncertainty should also be noted on the list.

4) Conduct Organism Risk Assessments from the list of organisms developed in step 3.

Based on the number of organisms identified and the available resources, it may be necessary to focus on fewer organisms than those identified using the above table. When this is necessary it is desirable that the organisms chosen for complete risk assessments be representative of all the organisms identified. A standard methodology is not available because the risk assessment process is often site or species specific. Therefore, professional judgement by scientists familiar with the aquatic organisms of concern is often the best tool to determine which organisms are necessary for effective screening.

This screening has been done using alternative approaches. Different approaches can be found in each of the three log commodity risk assessments (USDA, Forest Service, 1991, 1992, 1993).

Organism Risk Assessments

The Organism Risk Assessment element in figure 1 (page 8) is the most important component of the Review Process used in evaluating and determining the risk associated with a pathway. The Organism Risk Assessment can be independent of a pathway assessment if a particular nonindigenous organism needs to be evaluated. Figure 2, on page 11, represents the Risk Model which drives the Organism Risk Assessment.

The Risk Assessment Model is divided into two major components the "probability of establishment" and the "consequence of establishment". This division reflects how one can evaluate an nonindigenous organism (e.g. more restrictive measures are used to lower the probability of a particular nonindigenous organism establishing when the consequences of its establishment are greater).

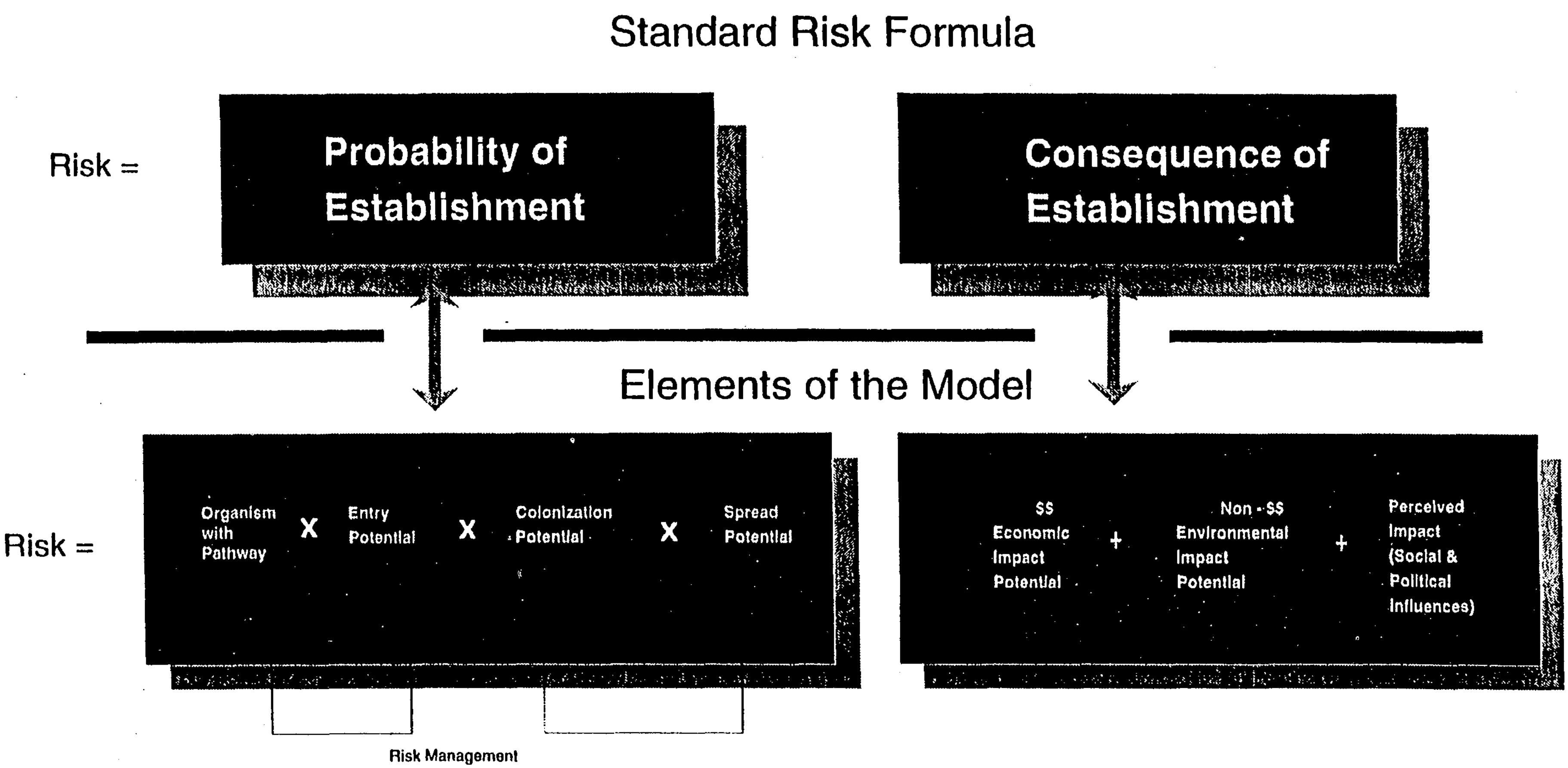
The Risk Assessment Model is a working model that represents a simplified version of the real world. In reality the specific elements of the Risk Model are not static or constant, but are truly dynamic showing distinct temporal and spatial relationships. Additionally, the elements are not equal in weighing the risk nor are they necessarily independent. The weight of the various elements will never be static because they are strongly dependent upon the nonindigenous organism and its environment at the time of introduction.

