# IDENTIFICATION, CONTROL, AND PREVENTION OF DISEASES ON FISH FARMS IN GUAM

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## Introduction

Aquaculture is a growing industry on Guam; and fish production, mainly hybrid tilapia and milkfish, is the most important commercial aquaculture activity on the island (FitzGerald and Nelson, 1979; Nelson, 1990). Most production occurs in freshwater and brackish ponds, but there have been recent efforts to encourage production of both species in high-density, recirculating systems. Historically, tilapia has constituted the bulk of Guam's aquaculture production, but more than 3 million milkfish were marketed as bait during 1996. The expansion of the aquaculture industry has resulted in increased importation of fish for stocking and in intensification of production, both of which increase the risk of losses from introduced diseases.

This report is part of a project with the overall goals of identifying the pathogens of tilapia and milkfish on Guam and of recommending methods of prevention and treatment of their diseases. The specific objectives of this project were to: 1) investigate reported mortalities suspected to have resulted from disease and identify the pathogens via observation of gross clinical signs, histopathology, and microbial cultures; and 2) to provide information on diagnosis and prevention of diseases, for use of aquaculturists and extension personnel on Guam. It is hoped that this will aid in the establishment of strategies for reducing the potential for the transferal of pathogens and parasites to Guam from other areas.

## Background on Fish Diseases on Guam

### Tilapia

Prior to undertaking this study we had received reports of recurring problems with diseases in some of the commercial tilapia ponds on Guam. Some of the problems were reported to be most severe during the dry season, when declining water quality is a problem. The problems with water quality were the result of the lack of water for flushing the ponds.

In the affected ponds, diseased fish often are afflicted with numerous unsightly lesions on the head, fins, and body. Lesions may also be present in the gills. At early stages of the disease, there are swollen spots or foci on the body surface. Later, scales in the affected areas become loose and gradually detach. At this stage the foci appear necrotic and reddish. Eventually, the skin in these areas peels off. These necrotic foci seem to be randomly located on the body. Common diseases with similar gross clinical signs may be caused by either bacterial or viral infections. However, there have been no systematic studies of the disease on Guam, and the causative agents and predisposing factors are still unknown.

The local tilapia farmers report reduction of the disease when the pond water is changed. They believe that the increasing salinity of the pond water during the dry season when less fresh water is available may be associated with the outbreak of the disease. Although, tilapia are known to be extremely tolerant of seawater (Suresh and Lin, 1992; Watanabe, 1997), and breeding populations have even become established in some brackish waters of Micronesia (Nelson and Eldredge, 1991), abrupt changes in salinity can weaken the fish. Also, there are likely to be other changes in water quality, in addition to salinity, that make the fish more susceptible to disease outbreaks. It is well known that the fish can be adversely affected by water quality, with poor water quality resulting in suppression of the immune system (Wedemeyer, 1996).

Potential pathogens are always present in aquatic environments and even as part of the array of normal intestinal microbes (Sakata and Koreeda, 1986). In Arizona, significant tilapia mortalities of cultured tilapia resulted from infection with a salt-tolerant strain of the bacteria *Aeromonas hydrophilia* (Lightner et al., 1988); and similar bacteria are almost certainly present on Guam. To date, there have been no investigations on Guam of potential predisposing factors related to the environment, such as temperature, salinity, or water quality. We also have no information on the environmental reservoir for the disease, its asymptomatic carriage, or the pathogen life cycle. Such information would be useful in treating and preventing outbreaks of this disease and helping to prevent its spread to other ponds.

In addition to the problems faced by the local farmers, there have been other incidences of diseased tilapia on Guam. For example, in 1984, striking mass mortalities of one species of tilapia occurred in Fena reservoir. Thousands of dead fish were removed from the reservoir by United States Navy personnel. By culturing samples from moribund fish, Leith et al. (1984) were able to determine that the disease was associated with a *Pseudomonas* infection. The disease only affected one species: *Oreochromis mossambicus*. Neither *Tilapia zillii*, the other tilapia species in the reservoir, nor the predatory cichlid *Cichla ocellaris* were affected. The disease apparently ran its course and ended rather abruptly, before any further studies could be conducted. Mortalities from such diseases could be occurring routinely, both in the wild and in aquaculture ponds, but they would not likely attract attention unless they reach levels near those of the severe outbreak of 1984.

