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Project Summary

Bacteria of Public Health Significance Associated with Fish Reared in Treated Wastewater

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The suitability of tertiary wastewater ponds for aquaculture was evaluated in terms of fish productions obtained and the significance of bacteria in the wastewater and from fish. Silver carp *Hypophthalmichthyes molitrix* were stocked into tertiary wastewater ponds at 10,000 fish per hectare (ha) and cultured for five months. Fish growth was determined monthly. Bacterial evaluations of water and fish were made monthly. Pour plates of the appropriate media were made to determine total plate count, fecal coliforms, and fecal streptococci. Enrichment procedures were used to isolate *Salmonella*. The relative abundance of selected bacterial colony types was found over the course of the experiment. Selected isolates were identified.

Net production of fish was 1,309.2 kg/ha with the average weight of fish produced being 204.6 grams. Fecal coliforms and fecal streptococci were commonly found from both the water and fish. *Salmonella* was rare in the wastewater ponds and very rare from the fish. Changes over time in fecal coliform or fecal streptococci from water generally were not represented by a corresponding response for that bacterial count from fish. Corresponding trends were more evident in total counts from water and fish. Certain colony types were much more common in water than fish, while others were more common from fish. *Klebsiella pneumoniae* was the most common fecal coliform from the water while *Aeromonas hydrophila* was the most common from fish. *Streptococcus faecalis* was the most common fecal streptococcus from both water and fish.

This Project Summary was developed by EPA's Health Effects Research Laboratory, Research Triangle Park, NC, to announce key findings of the research project that is fully documented in a separate report of the same title (see Project Report ordering information at back).

Aquaculture is an effective way to provide quality protein. It is taking on a more important role in the world food supply as the traditional capture fisheries are reaching their production limits, while the human population continues to grow. In the United States, aquaculture is a rapidly growing industry meeting the American consumers' demand for more lean, nutritious meats.

The basic objectives of aquaculture are to provide a suitable environment for growing the organism and producing an acceptable product. To meet these objectives requires an adequate availability of land, water, and nutrients, and culture systems that will produce an acceptable product. The competition in the United States for land, water, and nutrients has resulted in culture systems with high input costs aimed at producing luxury products. Where input costs can be lowered, other aquaculture systems can be developed to produce products for other markets.

A rich source of water and nutrients that is going underutilized in the United

States is domestic wastewater. The use of this water would allow the development of aquaculture systems that otherwise would not be practical. An appropriate use of wastewater is the culture of filter feeding fishes in tertiary wastewater lagoons. Tertiary wastewater lagoons are rich environments for aquaculture with an abundance of natural food organisms as a result of the natural food chain based on photosynthesis. The wastewater held in ponds is further purified through biological action while at the same time stimulating the production of algae and other food organisms. Planktonic algae are the primary producers in a wastewater pond. Other organisms feed on this production and pass this energy on through the food chain. Each time that energy is passed through the food chain there is a significant loss in efficiency. Ideally in aquaculture a desirable organism can be produced that takes direct advantage of the primary production without having to pass energy several steps, thus increasing the efficiency of the system by reducing the steps in the food chain.

There are several species of fish which can directly utilize the primary production of planktonic algae in wastewater ponds. A species showing a great deal of promise is the silver carp *Hypophthalmichthyes molitrix*. It is a filter feeding fish with very closely set gill rakers that form a matrix capable of removing 8 μm algae and other suspended matter from the water column. It has the potential for rapid growth and is able to convert a less usable biomass, i.e., algae into a more usable one, i.e., fish.

The culture of silver carp or other fishes in wastewater could become widespread if the product were considered acceptable. A major factor in determining whether such a product is acceptable and to what use it could be put, is what public health dangers might exist. These dangers could include the presence of significant amounts of pathogenic viruses, bacteria, parasites, or toxic chemicals. Secondarily treated domestic wastewater will contain low levels of human pathogens and toxic elements. But the presence of these agents in the water supply or the pond environment does not necessarily imply that aquaculture organisms produced in that environment would contain these agents in hazardous levels or be of unacceptable quality.

One of the indices of the quality of a product is the type and abundance of bacteria associated with the product. The bacterial flora of fish is often thought to be a reflection of that of the environment. In a domestic wastewater pond, that environment would include human fecal bacteria. The degree in which silver carp reared in wastewater are a reflection of the bacteria in the water column will strongly influence how the fish produced from such an environment can be used.

This study focused on the culture of silver carp, their bacterial flora, and how it reflects the bacterial flora of the environment. Several issues were addressed: the suitability of ponds receiving secondarily treated wastewater for the culture of silver carp; the nature of the bacterial flora of the water and fish and their interactions; antibody production by silver carp; the effect of exposing silver carp to water containing *Salmonella*; and a comparison of bacterial flora before and after processing from wastewater and non-wastewater cultured fish.

Tertiary wastewater lagoons are good environments for the culture of silver carp. The fish grew well and were able to tolerate the environmental stresses encountered. No disease outbreaks among the fish occurred although such fish pathogens as *Aeromonas* were present.

Fecal coliforms, fecal streptococci, and *Salmonella* were present from fish cultured in the wastewater environment. The levels of bacteria were similar to those reported from domestic livestock with the exception of fecal coliforms which were higher. *Aeromonas*, a saprophytic water bacteria, was a common component of the fecal coliform counts from fish. Thus the fecal coliform counts were distorted from the more classically considered components. Bacterial counts from both wastewater and non-wastewater cultured fish were similar after the fish had been processed. The levels found were similar to those reported for other forms of processed meats.

Silver carp are capable of being carriers of *Salmonella* when experimentally exposed to high concentrations in the water column. The fish can retain *Salmonella* in the intestine for 15 days after the fish are transferred to freshwater. *Salmonella* was rare from the fish cultured in the wastewater and was confined to the intestine.

In general, the levels of bacteria encountered in this study were within the range of levels encountered from commercially sold fish. The absence of *Salmonella* from the mucous or flesh, after processing of fish which carried *Salmonella* in the intestine, suggests that such fish could be successfully processed for consumption.

The relationships of total plate count, fecal coliform, and fecal streptococci from the wastewater and the fish are not simple. Bacterial counts from influent waters had little relationship to those from fish. Total plate counts of mid and effluent waters had some relationship to mucous and intestine counts of fish. This was not apparent for the fecal coliform or fecal streptococci. Various genera of bacteria would be present at more than one site, but its relative abundance would be different. Data obtained in this study would suggest that the bacterial flora of fish is not a good reflection of its aquatic environment.

The full report was submitted in fulfillment of CR-810418 by Auburn University under the sponsorship of the U.S. Environmental Protection Agency. This report covers a period from November 1982 to December 1984, and work was completed as of December 1984.

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The complete report, entitled "Bacteria of Public Health Significance Associated with Fish Reared in Treated Wastewater," (Order No. PB 85-217 677/AS; Cost: $11.50, subject to change) will be available only from:
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