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ENVIRONMENTAL RESEARCH BRIEF

Critical Responses of Populations of Crustacea to Toxicants

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Introduction

To adequately assess the biotic hazard of contaminants entering the marine environment, suitable criteria must be determined for detecting the ecological damage resulting from long-term exposure to low concentrations of contaminants. Considering the close phylogenetic relationship between crustacea and the insect target for which pesticides are directed, it is not surprising that crustaceans often are more sensitive to pesticides than are other marine organisms (Williams and Duke, 1979; Costlow, 1982). Research at the Environmental Research Laboratory (ERL) Gulf Breeze is directed toward determining appropriate measurements for assessing the long-term effects of pesticides on estuarine crustacean populations based on both laboratory and field experimentation.

Toxicity tests in the last decade have established the mysid shrimp, *Mysidopsis bahia*, as one of the most sensitive members of the estuarine community to pesticide exposure (for a review see Nimmo and Hamaker, 1982) when simple mortality is viewed as the major criterion. For the majority of pesticides examined in life-cycle toxicity tests using this estuarine crustacean, a sublethal reduction in reproductive potential has proven to be the most sensitive criterion yet determined for chronic biological effects in this zooplankton species (Nimmo *et al.*, 1977, 1979, 1980, 1981).

Mysid reproduction, culminating in the release of fully developed juveniles by brooding females, is, however, far different from the reproductive process seen in benthic crustacean populations in the estuarine community. These benthic populations, including both commercially and ecologically important crabs and shrimp, reproduce by releasing free-swimming pelagic larvae which undergo a complex metamorphic process prior to settling into the parental benthic population as young juveniles. Further-

more, larvae of estuarine crustaceans have been shown to be particularly sensitive to environmental stress.

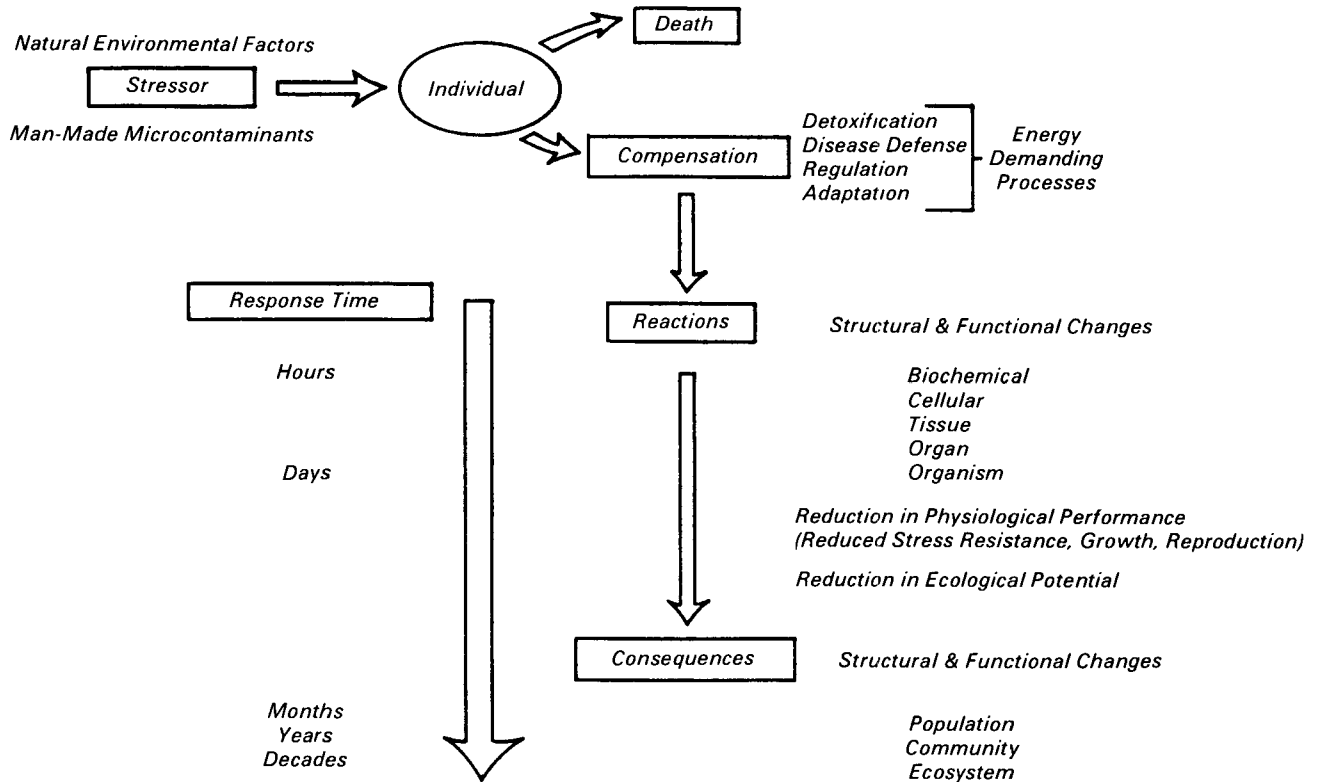
It is generally recognized that lower levels of biological organization, the cellular and organismal levels, respond to environmental stress long before higher levels such as community and ecosystem levels (Figure 1) (National Academy of Science, 1971; Waldichuk, 1979; Bayne *et al.*, 1980; Kinne, 1980). Furthermore, ecologists have for many years examined the productive processes of communities and ecosystems by studying the underlying physiological patterns of response of a species to environmental variables throughout its life cycle (Mann, 1969; Winberg, 1971; Steel, 1973, 1974; Grodzinski *et al.*, 1975).