There have been numerous, recent outbreaks of tilapia diseases in Japan, Taiwan, Thailand, and other Asian countries. Histopathological studies of the diseases of pond-cultured fishes in Asia commonly revealed lesions in the pancreas, kidney, heart, and liver. One of the main pathogens implicated in these diseases was a bacterium of the genus *Streptococcus* (Plumb, 1997). Species of the bacterial genus *Vibrio* are important pathogens in Asia (Ishimaru and Muroga, 1997). Also, a *Rickettsia*-like microorganism, a parasitic microbe that has both RNA and DNA (Fiest et al., 1989), has been linked to mass mortalities of tilapia in Taiwan (Chen et al., 1994). It has been reported that disease outbreaks in tilapia occur more frequently during the winter months in Taiwan.

Milkfish

There is virtually no information on the diseases of milkfish raised on Guam. During interviews to gather information for this proposal, farmers informed us of incidences of mass mortalities of milkfish where the cause of the mortality was unknown. However, the farmers presently do not report such incidences to anyone; and they do not have the expertise or the facilities to determine whether or not diseases are involved. The milkfish fry imported to Guam are all from Taiwan. In Taiwan the most serious disease in milkfish culture is red spot disease, which is caused by *Vibrio anquillarum* (Huang, 1977).

Disease-related mortalities of milkfish cultured on Guam have already led to substantial losses to the farmers. There is still a great potential for continued disease and parasite problems given the increased number of fish being imported and the intensification of culture that is occurring. Because of the greatly increased volume of imports, it would be prudent to monitor the milkfish in Guam for diseases that are common in Taiwan.

**Protection of Local Stream Fishes**

In the long run, the broad issue of disease control and prevention must be addressed to protect the aquaculture industry and to safeguard the health of the populations of indigenous stream fishes. For example, all of the tilapia and milkfish juveniles that are stocked in the ponds of Guam are imported from Taiwan. The Guam Department of Agriculture requires imported fry to have a certificate from the point of origin stating that they are free of disease; however, because of the sheer volume of imports, there is still considerable potential for introducing parasites. There is already evidence that fish parasites have been inadvertently introduced to Guam with fish imported for stocking and have become established in the wild, affecting indigenous fishes. The transfer of parasites from introduced fishes to native stream fishes has been well documented for the Hawaiian islands (Font, 1997). Many of Guam streams dry up seasonally, and especially during droughts. This results in fish being crowded into pools and increases the likelihood of parasites being transferred between species (Dr. W. Font, Southwestern Louisiana State University, personal communication). Kabata (1985) emphasized that, to protect the farmed fish from disease, "under no circumstances should contact between cultured fish and wild fish be permitted," and adherence to this dictum would also serve to prevent the transfer of pathogens and parasites from imported fish to wild stocks.

## Diseases of Tilapia and Milkfish That Are Prevalent in Taiwan

### Parasite-related Diseases

Factors that normally influence the incidence of parasite populations are water quality and temperature. One specialist in the Taiwan Fisheries Research Institute informed us that many parasites have been found to infect tilapia in Taiwan, but many of these reports were not published. These main species of parasites infecting tilapia in Taiwan are: *Trichodina* sp., *Dactylogyrus* sp., *Pseudodactylogyrus* sp., *Gyrodactylus* sp., *Glossatella* sp., *Argulus japonica*, *Caligus* sp., and *Lernaea cyprinus*. Among these, the ectoparasitic protozoans and mongenea are potentially the most pathogenic.

Bacterial Diseases

Diagnosis of bacterial diseases is based on the isolation and identification of the etiological agents. Diagnosis is complicated because issues in the taxonomy of bacteria are still being resolved and because similar diseases, such as septicemias, can be caused by different bacterial species (Roberts, 1993). Antisera against specific bacterial pathogens of fish are available, but only for a few species.