The two major components of the Risk Assessment Model are further divided into 7 basic elements which serve to focus scientific, technical, and other relevant information into the assessment. Each of these 7 basic elements are represented on the Risk Assessment Form (Appendix A, page 22) as probability or impact estimates. These may be determined using quantitative or subjective methods. See Appendix B (page 25) for a minimal subjective approach.

The strength of the assessment is that the information gathered by the assessor(s) can be organized under the seven elements. The cumulative information under each element provides the data to assess the risk for that element. Whether the methodology used in making the risk judgement for that element is quantitative, qualitative, or a combination of both; the information associated with the element (along with its references) will function as the information source. Placing the information in order of descending risk under each element will further communicate to

FIGURE 2

Risk Assessment Model



- For model simplification the various elements are depicted as being independent of one another
- The order of the elements in the model does not necessarily reflect the order of calculation

reviewers the thought process of the assessor(s).

Adequate documentation of the information sources makes the Review Process transparent to reviewers and helps to identify information gaps. This transparency facilitates discussion if scientific or technical disagreement on an element-rating occurs. For example, if a reviewer disagrees with the rating that the assessor assigns an element the reviewer can point to the information used in determining that specific element-rating and show what information is missing, misleading, or in need of further explanation. Focusing on information to resolve disagreements will often reduce the danger of emotion or a preconceived outcome from diluting the quality of the element-rating by either the assessors or the reviewers.

The characteristics and explanations of the seven elements of the Risk Assessment Model are as follows:

A. Elements -- Group 1: Assess Probability of Organism Establishment

When evaluating an organism not associated with a pathway, or an organism recently introduced, the first 2 elements under Group 1 would automatically be rated as high because entry into the new environment is either assumed or has already occurred.

1. <u>Nonindigenous Aquatic Organisms Associated with Pathway (At Origin)</u> -- Estimate probability of the organism being on, with, or in the pathway.

The major characteristic of this element is: Does the organism show a convincing temporal and spatial association with the pathway.

2. <u>Entry Potential</u> -- Estimate probability of the organism surviving in transit.

Some of the characteristics of this element include: the organism's hitchhiking ability in commerce, ability to survive during transit, stage of life cycle during transit, number of individuals expected to be associated with the pathway; or whether it is deliberately introduced (e.g. biocontrol agent or fish stocking).

3. <u>Colonization Potential</u> -- Estimate probability of the organism colonizing and maintaining a population.

Some of the characteristics of this element include: the organism coming in contact with an adequate food resource, encountering appreciable abiotic and biotic environmental resistance, and the ability to reproduce in the new environment.

4. Spread Potential -- Estimate probability of the organism spreading beyond the colonized area.

Some of the characteristics of this element include: ability for natural dispersal, ability to use human activity for dispersal, ability to readily develop races or strains, and the estimated range of probable spread.

B. Elements -- Group II: Assess Consequence of Establishment

5. Economic Impact Potential -- Estimate economic impact if established.

Some of the characteristics of this element include: economic importance of hosts, damage to crop or natural resources, effects to subsidiary industries, exports, and control costs.

6. Environmental Impact Potential -- Estimate environmental impact if established.

Some of the characteristics of this element include: ecosystem destabilization, reduction in biodiversity, reduction or elimination of keystone species, reduction or elimination of endangered/threatened species, and effects of control measures. If appropriate, impacts on the human environment (e.g. human parasites or pathogens) would also be captured under this element.

7. Perceived Impact (Social & Political Influences) -- Estimate impact from social and/or political influences.

Some of the characteristics of this element include: aesthetic damage, consumer concerns, and political repercussions.

Often the assessor feels uncomfortable dealing with the categories of Economic and Perceived Impact. However, information found by an assessor relating to these categories maybe helpful in making risk management decisions. The assessor should not be expected to reflect, or second guess, what an economist or politician would conclude but rather to present information gathered on the organism that would (or could) have an affect in these areas.

The elements considered under Consequences can also be used to record positive impacts that a nonindigenous organism might have for example its importance as a biocontrol agent, aquatic pet, sport fish, scientific research organism, or based on its use in aquaculture. The elements in the case of deliberate introductions would record information that will be useful in determining the element-rating that would be a balance between the cost, the benefit, and the risk of introducing the nonindigenous organism.

The Risk Assessment Form (Appendix A, page 22) should be flexible. Each nonindigenous organism is unique. The assessor needs to have the freedom to modify the form to best represent the risk associated with that particular organism. The seven elements need to be retained to calculate the risk but other sections may be added or subtracted. If the assessor feels that information, ideas, or recommendations would be useful, they should be included in the assessment. The assessor can combine "like" organisms into a single assessment if their biology is similar (e.g. tropical aquarium fish destined to temperate North America).

The number of risk assessments to be completed from the list of nonindigenous organisms in a particular pathway depends on several factors. These include the amount of individual organism information, available resources, and the assessor's judgement concerning whether the completed assessments effectively represent the pathways' nonindigenous organism risk.

The source of the statements and the degree of uncertainty the assessor associated with each element needs to be recorded in the Risk Assessment. The use of the Reference Codes at the end of each statement, coupled with the use of the Uncertainty Codes for each element, fulfill these requirements. Both the Reference Codes and the Uncertainty Codes are described in Appendix A on page 24.