The objective of the research summarized herein was to provide information necessary to determine appropriate responses for assessing the long-term effects of various classes of pesticides on estuarine crustacean populations. Dose-response relationships of pesticide toxicity and individual physiological functions were examined and compared for various life stages of estuarine mysids (*Mysidopsis bahia*), grass shrimp (*Palaemonetes pugio*), and mud crabs (*Eurypanopeus depressus*). Correlations between physiological dysfunction of discrete life stages and alterations in the ecological fitness of the population should aid in the selection of sensitive, rapid, and inexpensive monitoring tools for predicting chronic effects of pesticides on pesticide-sensitive estuarine populations.

This research represents an ongoing effort by the ERL-Gulf Breeze to identify particularly sensitive members of the estuarine community to potential microcontamination by specific classes of compounds and to provide the U.S. Environmental Protection Agency (EPA) with ecologically-sound test methods for assessing the ecotoxicological hazard of these compounds to this vulnerable community.

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Figure 1. Rationale for the usefulness of monitoring sublethal physiological parameters as short-term predictive indicators of long-term ecological damage by potential marine pollutants.



Earlier efforts at this laboratory produced the first marine invertebrate life-cycle toxicity test using the estuarine mysid, *Mysidopsis bahia* (Nimmo *et al.*, 1977, 1979, 1980, 1981; Nimmo and Hamaker, 1982). Recent emphasis has shifted toward an appreciation of the functional role of ecological indicator species and examination of the impact of pollutant stress on community processes, resulting from dysfunction among populations of these species both in laboratory and field experimentation. Furthermore, an increased awareness of environmental modification in the toxic expression of potential microcontaminants has resulted in laboratory examination of the mediation of toxicity by physicochemical environmental factors.