A wide range of bacterial pathogens is known to infect tilapia and milkfish cultured in Taiwan. Red spot disease of milkfish is caused by the highly contagious *Vibrio anguillarum* and is the most serious disease for milkfish in Taiwan. Low temperature seems to be the major risk factor (Huang, 1977). The gross signs of *Vibrio parahaemolyticus* infection of tilapia include red spots similar to those seen in milkfish that are infected with *Vibrio anguillarum*. Another species of *Vibrio* causes an eye disease of milkfish (Ishimaru and Muroga, 1997). A species of *Pseudomonas* was found to responsible for the mortalities of tilapia fry in Taiwan (Duremdez and Lio-Po, 1985). Other prevalent pathogenic bacteria of milkfish and tilapia in Taiwan include species of the genera: *Aeromonas*, *Edwardsiella*, *Flavobacterium* (formerly *Flexibacter*), *Pseudomonas*, and *Streptococcus* (Tang et al., 1985). A ricketssia-like bacteria has also been identified as a causative agent for disease outbreaks in tilapia cultured in Taiwan (Chen et al., 1994).

Fungal Diseases

Fungus rarely attacks healthy fish, but commonly infects fish that have suffered stress from temperature shock, mechanical injury, or lesions from other diseases. Two of the main fungal diseases known to affect tilapia and milkfish cultured in Taiwan are saprolegniasis and gill rot. Saprolegniasis is a localized disease caused by fungi of the orders of Saprolegniales (water molds), Peronosporales, and Leptomitales. Infected fish are lethargic, and do not eat. Gross signs of the disease include white cottonlike masses on the body surface. Gill rot is caused by Branchiomyces sp., a fungus that grows within the branchial vessels.of the gill, causing respiratory stress.

Viral Diseases

There have been few reports of viral diseases of either tilapia or milkfish. However, Smith and Speer (1998) recently documented severe mortalities of tilapia fingerlings that were associated with viral infections of various organs, but particularly the spleen and kidney. Chen et al. (1985) isolated a virus from tilapia in Taiwan that was similar to infectious pancreatic necrosis virus (IPNV), which is known to occur in eels. From studies that involved experimentally infecting tilapia with IPNV, Mangunwiryo and Agius (1987) concluded that tilapia could serve as a reservoir of this virus, which could then be passed to fish species that are more susceptible.

## Identification of Pathogens from Diseased Fish in Commercial Ponds on Guam

The operation in southern Guam, where the affected fish for this study were collected, was raising milkfish for bait. They had a very severe disease problem, but the workers did not recognize it as such. Most of the diseased fish were much smaller than the healthier individuals. For example, the healthier fish had grown to 12 cm in Standard Length, while the most clearly affected individuals remained at approximately 3 cm SL. Many of the smaller fish had wasted bodies, which gave the appearance that the head was large. Many of the diseased fish had difficulty swimming. Red spots (petechiae) were obvious on their bodies, there was red coloration around the base of the fins, and the abdominal area was soft and pale, indicating subepidermal edema. Mortalities were occurring daily.

Moribund or afflicted fish were collected from the culture tanks by net. The fish were returned to the University of Guam where they were examined, dissected, and preserved in 10% buffered formalin. After 24-72 hours, preserved tissues were dehydrated through a graded series of ethyl alcohol, cleared in xylene, and embedded. Six to eight micron-thick sections were made on a rotary microtome. The sections were mounted on slides and stained with hematoxylin and eosin or Giemsae, and observed under the light microscope. Dr. S.N. Chen, a disease specialist from the Department of Zoology at the National Taiwan University allowed us to work in his laboratory to identify the pathogens.

For investigation of bacterial infections, isolates were obtained from necrotic foci on the body surface and from internal organs. All cultures were incubated at 28 ˚ C. After 24 hours, positive cultures were re-streaked and incubated an additional 24 hrs. Bacterial isolates were diagnosed through use of the API multi-test system (Analytab Products, Plainview, New York). While the API system is not designed specifically for aquatic diseases, it commonly used for rapid partial identifications by diagnostic laboratories dealing with fish disease.

Effects of the disease were also evident from the histological examinations. Inflammatory cells were found in the liver around the hepatic ducts (Figure 1). Also in the liver, inflammatory cells were seen in the parenchyma, and many of the parenchymal cells contained pyknotic nuclei, indicating multifocal necrosis. In addition, some cells around the lumen were lysed (Figure 2). The gill lamellae were thin and pale, indicating necrosis, and there were unidentified cyst-like structures scattered throughout the gills (Figure 3). In the kidney, although most of the tubules appeared to be normal, some showed degeneration of the tubular epithelium with debris in the lumina (Figure 4).