If a federal agency uses the Review Process for potential environmental problems, much of the information may contribute to meeting that agency's National Environmental Policy Act(NEPA)requirements. When both NEPA documentation and a risk assessment are warranted, the two should be coordinated so that resources are not duplicated. Although a risk assessment is similar to an Environmental Impact Statement (EIS) the risk assessment differs by focusing on the probability of occurrence and the impact of that occurrence, while an EIS generally places its emphasis on who or what will be impacted. Therefore, a risk assessment is more likely to clarify possible outcomes, determine or estimate their probabilities of occurrence, and succeed in recording the degree of uncertainty involved in making the predictions.

Summarizing Organism and Pathway Risk

An estimate of risk is made at three levels in the Review Process. The first, places a risk estimate on each of the seven elements within the Risk Assessment (element-rating). The second, combines the seven risk element estimates into a Organism Risk Potential (ORP) which represents the overall risk of the organism being assessed. The third, links the various ORPs into a Pathway Risk Potential (PRP) which will represent the combined risk associated with the pathway.

The assigning of either a quantitative or a qualitative estimate to an individual element, and determining how the specific elements in the Model are related, and how the estimates should be combined are the most difficult steps in a risk assessment. There is not a "correct" formula for completing these steps. Various methodologies such as geographical information systems, climate and ecological models, decision-making software, expert systems, and graphical displays of uncertainty may potentially increase the precision of one or more elements in the Risk Assessment Model. Indeed, risk assessments should never become so static and routine that new methodologies can not be tested and incorporated.

When evaluating new technologies and approaches it is important to keep in mind that the elements of the Risk Assessment Model are dynamic, chaotic, and not equal in value. New technologies or approaches which may be appropriate for assessing one organism may be immaterial or even misleading in evaluating another organism.

The high, medium, and low approach presented in Appendix B page 25 for calculating and combining the various elements is judgmental. The process in Appendix B is a generic minimum for determining and combining the element estimates and not necessarily "the best way it can be done".

The strength of the Review Process is that the biological statements under each of the elements provide the raw material for testing various approaches. Therefore, the risk assessments will not need to be re-done to test new methods for calculating or summarizing the ORP and PRP.

On risk issues of high visibility, examination of the draft assessment should be completed by pertinent reviewers not associated with the outcome of the assessment. This is particularly appropriate when the risk assessments are produced by the same agency, professional society, or organization that is responsible for the management of that risk.

ELEMENTS OF RISK MANAGEMENT AND OPERATIONAL REQUIREMENTS

The previous sections dealt with assessing the level of risk associated with a particular pathway or organism. Once the risk assessment is completed, it is the responsibility of risk managers to determine appropriate policy and operational measures.

A. Elements To Consider In Risk Management Policy:

- Risk assessments (including uncertainty and quality of data)
- Available mitigation safeguards (i.e., permits, industry standards, prohibition,inspection)
- Resource limitations (i.e., money, time, locating qualified experts, needed information)
- Public perceptions/perceived damage
- Social and political consequences
- Benefits and costs should be addressed in the analysis

B. The following four risk management operational steps should be accomplished:

- Step 1: Maintain communication and input from interested parties;
- Step 2: Maintain open communication between risk managers and risk assessors;
- Step 3: Match the available mitigation options with the identified risks;
- Step 4: Develop an achievable operational approach that balances resource protection and utilization.

STEP 1: Participation of interested parties should be actively solicited as early as possible. All interested parties should be carefully identified because adding additional interested parties late in the assessment or management process can result in revisiting issues already examined and thought to have been brought to closure. All identified interested parties should be periodically brought up-to-date on relevant issues.

STEP 2: Continuous open communication between the risk managers and the risk assessors is important throughout the writing of the risk assessment. This is necessary to ensure that the assessment will be policy relevant when completed. Risk Managers should be able to provide detailed questions about the issues that they will need to address to the risk assessors before the risk assessment is started. This will allow the assessors to focus the scientific information relevant to the questions (issues) that the risk managers will need to address.

As important as open communication is between risk managers and risk accessors, it is equally important that risk managers do not attempt to drive, or influence, the outcome of the assessment. Risk assessments need to be policy-relevant not policy-driven.

STEP 3: Matching the available mitigation options with the identified risks can sometimes be done by creating a mitigation matrix placing the organisms, or groups of organisms, identified in a specific pathway along one axis and the available mitigation options along the other. Where a specific organism, or group of organisms, meets a specific mitigation process in the matrix, the efficacy for control is recorded. Using this process it becomes apparent which mitigation or mitigations are needed to reduce the risk to an acceptable level. The mitigation matrix (page 18) was used in the mitigation report on New Zealand log imports (USDA, APHIS, 1992) which addresses the nonindigenous organisms identified in the New Zealand log risk assessment (USDA, FS, 1992).

STEP 4: Developing a realistic operational approach is not easy. Each new operational decision must consider a number of management, agency, and biological factors that will always be unique to any specific organism or pathway. However, at an operational risk management level each step in the operational pyramid (page 19) is a process that needs to be examined before approval of the importation, or release, or action against, a nonindigenous organism or pathway is taken. These include the risk assessment, the development of conditions for entry to meet current industry or regulatory standards, effective mitigation of any identified potential nonindigenous aquatic organisms, feasibility of achieving the mitigation requirements, and finally, a system of monitoring to ensure that all mitigation requirements are maintained.