Measurements of Chronic Toxicity to Endrin in *Mysidopsis bahia* Populations

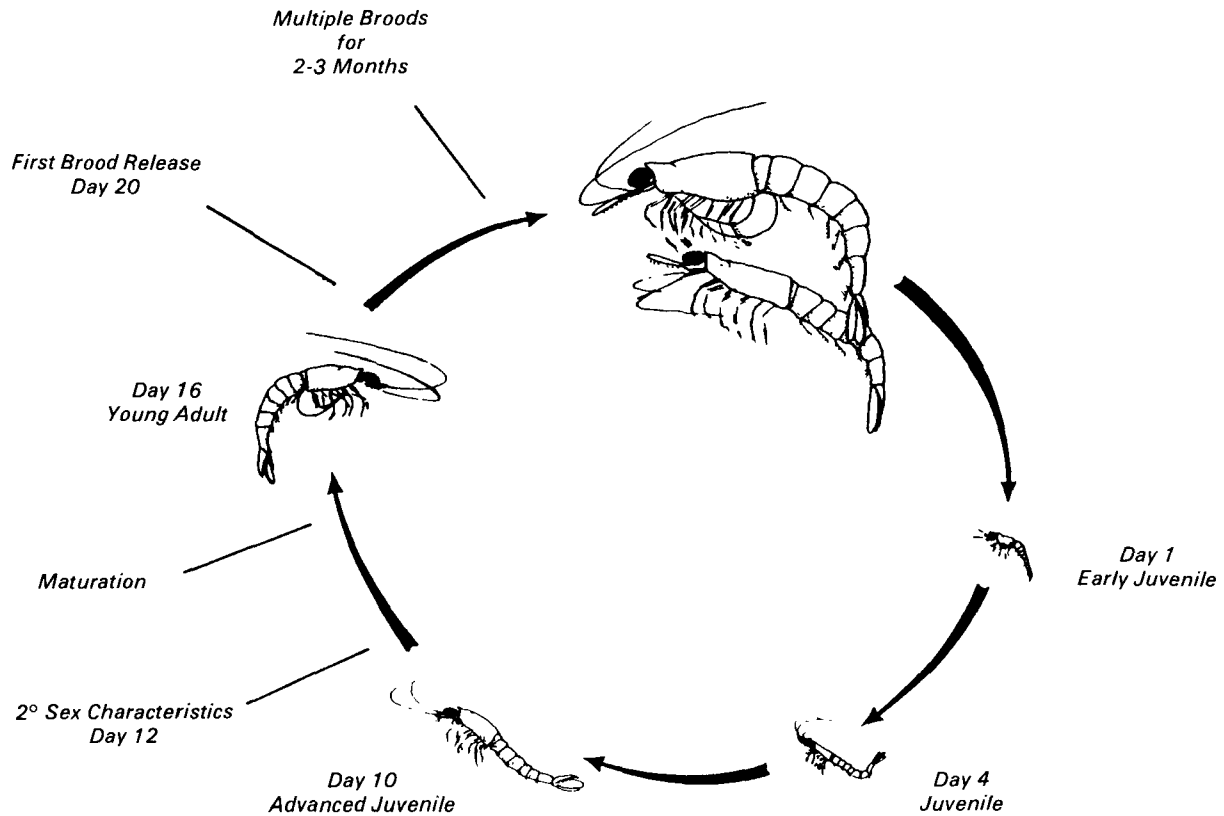
During a life-cycle toxicity test with *Mysidopsis bahia* exposed to the organochlorinated insecticide, endrin, various life stages were sub-sampled and measurements made of metabolic and growth functions (Figure 2). These individual physiological measurements were combined into bioenergetic and physiological indices and compared with alterations in survival and reproduction during the chronic exposure period.

Concentrations of endrin that were acutely lethal (120 ng/L) stimulated respiration rates of newly released

juveniles after only one day of exposure (Figure 3). Exposure to chronically lethal concentrations (60 ng/L) similarly resulted in higher metabolic rates, but to a lesser extent. Sublethal endrin exposure significantly reduced growth rates of rapidly growing juveniles after four days exposure to concentrations which were lethal after nearly three weeks exposure (60 ng/L). In accordance with lower net growth efficiencies (K_2 values), increased metabolic demands accompanying endrin exposure reduced the amount of assimilated energy available for production of new tissue.

Energy metabolism during juvenile stages of *M. bahia* was based primarily on utilization of lipid substrates (as indicated by the ratio of atoms of oxygen consumed to atoms of nitrogen excreted). During the maturation period, higher ammonia excretion rates and lower O:N ratios suggested increased amounts of protein used as an energy source. Reduced young production following chronic exposure to endrin concentrations of 30 ng/L or greater was correlated with increased lipid catabolism during maturation of young mysids into adults (Figure 3). It was postulated that enhanced energy demands, associated with higher metabolic rates during sublethal endrin exposure, favored usage of energy-rich lipid material at the cost of shunting lipids away from gametogenesis and preparation for reproduction.

Figure 2. Life cycle of the estuarine mysid shrimp, *Mysidopsis bahia*, depicting the various life stages subsampled for physiological measurements during chronic pesticide exposure.