The bacteria (Figure 5) isolated from milkfish internal organs was a Gram-negative rod that is approximately 5 microns long and cytochrome oxidase positive. Results of the 24- and 48-hour tests with the various media used in the API rapid identification did not result in a species identification; this is not unusual in applying this system to pathogens of aquatic organisms. However, the tests indicated that the pathogen affecting the cultured milkfish on Guam was most likely a species of either the genus *Pseudomonas* or *Aeromonas*. Species of both of these genera are known to cause septicemias in a variety of fishes. These bacteria are almost always present in aquatic environments and usually only become pathogenic following some other form of stress, such as rough handling, poor water quality, or exposure to low temperatures.

**Review of Diagnostic Tools for Tilapia and Milkfish Diseases**

The Center for Tropical and Subtropical Aquaculture sponsored the development of the Hawaii Aquaculture Module Expert System, a computer software package that provides assistance with diagnosing and treating diseases of tilapia. The system was developed by Dr.

James Brock, Dr. Stephen Itoga, and Mr. David Coleman. The program may be downloaded, free of charge from their world wide web site (**http://Iibrary.kee.hawaii.edu/praise/hames/**) in Hawaii. The program provides general information useful in solving disease problems. The program includes such topics as how to conduct examinations of fish, how to preserve organs for histological examination, and how to prepare and ship fish for analysis. Addresses are also given for extension agents in Hawaii who can be contacted for assistance.

Another computer program that may be of assistance in diagnosing fish diseases in some cases is Fish-Vet (Fish Vet, Inc., 12620 Ivy Mill Road, Resiterstown, Maryland 21136). The program allows the user to enter information regarding disease symptoms and the pond conditions, then suggests the possible cause. Pictures of diseased fishes are included to help guide the user through the diagnostic procedure. This program provides some useful information, but it will not be able to provide a positive identification of pathogens, and the diagnosis will usually not be as reliable as that made by a veterinarian or fish-disease specialist at a diagnostic center. A copy of the program is available for use at the at the University of Guam Marine Laboratory, and additional information about the program, including orders and updates, can be found at the Fish Vet web site (**http://www.jagunet.com/-rishvet/**).

**The Lack of Approved Therapeutics**

Any drugs that are legally used in the prevention or treatment of diseases of fish raised for human consumption must first be approved by the United States Federal Drug Administration (FDA). To date, few drugs have been approved for any fish species, and none has been approved for any of the species being cultured commercially on Guam (i.e., tilapia, milkfish, and Asian catfish). Complete information regarding the drugs that are approved and the exemption process can be found on the internet at the web site of the Center for Veterinary Medicine (CVM) of the FDA at their web site (http://ww.cvm.fda.gov/fda/infores/other/aqua/aquadrugs/html). Exemptions for emergency use are possible under certain situations, but these procedures are not practical for the commercial fish farmers on Guam. Therefore, fish farmers on Guam must focus on the prevention of diseases rather than on treatment.

**Strategies for Disease Prevention**

Because there are no approved treatments for the diseases of either tilapia or milkfish, aquaculturists on Guam would be prudent to pay strict attention to the prevention of fish diseases, through a combination of good husbandry and pond management practices. Literature on the key aspects of health management for aquatic organisms is available (e.g. Austin and Austin, 1993; Plumb, 1994). The major objectives in health maintenance in aquaculture facilities are reducing the probability of exposing the fish to pathogens and minimizing stressors that may make the fish more susceptible to infection by disease-causing organisms.

Austin and Austin (1993) provide some simple means to aid in maintaining the health of fish in culture systems. The aquaculture production units (i.e., ponds, raceways, or tanks) should be kept clean so that there is no accumulation of organic materials such as fecal materials and uneaten feed. Care should be taken to remove any dead or moribund fish as soon as possible, as fish that have died from disease may release pathogens that spread to others. There should be no accumulation of attached algae or algal slimes in the aquaculture units. Oxygen content should be maintained near saturation and dissolved nitrogen, particularly ammonium, must be kept a low levels. Disinfection of nets, footwear, and other items that may transfer pathogens between production units should be routinely practiced.

In the intensive culture operations on Guam, such as milkfish being grown out for bait and tilapia being grown out in recirculating systems, it is possible to reduce or eliminate the pathogen load in the systems by filtering and treating the water. Water treatment with UV light, ozone, chlorine, or other means can be used. Details of a variety of such water treatment systems have been described by Wedemeyer (1996) in his book dealing with fish physiology in intensive culture systems.