MITIGATION MATRIX

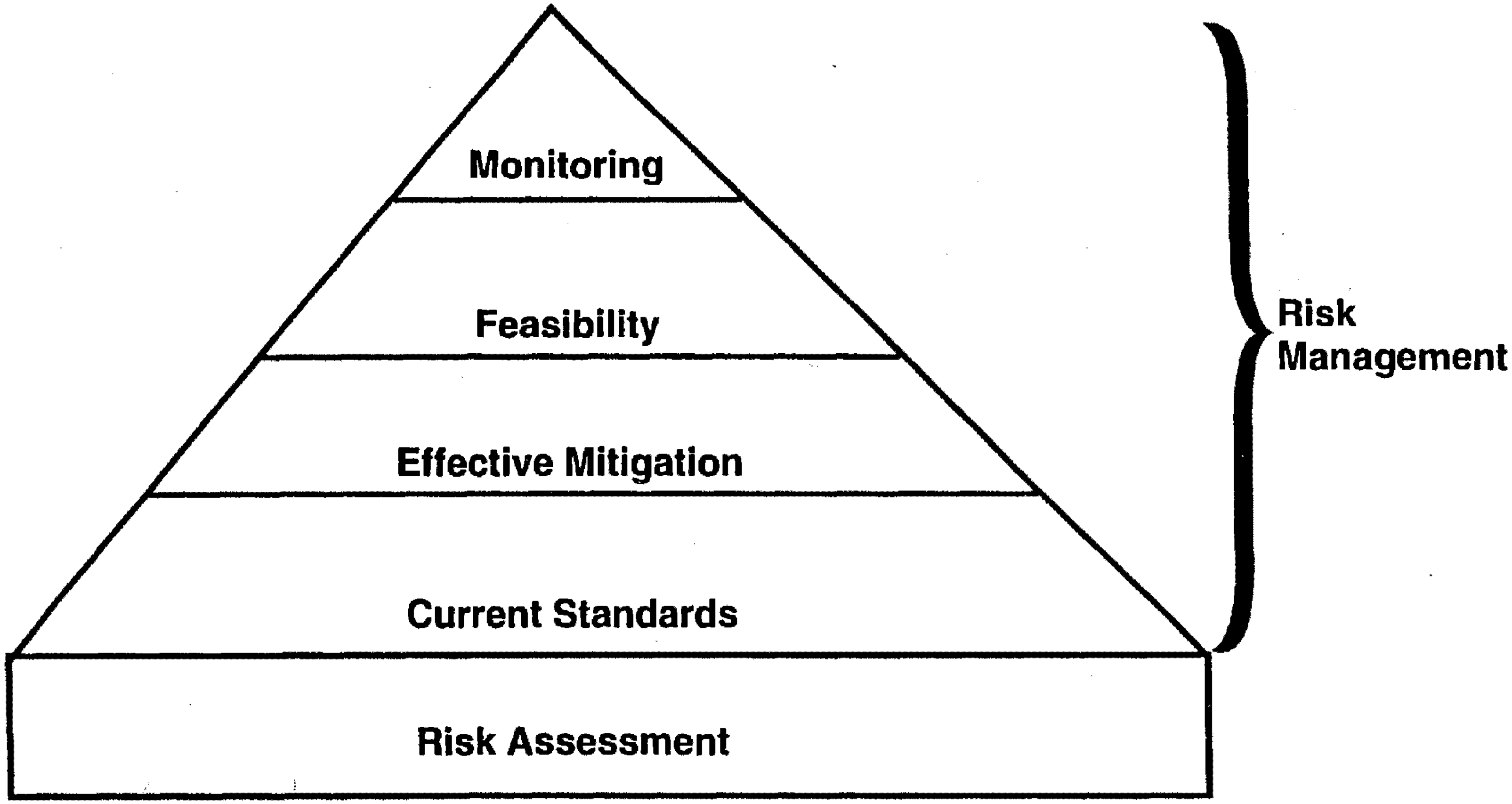
Pinus radiata logs from New Zealand
(Pathogens & Plant Feeding Insects vs. Mitigation)

Mitigation Procedures in **NEW ZEALAND**					In **USA**	
ORGANISM	30 DAY LIMIT	SAWLOG QUALITY ONLY	DE-BARKING	MB FUMI-GATION	AGENCY ENTRY REQ.	HEAT PROCESS SAWMILL
Bark Beetles	S	S	E	T	S	T
Platypus spp.	S	S	S	T	S	T
Sirex/ Fungus	S	E	S	E	S	T
Lepto-graphium	S	E	S	E	S	T
Kaloterm es	S	E	S	T	S	T
Huhu beetles	S	E	S	E	S	T
Hitch hikers	S	S	E	T	S	T
Unknown Pests	S	S	S	E	S	T

Key:

(S)ome reduction of pest risk expected (less than 95%)
(E)xtensive reduction (95 percent or more) of pest risk expected
(T)otal (100 percent or nearly 100 percent) reduction of pest risk expected

Operational Pyramid (Risk Management)



Components of the Final Analysis

A completed Risk Analysis may contain the following:

- Tracking/Information Form or Section

This documents the analysis process and records information about why the assessment was done, who the assessment was done for, and information which might not be found in the assessment itself but could be useful background information for future reviewers. It also would contain information that would be helpful in determining (at a later date) the depth of the review, which resources were used and which methodologies were tried but not used in the final assessment. The main function of this form or section would be to provide additional transparency to the analysis and to provide a historical record for future reviewers.