Measurements of Chronic Toxicity to Thiobencarb in *Mysidopsis bahia* Populations

A number of vital life processes of the estuarine mysid, *Mysidopsis bahia*, were examined throughout its life cycle during exposure to the carbamate herbicide, thiobencarb (Figure 4). Initial exposure of juvenile mysids to thiobencarb resulted in elevated respiration rates. Concentrations of thiobencarb which produced significant reductions in population survival through a complete life cycle in approximately 24 days (181 ng thiobencarb/L) significantly stimulated respiration rates of juveniles after only four days of exposure. Increased metabolic demands with sublethal thiobencarb exposure (≥ 22 ng/L) reduced the amount of assimilated energy available for production of new tissue by juvenile mysids, resulting in retarded juvenile growth rates after only four days of exposure. Modifications in the energy metabolism of individual mysids exposed to thiobencarb were related to decreases in total young production of discrete populations. Greater usage of proteinaceous substrates for energy metabolism during the maturation of *M. bahia* juveniles was altered by exposure to high sublethal concentrations of thiobencarb. Higher O:N ratios during the maturation of these thiobencarb-exposed mysids suggest a greater reliance on the more energy-rich lipid substrates in order to support the elevated rates of oxidative metabolism,

resulting in less lipid material being available for gamete production.

From the responses of *M. bahia* to chronic thiobencarb exposure through an entire life cycle, it may be concluded that metabolic dysfunction in individual mysids preceded population responses important in determining community trophic patterns. Lower secondary production in a crustacean population, as indicated in this study by retarded mysid growth rates and inhibited reproductive rates, would alter the energy-flow patterns between connected trophic levels in the ecosystem. Although thiobencarb is directly toxic to fish in low ng/L concentrations (Johnson and Fenley, 1980; Schimmel *et al.*, 1983), the reduced secondary production of mysid populations with exposure to low ng/L concentrations of thiobencarb could indirectly affect fish populations dependent upon mysids as a food source (Darnell, 1958; Odum, 1971; Chao and Musick, 1977; Mauchline, 1980) and disrupt the balance of estuarine food webs. Short-term measurements of altered metabolic patterns in contaminated zooplankton, therefore, may offer the potential of monitoring for and predicting ecological disruptions in the estuarine ecosystem at higher levels of biological organization, i.e., at the population or community level.

Figure 3. Summary of the effects of endrin on *Mysidopsis bahia* exposed through an entire life cycle. VO_2 = weight-specific oxygen consumption rate; K_2 = net growth efficiency (percentage of assimilated energy used for growth); O:N = atoms of oxygen consumed to atoms of nitrogen excreted; VNH_3 = weight-specific ammonia excretion rate.