The nutrition of the fish is also important in their resistance to disease. The quality of artificial diets is especially important in tank and raceway culture systems, where the fish to not have access to natural food items. Also, many aspects of the quality of diets can deteriorate with prolonged or improper storage. Most commercially prepared fish feeds contain polyunsaturated fats from fish oils, and these react with the oxygen in the air to form a variety of toxic compounds that result in the feed becoming rancid (Wedemeyer, 1996). Molds that grow on improperly stored feeds also produce toxins. Fish farmers should provide diets of high quality, avoid storage times over 90 days for dry feeds, and take care to properly store the feeds in order to avoid declines in nutritional quality.

The fish culture operations that we observed on Guam all neglected one or more of the above safeguards. For example, some tanks used to raise milkfish had accumulated organic materials, and the same nets were used for removing dead or dying fishes for several tanks. In addition, there was heavy algal growth in the tanks, and power outages had resulted in oxygen depletion. On another farm, dead catfish remained floating in the pond; the deaths were said to be from water quality problems. At one facility a pond fill of fish was not being fed at all since the fish could not be marketed. Any of these practices could result in disease outbreaks.

## Recommendations

The farmers on Guam could benefit by having more information about the importance of good husbandry and pond management in the prevention of disease. Extension information should be disseminated through small workshops and written materials in an appropriate language. Having basic information available in Chamorro, English, and Chinese would help insure that the aquaculture personnel on Guam would be able to make use of the extension materials. Because of the differences in languages used on different farms and the small number of farms, workshops and demonstrations pertaining to health management would be most effective if held at farm sites. This would also allow specific problems to be addressed.

In the long run, the possibility of importing diseased fish would be reduced if Guam farmers had a source of locally produced juveniles. Roberts and Sommerville (1982), in their review of the diseases in species of tilapia, pointed out the dangers of transferring pathogens between areas and recommended that, ideally, a country should be self-sufficient in fry production. The Guam Aquaculture Development and Training Center facility should explore the feasibility of producing high-health tilapia and/or milkfish juveniles for local farmers. If careful attention is paid to water quality, nutrition, shipping, and handling of the fish at the territorial hatchery, the local farmers could be supplied with fry that are healthy and exposed to a minimum of stress. However, without additional support and better management of the Guam

Aquaculture Development and Training Center, the large scale production of juveniles may not be possible.

With the recent push to develop new diagnostic methods for aquaculture diseases (Reddington and Lightner, 1994), it would be prudent for the responsible agencies on Guam to keep abreast of these development with the aim of eventually developing the capabilities to screen imported juveniles to confirm that pathogens or parasites are not being introduced. Many of the pathogens of tilapia and milkfish are common in almost all aquatic environments, but they are usually either free-living or innocuous. Therefore, the primary problem of disease prevention for the fish farmer is one of minimizing the susceptibility of the fish to pathogens. However, the issue of preventing pathogens and parasites from being introduced with imported juveniles is also important, and the cases where pathogens have been transferred between areas have been known for some time (Roberts and Sommerville, 1982). The potential importance of imported pathogens, particularly viruses, is also high for other cultured aquatic species.