- Pathway information form or section
- A complete list of the organisms of concern
- The individual Organism Risk Assessments
- Response to specific questions requested by risk managers
- Summation of the methodology used in determining the ORPs and PRPs
- Mitigation/risk matrix
- Detailed discussion associated with each level of the operational pyramid
- Summation and responses to outside reviewers

III. REFERENCES

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APPENDIX A:

ORGANISM RISK ASSESSMENT FORM
(With Uncertainty and Reference Codes)

ORGANISM ______________________	**FILE NO.** _______
ANALYST ______________________	**DATE** ___________
PATHWAY ______________________	**ORIGIN** _________

I. **LITERATURE REVIEW AND BACKGROUND INFORMATION**
(Summary of life cylce, distribution, and natural history):

II. **PATHWAY INFORMATION** (include references):

III. **RATING ELEMENTS:** Rate statements as low, medium, or high.Place specific biological information in descending order of risk with reference(s) under each element that relates to your estimation of probability or impact. Use the reference codes at the end of the biological statement where appropriate and the Uncertainty Codes after each element rating.

PROBABILITY OF ESTABLISHMENT

Element Rating (L,M,H)	Uncertainty Code (VC - VU)	
______ ,	______	Estimate probability of the nonindigenous organism being on, with, or in the pathway. (Supporting Data with reference codes)
______ ,	______	Estimate probability of the organism surviving in transit. (Supporting Data with reference codes)
______ ,	______	Estimate probability of the organism successfully colonizing and maintaining a population where introduced. (Supporting Data with reference codes)
______ ,	______	Estimate probability of the organism to spread beyond the colonized area. (Supporting Data with reference codes)

CONSEQUENCE OF ESTABLISHMENT

Element Rating (L,M,H)	Uncertainty Code (VC - VU)	
______ ,	______	Estimate economic impact if established. (Supporting Data with reference codes)
______ ,	______	Estimate environmental impact if established. (Supporting Data with reference codes)
______ ,	______	Estimate impact from social and/or political influences. (Supporting Data with reference codes)

IV. ORGANISM/PATHWAY RISK POTENTIAL: (ORP/PRP) __________

Probability of Establishment ¦ Consequence of Establishment = ORP/PRP RISK

V. SPECIFIC MANAGEMENT QUESTIONS:

VI. RECOMMENDATIONS:

VII. MAJOR REFERENCES:

REFERENCE CODES TO ANSWERED QUESTIONS

Reference Code	Reference Type
(G)	General Knowledge, no specific source
(J)	Judgmental Evaluation
(E)	Extrapolation; information specific to pest not available; however information available on similar organisms applied
(Author, Year)	Literature Cited

UNCERTAINTY CODES TO INDIVIDUAL ELEMENTS

Uncertainty Code	Symbol	Description
Very Certain	VC	As certain as I am going to get
Reasonably Certain	RC	Reasonably certain
Moderately Certain	MC	More certain than not
Reasonably Uncertain	RU	Reasonably uncertain
Very Uncertain	VU	A guess

APPENDIX B: JUDGMENTAL CALCULATION OF ORGANISM RISK AND PATHWAY RISK

Step 1. Calculating the elements in the Risk Assessment

The blank spaces located next to the individual elements of the risk assessment form (Appendix A) can be rated using high, medium, or low. The detailed biological statements under each element will drive the judgmental process. Choosing a high, medium, or low rating, while subjective, forces the assessor to use the biological statements as the basis for his/her decision. Thus, the process remains transparent for peer review.

The high, medium, and low ratings of the individual elements cannot be defined or measured -- they have to remain judgmental. This is because the value of the elements contained under "probability of establishment" are not independent of the rating of the "consequences of establishment". It is important to understand that the strength of the Review Process is not in the element-rating but in the detailed biological and other relevant information statements that motivates them.

Step 2. Calculating the Organism Risk Potential

The Organism Risk Potential and the Pathway Risk Potential ratings of high, medium, and low should be defined (unlike the element rating in step 1 which have to remain undefined). An example is provided of these definitions at the end of Appendix B page 29.

The following 3 steps must be completed in order to calculate the Organism Risk Potential.

Step 2a. Determine Probability of Establishment

Probability of Establishment	=	[Organism with Pathway]	[Entry Potential]	[Colonization Potential]	[Spread Potential]

****

The probability of establishment is assigned the value of the element with the lowest risk rating (example: a high, low, medium, and medium estimate for the above elements would result in a low rating).

Because each of the elements must occur for the organism to become established, a conservative estimate of probability of establishment is justified. In reality (assuming the individual elements are independent of each other) when combining a series of probabilities (such as medium - medium - medium) the probability will become much lower than the individual element ratings. However, the degree of biological uncertainty within the various elements is so high that a conservative approach is justified.

Step 2b. Determine Consequence of Establishment

Consequence of Establishment = $\boxed{\text{Economic}}$ $\boxed{\text{Environmental}}$ $\boxed{\text{Perceived}}$

Consequence of Establishment =

Economic	Environmental	Perceived	
H	L,M,H	L,M,H	= H
L,M,H	H	L,M,H	= H
M	M	L,M,H	= M
M	L	L,M,H	= M
L	M	L,M,H	= M
L	L	M,H	= M
L	L	L	= L

Note that the three elements that make up the Consequence of Establishment are not treated as equal. The Consequence of Establishment receives the highest rating given either the Economic or Environmental element. The Perceived element does not provide input except when Economic and Environmental ratings are low (see next to the last column on the above table).