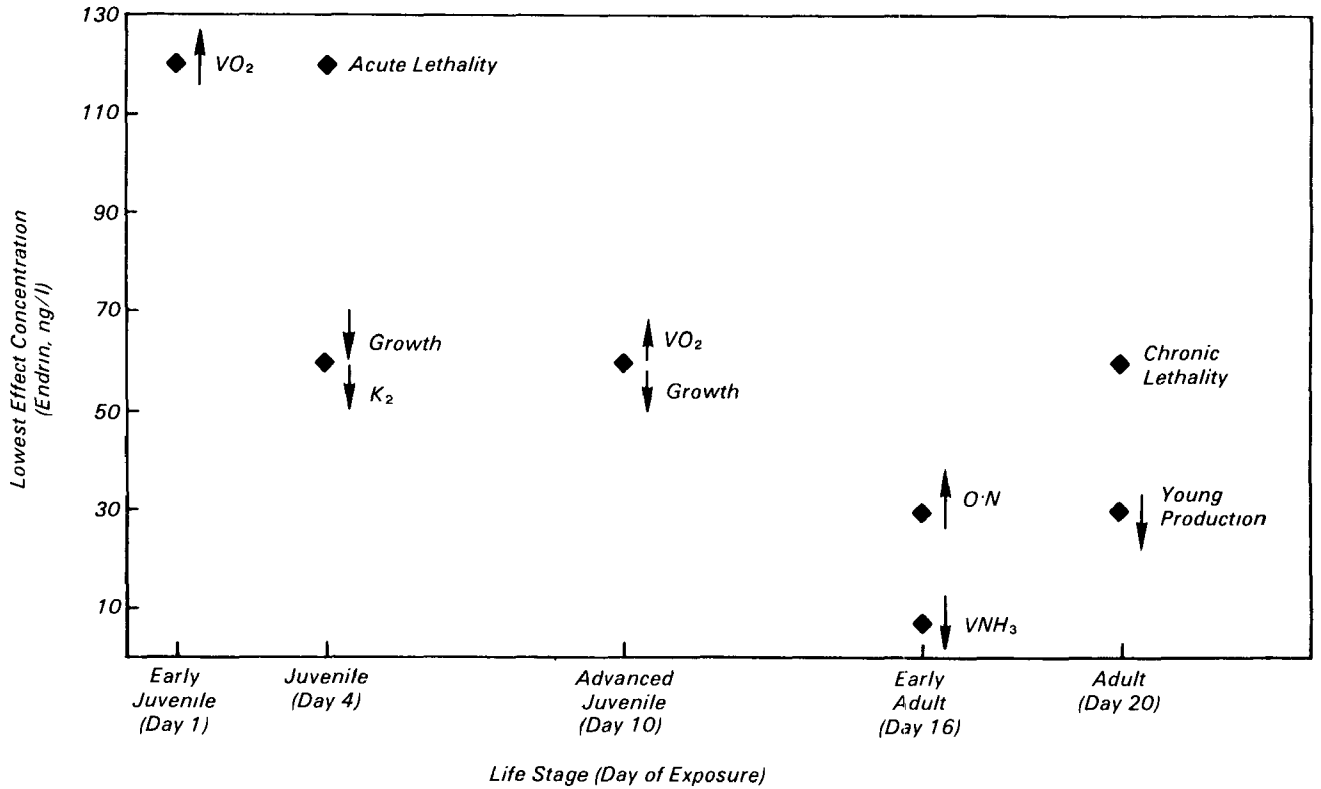
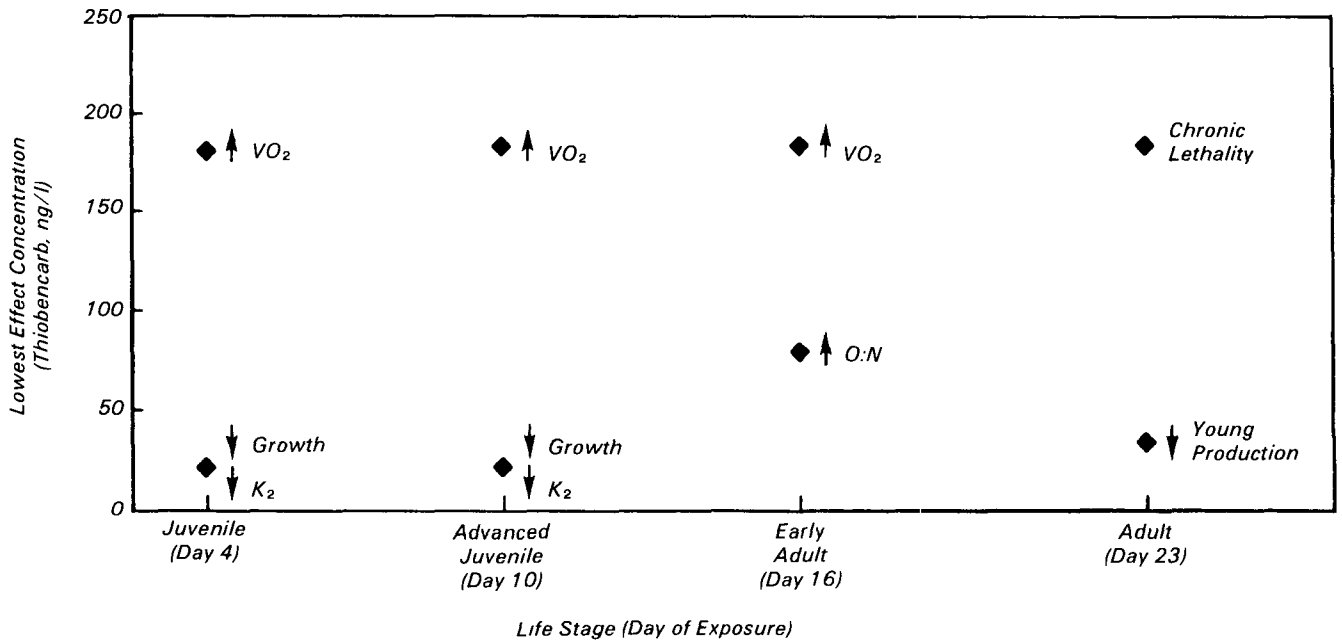