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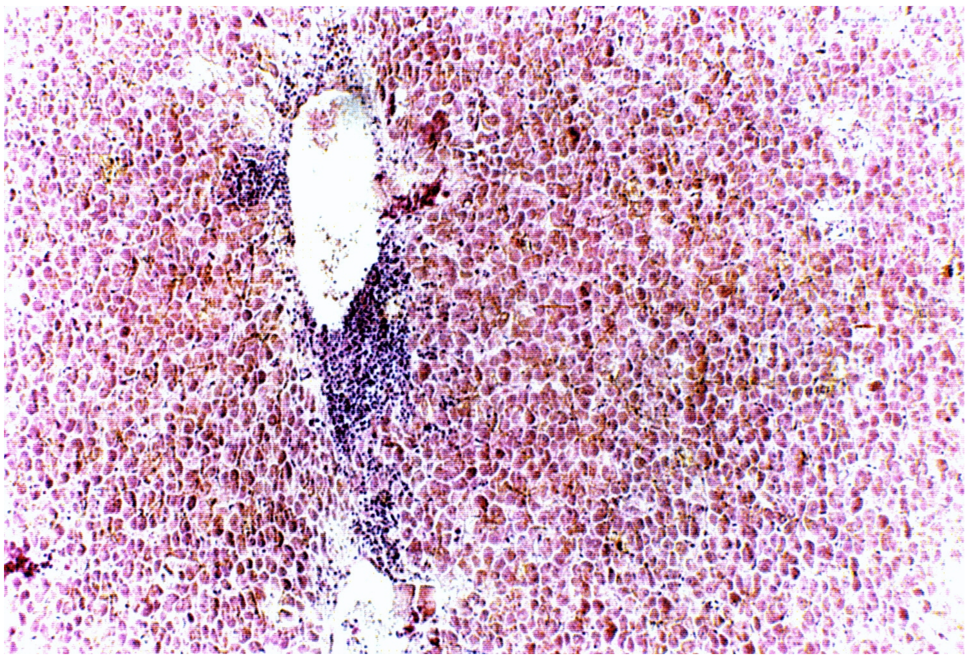

Figure 1. Inflammatory cells in the liver of a diseased milkfish (*Chanos chanos*) from a commercial aquaculture facility on Guam. Accumulation of inflammatory cells can be seen in the hepatic ducts.

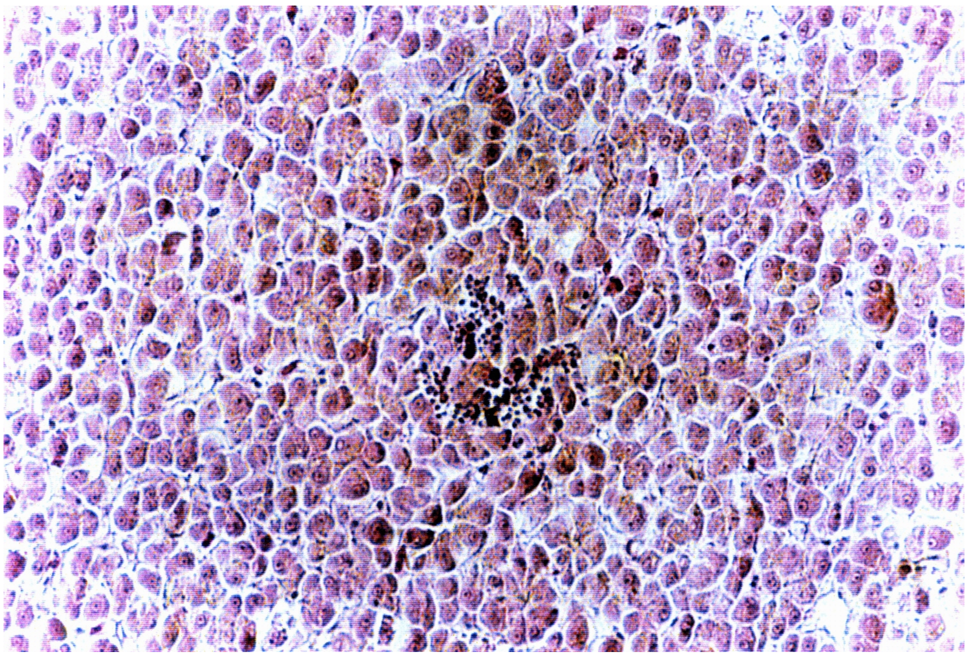

Figure 2. Section of parenchyma tissue of a diseased milkfish (*Chanos chanos*) from a commercial aquaculture facility on Guam. Inflammatory cells are present, some cells have pyknotic nuclei (multifocal necrosis), and some cells around the lumen appear lysed.