Step 2c. Determine Organism Risk Potential (ORP)

ORP RISK = [PROBABILITY OF ESTABLISHMENT] [CONSEQUENCE OF ESTABLISHMENT]

ORP RISK =	Probability of Establishment	Consequence of Establishment	ORP Risk
	High	High	= High
	Medium	High	= High
	Low	High	= Medium
	High	Medium	= High
	Medium	Medium	= Medium
	Low	Medium	= Medium
	High	Low	= Medium
	Medium	Low	= Medium
	Low	Low	= Low

Here the conservative approach is to err on the side of protection. When a borderline case is encountered (lines 2, 4, 6, 8 on the above chart) the higher rating is accepted. This approach is necessary to help counteract the high degree of uncertainty usually associated with biological situations.

Step 3. Determine the Pathway Risk Potential (PRP)

ORP		PRP
Rating	Number	Rating
High	1 or more	High
Medium	5 or more	High
Medium	>0 but <5	Medium
Low	All	Low

The PRP reflects the highest ranking ORP. The only exception is when the number of medium risk organisms reaches a level at which the total risk of the pathway becomes high. The number, 5 or more, used in the above table is arbitrary.

Definition of Ratings used for Organism Risk Potential and Pathway Risk Potential:

Low = acceptable risk - organism(s) of little concern (does not justify mitigation)

Medium = unacceptable risk - organism(s) of moderate concern (mitigation is justified)

High = unacceptable risk - organism(s) of major concern (mitigation is justified)

When assessing an individual organism, a determination that the ORP is medium or high often becomes irrelevant because both ratings justify mitigation. When evaluating a pathway, the potential "gray area" between a PRP of medium and high may not be a concern for the same reason.

APPENDIX C: DEFINITIONS (Aquatic Nuisance Species Act definitions in bold type)

AQUATIC NUISANCE SPECIES - A nonindigenous species that threatens the diversity or abundance of native species or the ecological stability of infested waters, or commercial, agricultural, aquacultural or recreational activities dependent on such waters. Aquatic nuisance species include nonindigenous species that may occur in inland, estuarine and marine waters and that presently or potentially threaten ecological processes and natural resources. In addition to adversely affecting activities dependent on waters of the United States, aquatic nuisance species adversely affect individuals, including health effects.

AQUATIC SPECIES - All animals and plants as well as pathogens or parasites of aquatic animals and plants totally dependent on aquatic ecosystems for at least a portion of their life cycle. Bacteria, viruses, parasites and other pathogens of humans are excluded.

BALLAST WATER - Any water and associated sediments used to manipulate the trim and stability of a vessel.

CONTROL - Activities to eliminate or reduce the effects of aquatic nuisance species, including efforts to eradicate infestations, reduce populations of aquatic nuisance species, develop means to adapt human activities and facilities to accommodate infestations, and prevent the spread of aquatic nuisance species from infested areas. Control may involve activities to protect native species likely to be adversely affected by aquatic nuisance species. Preventing the spread of aquatic nuisance species is addressed in the Prevention Element of the proposed Program; all other control activities are included in the Control Element.

ECONOMIC IMPACT POTENTIAL - The expected net change in society's net welfare which is the sum of the producers' and consumers' surpluses arising from changes in yield and cost of production caused by the pest.

ECOSYSTEMS - In the broadest sense, these are natural or "wild" environments as well as human environments, including infrastructure elements. An ecosystem may be an animal or plant in the case where the species involved is a pathogen or parasite.

ENTRY POTENTIAL - The relative ability of an organism to penetrate the borders of a given area within a time interval.

ENVIRONMENTALLY SOUND - Methods, efforts, actions or programs to prevent introductions or control infestations of aquatic nuisance species that minimize adverse impacts to the structure

and function of an ecosystem and adverse effects on non-target organisms and ecosystems and emphasize integrated pest management techniques and nonchemical measures.

ESTABLISHED - When used in reference to a species, this term means occurring as a reproducing, self-sustaining population in an open ecosystem, i.e., in waters where the organisms are able to migrate or be transported to other waters.

EXCLUSIVE ECONOMIC ZONE - The Exclusive Economic Zone of the United States established by Proclamation Number 5030 of March 10, 1983, and the equivalent zone of Canada.

INDIGENOUS - The condition of a species being within its natural range or natural zone of potential dispersal; excludes species descended from domesticated ancestors (OTA, 1993).

INTENTIONAL INTRODUCTIONS - The knowing import or introduction of nonindigenous species into, or transport through, an area or ecosystem where it was not previously established. Even when there is no intent to introduce an aquatic organism into an ecosystem, escapement, accidental release, improper disposal (e.g., "aquarium dumps") or similar releases are the virtual inevitable consequence of an intentional introduction, not an unintentional introduction.

Synonyms: Purposeful, Deliberate.

INTEGRATED PEST MANAGEMENT - The control of pests utilizing a practical, economical, and scientifically based combination of chemical, biological, mechanical or physical, and cultural control methods. Coordinated application of non-chemical control methods is emphasized in order to reduce or eliminate the need for pesticides. Integrated pest management is a balanced approach which considers hazard to the environment, efficacy, costs, and vulnerability of the pest. It requires: (1) identification of acceptable thresholds of damage; (2) environmental monitoring; and (3) a carefully designed control program to limit damage from the pest to a predetermined acceptable level.