Figure 4. Summary of the effects of thiobencarb on *Mysidopsis bahia* exposed through an entire life cycle. Terms defined in legend for Figure 3.



Field-Study Confirmation of Toxicity Measurements of *Mysidopsis bahia* to Fenthion

Low-level exposure to fenthion, following ground ULV application of this organophosphate insecticide in various mosquito control programs in Florida, produced both increased mortality and sublethal growth retardation of *Mysidopsis bahia* juveniles. Production of juvenile populations without continuous recruitment is the outcome of two opposing processes: increased weight of individuals in the population and decreased numbers of individuals in the population due to mortality (Winberg, 1971). Increased mortality and sublethal growth retardation of mysids following low-level exposure to fenthion, therefore, would result in reduced population production of this crustacean, which serves as an important link in the estuarine food chain between primary producers and commercially important fish utilizing the estuary as a nursery.

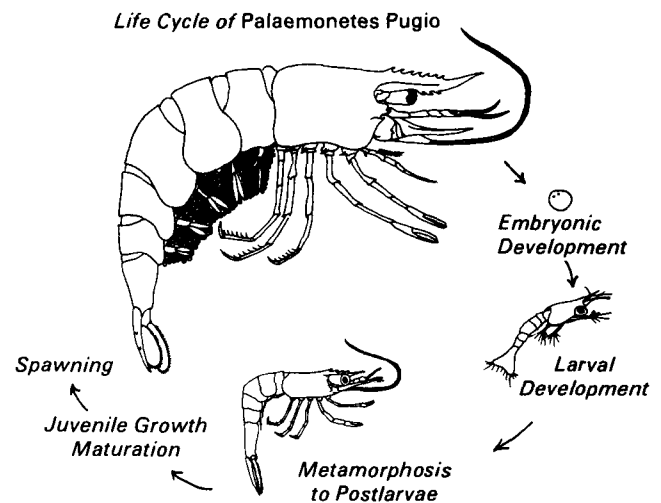
Significantly higher rates of oxygen consumption of fenthion-exposed mysids eight days after the field spray accompanied the reduced weights of the exposed mysids, suggesting bioenergetic disruptions in mysids exposed sublethally to fenthion in the field. Similar results have been reported for *M. bahia* exposed to sublethal concentrations of pesticides through an entire life cycle in the laboratory (McKenney, 1982, 1985). Increased metabolic demands on mysids exposed in the laboratory to pesticides, as indicated by higher respiration rates, reduced the amount of assimilated energy available for production of new tissue, resulting in lower juvenile growth rates. These field study results, therefore, confirm those of earlier laboratory studies; short-term measurements of metabolic dysfunction in mysids may predict altered production rates in mysid populations.

Measurements of Chronic Toxicity to Fenvalerate in *Palaemonetes pugio* Populations

Larvae of the estuarine grass shrimp, *Palaemonetes pugio*, were reared in the laboratory from hatch through metamorphosis (Figure 5) under optimal salinity conditions (20 ‰) in a range of lethal and sublethal concentrations of the pyrethroid insecticide, fenvalerate. Greater than 50 percent of the larvae exposed to measured concentrations averaging 0.8 ng/L of fenvalerate died within four days of continuous exposure. This concentration of fenvalerate represents the lowest level of fenvalerate reported to produce toxic effects on nontarget aquatic organisms. Furthermore, since no mortality occurred for adult *P. pugio* exposed to this same fenvalerate concentration, early larval stages of estuarine crustaceans may be the most sensitive to pesticide toxicity.