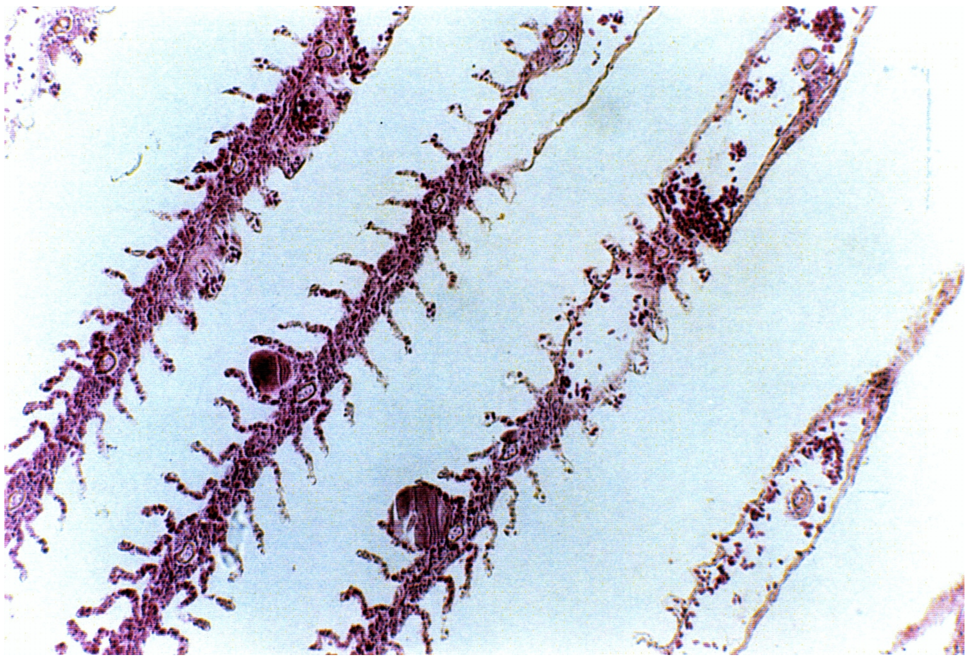

Figure 3. Section of the gill lamellae of a diseased milkfish (*Chanos chanos*) collected from a commercial aquaculture facility on Guam. The lamellae appear thin and pale (necrotic). Unidentified cyst-like structures are scattered in the gills.

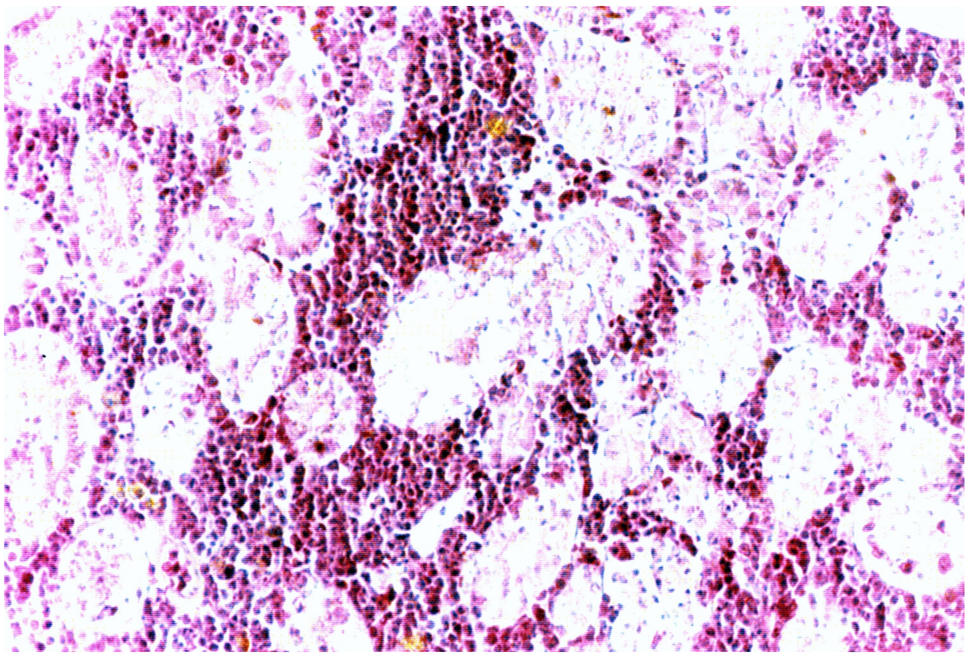

Figure 4. Section of the kidney of a diseased milkfish (*Chanos chanos*) from a commercial aquaculture facility on Guam. Most of the tubules are normal, but a few show degeneration of the tubular epithelium and debris in the lumen.

Figure 5. Gram negative, cytochrome oxidase positive bacteria isolated from the internal organs of diseased milkfish (*Chanos chanos*) from a commercial aquaculture facility on Guam. The rods are approximately 5 microns long.