NATIVE - Indigenous.

NONINDIGENOUS SPECIES - Any species or other viable biological material that enters an ecosystem beyond its historic range, including any such organism transferred from one country into another [Nonindigenous species include both exotics and transplants].

Synonyms: Introduced, Exotic, Alien, Foreign, Non-native, Immigrant, Transplants.

ORGANISM - Any active, infective, or dormant stage of life form of an entity characterized as living, including vertebrate and invertebrate animals, plants, bacteria, fungi, mycoplasmas, viroids, viruses, or any entity characterized as living, related to the foregoing.

PATHWAY - The means by which aquatic species are transported between ecosystems.

PREVENTION - Measures to minimize the risk of unintentional introductions of nonindigenous aquatic species that are, or could become, aquatic nuisance species into waters of the United States.

PUBLIC FACILITIES - Federal, State, regional and local government-owned or controlled buildings, structures and other man-made facilities, including water intakes, boat docks, electrical power plants, locks and dams, levees, water control structures, and publicly-owned fish culture facilities. Electric generating stations, water supply systems and similar facilities operated by public utilities or other non-governmental entities are also considered public facilities.

RISK - Is the likelihood and magnitude of an adverse event.

RISK ANALYSIS - The process that includes both risk assessment and risk management.

RISK ASSESSMENT - The estimation of risk.

RISK COMMUNICATION - The act or process of exchanging information concerning risk.

RISK MANAGEMENT - The pragmatic decision-making process concerned with what to do about the risk.

SPECIES - A group of organisms, all of which have a high degree of physical and genetic similarity, can generally interbreed only among themselves, and show persistent differences from members of allied species. Species may include subspecies, populations, stocks, or other taxonomic classifications less than full species.

TRANSPLANTS - Species native to North America which have been introduced into ecosystems where they did not occur prior to European colonization. In other words, such species did not historically occur in the location in question.

UNINTENTIONAL INTRODUCTION - **An introduction of nonindigenous species that occurs as a result of activities other than the purposeful or intentional introduction of the species involved, such as the transport of nonindigenous species in ballast or in water used to transport fish, mollusks or crustaceans for aquaculture or other purpose. Involved is the release, often**

unknowingly, of nonindigenous organisms without any specific purpose. The virtually inevitable escapement, accidental release, improper disposal (e.g., "aquarium dumping") or similar releases of intentionally introduced nonindigenous species do not constitute unintentional introductions.

Synonyms: Accidental, Incidental, Inadvertent.

UNITED STATES - The 50 States, the District of Columbia, Puerto Rico, Guam, and all other possessions and territories of the United States of America.

VECTOR - A biological pathway for a disease or parasite, i.e., an organism that transmits pathogens to various hosts. Not a synonym for **Pathways** as that term is used in the proposed Aquatic Nuisance Species Program.

WATERS OF THE UNITED STATES - The navigable waters and the territorial sea of the United States. Since aquatic nuisance species can move or be transported by currents into navigable waters, all internal waters of the United States, including its territories and possessions, are included. The Territorial Sea of the United States is that established by Presidential Proclamation Number 5928 of December 27, 1988.

Synonyms: United States Waters

APPENDIX H

OBSERVERS' COMMENTS AND LIST OF OBSERVERS

APPENDIX H. OBSERVERS' COMMENTS AND LIST OF OBSERVERS

The workshop agenda included an opportunity for observers to make public statements during the afternoon plenary sessions on January 7 and January 8. At the discretion of each breakout group chair, observers were also provided an opportunity to participate in discussions during breakout group sessions. A list of observers is provided at the end of this section.

Also included here are written comments received from Tony Amoriggi. Mr. Amoriggi's comments, submitted in July 1997 in connection with the stakeholder meetings on the report of the JSA Shrimp Virus Work Group, were inadvertently omitted from the minutes of the stakeholder meetings. Although Mr. Amoriggi was not present at the risk assessment workshop, his comments have been included here for reference.

James Heerin
Shrimp Culture, II, Inc.
Roswell, Georgia

Mr. Heerin commented about the composition of the peer review workshop panel. He expressed the concern that no one from the shrimp processing industry was represented on the panel or on the shrimp processing workgroup, and he commented that there were only two people on the panel with any significant involvement in aquaculture production.

Andrew Duda
A. Duda and Sons, Inc.
Oviedo, FL

Mr. Duda cautioned that the media will focus on the executive summary of the workshop report. He asked that the panel consider the media's likely reaction to the report, and its executive summary when applying the modified Aquatic Nuisance Species Task Force risk assessment methodology. He also stated that it is necessary to separate issues, and look at them pragmatically. Growers know that disease is a problem, and they want to be part of, and learn from the risk assessment process. He also suggested that the likelihood of virus colonization is low; if the likelihood were high, the virus would have wiped out the South Carolina shrimp fishery shortly after it was observed there in aquaculture farms.

David Whitaker
South Carolina Department of Natural Resources
Charleston, SC

Mr. Whitaker stated that workshop participants need to consider that the risk of an event leading to the long-term, total annihilation of a fishery is an entirely different matter than the risk of an event in which the disease spreads, runs its course, and the population recovers.

Mark Frischer
Skidaway Institute of Oceanography
Savannah, GA

Mr. Frischer commented that shrimp viruses are a global issue, and shrimp represent a global industry. He noted that it is unwise not to consider the practices in the shrimp industry worldwide.