Continual exposure to the sublethal concentration of fenvalerate (a nominal concentration of 1.6 ng/L) delayed completion of metamorphosis for developing grass shrimp larvae by nearly two days. Extension of this particularly vulnerable pelagic phase in the life cycle may increase predation pressure on the species. Increased predation on larvae would reduce the number available for recruitment into the parental benthic population or for dispersal and establishment of new populations in less severe environments.

Figure 5. Life cycle of the ecologically important estuarine grass shrimp, *Palaemonetes pugio*. This species has been used successfully at ERL-Gulf Breeze both in life-cycle and larval toxicity tests.



Oxygen consumption rates of newly-released *P. pugio* larvae were significantly higher after exposure for 24 hours to fenvalerate concentrations which, after continual exposure through the entire larval development, resulted in significantly fewer larvae completing metamorphosis. These results suggest that altered rates of respiration of marine crustacea may serve as rapid biological monitors of detrimental effects of pesticide exposure to important components of the estuarine community.

Upon completion of metamorphosis, postlarval *P. pugio* had significantly higher rates of oxygen consumption in sublethal concentrations of fenvalerate, which previously had not altered larval metabolism. This species shifts from a free-swimming pelagic larvae to benthic postlarvae when metamorphosis is complete. The highly adsorptive nature of fenvalerate (Schoor and McKenney, 1983) could have resulted in adsorption onto the glass surface of the exposure beakers during this study. Presumably benthic postlarvae could have been more readily exposed to fenvalerate adsorbed to the glass surface than larvae in the water column, thus affording greater bioavailability and increased metabolic sensitivity.

Responses of an estuarine organism to a toxicant are dictated by the simultaneous influences of a number of endogenous and exogenous variables (Figure 6). Tolerance of osmotic stress is the most essential adaptation required for a population to succeed in the fluctuating salinity conditions of an estuary (for reviews see Lockwood, 1976; Gilles and Jeuniaux, 1979). Moreover, metabolic compensation to salinity by developing larvae of *P. pugio* may be modified by sublethal toxicant exposure (McKenney and Neff, 1981). Therefore, as an indication of the ecological fitness of larval *P. pugio*, a secondary objective of this study was to measure larval metabolism during osmotic stress to

Figure 6. Responses of an estuarine organism to a toxicant are dictated by the simultaneous influences of a number of endogenous and exogenous variables, including fluctuating salinity conditions.

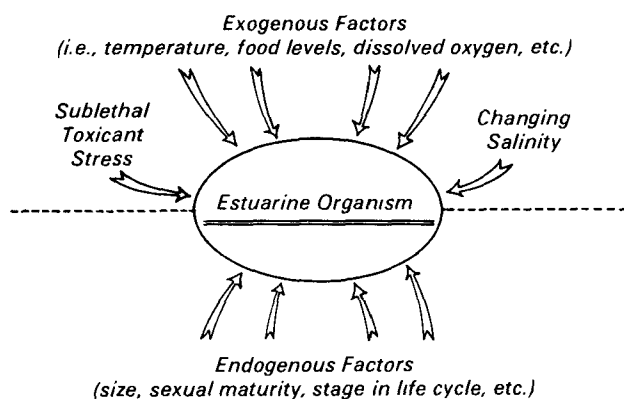
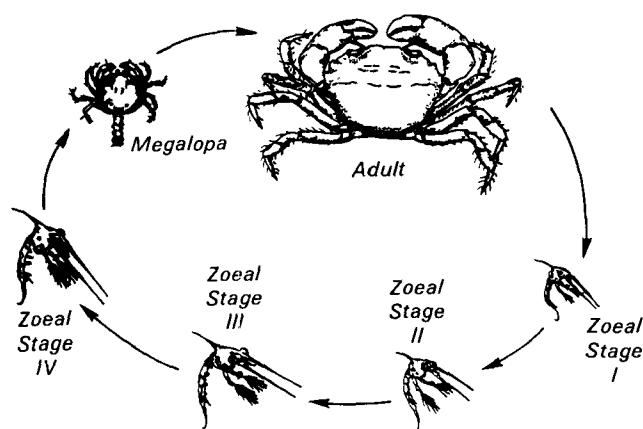


Figure 7. Complete larval development of the estuarine mud crab, *Eurypanopeus depressus*, through metamorphosis.



determine if the ability of larvae to adapt to fluctuating salinities was altered by sublethal exposure to fenvalerate.