Rolland Laramore
Bonney, Laramore, and Hopkins; Harbor Branch Oceanographic Institution
Vero Beach, FL

Mr. Laramore questioned the ability of diagnostic procedures, specifically the gene probe, and PCR, to detect differences in viral strains (i.e., to distinguish between native, and non-native species).

He added that aquaculture species can migrate across international borders, and he added that there is no "fence" between the waters of Mexico, and the United States.

Mr. Laramore stated that work he performed with Ralston-Purina determined that viruses, and bacteria are killed by high temperatures during feed processing. He noted, however, that farmed shrimp, particularly those in hatcheries, and maturation systems, are fed both "natural", and processed feeds. "Natural" feeds include frozen shrimp, squid, and krill, which could carry the viruses with them. Shrimp Culture, Inc., avoided this problem by irradiating "natural" feed.

Mr. Laramore also stated that, within 2 or 3 years, the discussion is likely to focus on different strains of these viruses, some of which may prove to be local or native rather than nonindigenous.

He added that, to date, industry, and academia have not worked well together. He noted that many of the larger shrimp farms have qualified scientists on staff, but, so far, collaboration between industry, and academia has not occurred.

Mr. Laramore commented that he is disturbed that research that has come out of Honduras has been relegated to "nondata" status. The Honduran data come from samples of approximately 300 million to 400 million shrimp. He urged those who have not read his paper, *"Shrimp Culture in Honduras Following the Taura Syndrome Virus,"* to do so, and stated that he would like to hear from people about any errors in the paper's assumptions.[1] He also stated that he believes that similar data from Panama and Ecuador may exist.

Craig Browdy
South Carolina Department of Natural Resources, Waddell Mariculture Center
Bluffton, SC

Dr. Browdy commented about the relevance of laboratory information in determining events that might occur in the wild. He urged the workshop participants to emphasize cell culture in its list of research needs. He suggested that cell culture methods for insects, and fish can determine the amount of virus in a sample, but he noted that these methods do not yet exist for crustacea. He also urged that time during the workshop be devoted to looking at the individual pathways of

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[1] Laramore, C.R. 1997. Shrimp culture in Honduras following the Taura syndrome virus. IV Central American Symposium on Aquaculture, Tegucigalpa, Honduras.

infection of aquaculture ponds in terms of the relative risks of infecting aquaculture stocks. Dr. Browdy concluded that this information will be very important for the risk management workshop.

Jerome Erbacher
National Marine Fisheries Service (NMFS), Office of Industry, and Trade
Silver Spring, MD

Mr. Erbacher stated that he worked for 3 years as the assistant to the NMFS aquaculture coordinator. He also explained that he was one of the authors of the report of the JSA Shrimp Virus Work Group.

Mr. Erbacher stated that aquaculture is "the canary in the coal mine." While aquaculture may be a partial cause of the introduction of nonindigenous viruses, he indicated that it is also the biggest victim of viral introductions, which have caused significant economic, and employment problems in the industry. Mr. Erbacher noted that the risk of introducing viruses from the wild to aquaculture operations is an important part of risk management for viral introductions, and that the upcoming NMFS management workshop will look extensively at this issue. He stated that any insight that the participants in the peer review workshop can provide about how these viruses are transferred from the wild to aquaculture will greatly assist the next phase of the risk management process.

Deyaun Boudreaux
Texas Shrimp Association
Port Isabel, TX

Ms. Boudreaux stated that it is important to identify the natural host of each nonindigenous virus, if possible. On behalf of the wild shrimp fishery, she thanked the workshop participants for helping to find ways in which we can be better stewards of the ocean, and the habitat of penaeid shrimp.

July 27, 1997

Ms. Kate Schalk
Vice President
Eastern Research Group
110 Hartwell St.
Lexington, Ma. 02173

Dear Ms. Schalk,

At the recent Virus Stakeholder meeting in Charleston, South Carolina, on July 15, 1997, an introductory statement was made by Dr. Kay Austin, stating that the only known study in which it was demonstrated that farmed raised shrimp were responsible for the decline of the blue shrimp, *P. Stylirostris* occurred in the Gulf of California. This study was alleged to be reported in a thesis prepared by Carlos R. Pantoja Morales while studying the incidence of IHHNV in populations of shrimp off the cost of Sonora, Mexico.

Since I am fluent in Spanish, I asked for a copy of the study that Dr. Austin quoted, unfortunately no copies were available at the time of the meeting. After requesting a copy of said thesis, Dr. Tom Siewicki with the National Marine Fisheries Service, was kind enough to forward a copy to me for my review.

After having read said masters thesis, by Carlos Roberto Pantoja Morales, I find no data that relates to the incidence of pond raised shrimp and IHHNV in the wild population of *P. stylirostris.* In fact, there were no analyses of IHHNV reported in any farm raised shrimp in his thesis. The only shrimp samples analyzed and reported in this thesis were wild caught shrimp taken from 39 stations along the coast of Sonora, Mexico, and it should be noted, that the species collected were *P. vannamei*, *P. stylirostris* and *P. californiensis*

Regards,

Tony Amoriggi

Shrimp Virus
Peer Review and Workshop

Crystal Gateway Marriott
Arlington, VA
January 7-8, 1998

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☆U.S. GOVERNMENT PRINTING OFFICE: 1999 - 750-101/00046