Acute osmotic stress modified the metabolism of larval *P. pugio* reared in sublethal, nominal fenvalerate concentrations of 0.1 and 0.2 ng/L and these metabolic responses varied with stage of development. After eight days of exposure to fenvalerate, oxygen consumption rates were elevated when larvae were acutely exposed to hypoosmotic stress (10 o/oo S). Metabolic responses of premetamorphic larvae to hyperosmotic stress (30 o/oo S) were also modified by sublethal fenvalerate exposure. Alterations in metabolic-salinity patterns of larval grass shrimp developing under sublethal fenvalerate concentrations suggest a reduction in the ecological fitness of this sensitive life stage manifested as a limitation in their capacity to adapt to the fluctuating salinity conditions of estuarine waters.

Measurements of Chronic Toxicity to Lindane in *Eurypanopeus depressus* Populations

The various life stages of an estuarine mud crab (*Eurypanopeus depressus*), including the zoea, megalopa, and adult stages (Figure 7), exhibited different response patterns to lindane exposure, hypoosmotic stress, and interactions between the pesticide and salinity stress. Larval stages of *E. depressus* were more sensitive to lindane exposure than adults. The larval 96-hour LC50 value for lindane exposure was 0.66 µg/L as opposed to 25 µg/L for adult crabs. Long-term exposure to sublethal concentrations of the organochlorinated insecticide, lindane, caused alterations in ionic and osmotic regulatory ability and related compensatory metabolic mechanisms in *E. depressus*. A lindane exposure concentration of 1.45 µg/L reduced the hemolymph osmotic concentrations in adult crabs. Chloride ion regulation, however, was a more sensitive criterion, being disrupted at a lindane exposure concentration of 0.70 µg/L. A lindane exposure concentration of 0.01 µg/L increased larval mortality and altered larval respiration and ammonia excretion rates directly and in combination with salinity stress. Increased larval sensitivity to pesticide

exposure may reduce larval survival/recruitment in pesticide contaminated areas, resulting in altered distributional patterns in adult benthic populations. Similarly, disruptions of osmo-regulatory mechanisms may limit the natural distribution of this species to areas with less salinity variability.

Conclusions

Short-term measurements of altered metabolic patterns in contaminated zooplankton offer the potential for monitoring and predicting disruptions in estuarine ecosystems. Bioenergetic events at the organismal level precede secondary production rate changes at the population level. Laboratory responses to both an organochlorinated pesticide (endrin) and a carbamate pesticide (thiobencarb) were characterized by modification of energy metabolism in individual mysids, and these results preceded lower secondary production rates caused by the same compounds. Sublethal exposure of estuarine mysids to concentrations of these pesticides, which initially elevated mysid respiration, eventually inhibited growth and reproductive capacity of isolated mysid populations in the laboratory. Results of these laboratory studies were confirmed in a field study, indicating that physiological measurements of metabolic dysfunction in mysids exposed sublethally to pesticides may be used to predict altered production rates in mysid populations.

Larvae of estuarine crabs and shrimp were more sensitive to pesticide exposure than adults. Larvae of the estuarine grass shrimp, *Palaemonetes pugio*, died upon exposure to the lowest concentrations of a synthetic pyrethroid insecticide (fenvalerate) found to produce toxic effects on non-target aquatic organisms. Extremely low, sublethal levels of fenvalerate (below the limit of analytical detection) reduced the ecological fitness of larval estuarine grass shrimp (*Palaemonetes pugio*) by limiting their capacity to adapt to naturally occurring salinity fluctuations in estuarine waters. Concentrations of the organochlorinated insecticide, lindane, which were toxic to larval mud crabs (*Eurypanopeus depressus*), were several orders of magnitude below those

toxic to adult crabs. Moreover, chronic exposure to sublethal concentrations of this pesticide altered ionic and osmoregulatory abilities and related compensatory metabolic mechanisms in larvae and adults of this estuarine crab, with larvae being more sensitive than adults.

The research is described in the following publications:

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