

Mass mortality of Oreochromis mossambicus (Pisces, Cichlidae) in
Fena Lake, Guam associated with a Pseudomonas infection.

by

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Abstract

An epizootic die-off of Oreochromis mossambicus (commonly referred to as Tilapia) occurred in Fena Lake, Guam during early April 1984. Over 2300 dead adult fish were collected from the lake. Fish of other species inhabiting the lake were not affected. Moribund fish showed cutaneous and internal hemorrhaging. Bacterial cultures taken from three afflicted fish revealed the presence of infection by Pseudomonas spp. and indicate that the fish died of Pseudomonas hemorrhagic septicemia. It is believed that stress caused by overcrowding and low oxygen levels in the early morning predisposed the fish to infection by this opportunistic pathogen. Reduced sunlight due to volcanic haze from the eruption of Mauna Loa in Hawaii may also have been a factor in the suspected reduction of oxygen levels. It is recommended that a monitoring program be set up for oxygen levels, fish abundance, nutrient levels, and sedimentation rates in the lake and that removal of aquatic weeds be considered to prevent further outbreaks of disease.

Introduction

Mass mortalities of fish were noted in Fena Lake, Guam in early April 1984. Of four common species of fish inhabiting the lake only Oreochromis mossambicus (Cichlidae) was affected by the epizootic. This species was introduced to the lake in 1955 in order to control the dense stands of aquatic vegetation (Brock and Yamaguchi, 1955; Brock and Takata, 1956).

The Fena Lake Reservoir is under the control of the U. S. Navy and provides drinking water for both military personnel and civilian users in southern Guam. We were asked by personnel at the Fena Lake Water Quality Control Laboratory to (1) assist in determining the cause of the mortalities, and (2) to advise whether or not there was a potential public health risk. The purpose of this report is to describe the progression of the epizootic, discuss the causes of the mortalities, and recommend a program of management for the lake so as to avoid recurrences.

Materials and Methods

Fish Specimens

Moribund and dead specimens of O. mossambicus from Fena Lake were obtained on several occasions beginning 5 April 1984. The fish were examined for the presence of obvious parasites and the condition of the skin and gills noted. An examination of their internal organs was also made.

Fish counts

Starting about April 6, 1984 informal counts of the relative abundances of the major fish species in the lake were made. Visual surveys were made throughout the lake but concentrated on the regions containing Hydrilla verticillata beds in the area between the Imong and the Almagosa rivers where most of the fish congregate. Counts were made of Oreochromis mossambicus, Tilapia zilli, Anguilla marmorata and Cichla ocellaris. Formal underwater visual surveys were made on May 4, May 25, June 1, and June 8, 1984. Counts of fish were made by divers and were continued until between 45 and 50 C. ocellaris, the most abundant of the fishes, had been counted. A 2.5 cm² mesh size gill net was set for 1.5 hours in a narrow section of the lake near the Almagosa River on May 4 and for another 2-hour period on May 17. This method was discontinued because underwater observations revealed that the O. mossambicus and T. zilli swam into the net without becoming captured. Apparently, most of these fish are too large for this size net.

Counts of dead and near-dead O. mossambicus were obtained from Navy personnel who were charged with removing such fish from the lake and disposing of them. Navy or Fena Lake Laboratory personnel also provided information on the date of onset of the epizootic and reports on the fish populations in the lake at that time.

Bacterial Culturing

Four specimens of O. mossambicus were collected from the lake on April 13. Three of the fish were still alive, although moribund, when collected.

They were transported to the Marine Laboratory on ice and samples were taken for bacterial culturing within five hours of their collection. Each of the four specimens was about 30 cm long.

The fish were sanitized by dipping them in a solution of 1000 ppm Hyamine disinfectant, placed on a sanitized operating tray, and sprayed with 70% ethanol. A longitudinal cut was made along the lateral surface of the abdomen starting near the gill and extending posteriorly for about 7 cm using aseptic technique. The cut was deepened with a second scalpel. The incision was opened with a surgical tissue spreader and the cut deepened to open up the swim bladder. The kidney, which lies dorsal and posterior to the swim bladder, was pierced with a bacterial culture loop. Kidney material was then inoculated onto the surface of trypticase soy agar (TSA) slants. Material from the body cavity dorsal to the swim bladder was similarly inoculated. The agar slants were incubated at 23°C and 37°C. Four slants were made from each fish specimen. Samples from eight cultures representing all four original fish were streaked onto TSA plates to obtain isolated colonies. Samples from all the colonial morphologies on the agar plates were restreaked again to obtain pure cultures.

Media necessary for the biochemical tests listed in Table I and II were prepared according to standard procedures (Warren, 1981). Gram stains were prepared from the pure cultures and hanging drop mounts were used to determine motility. Isolates were incubated both at 23°C and at 37°C. Cultures of Escherichia coli B and Staphylococcus epidermidis were carried through the various biochemical tests as controls.

Further Bacterial Culturing

A lethargic O. mossambicus was collected from the lake with a hand net on April 20, 1984. No other O. mossambicus were seen during this visit. The fish was killed by a blow on the head and samples were taken in the field within 10 minutes after the death of the fish. Six samples from kidney material and body fluids were obtained aseptically and inoculated onto TSA slants as described above.

A moribund specimen of O. mossambicus with a contorted body was collected on May 10 with a hand net. Four samples were taken for culturing within 30 minutes after the fish was killed.

Four apparently healthy Tilapia zilli were collected by gill net on May 4 from the southern end of the lake near the Imong River. These specimens were aseptically sampled within 20 minutes after the fish were killed. Three samples were taken from kidney material and fluids of the body cavity for each specimen.

Two apparently healthy specimens of C. ocellaris collected in a gill net near the southern end of the lake at the Imong River inlet on May 4 were also sampled for bacterial infection.

Eight specimens of emaciated or lethargic Bufo marinus were collected near the pier at the northern end of the lake on May 25. Duplicate samples were obtained aseptically from the body fluids of five of these toads and inoculated onto TSA slants.

Results and Discussion

Appearance of afflicted fish

Moribund fish obtained during early and mid April had red inflamed joints at the base of the fins and prominent red hemorrhagic splotches on the abdomen. Upon opening the fish, small hemorrhagic areas were found on the lining of the intestinal cavity and air sac. The stomach and the intestines were almost devoid of content, indicating that the fish had not eaten for some time. The kidneys were abnormally soft and enlarged. The gills sometimes showed regions of local inflammation and mucoid plaques. The afflicted fish often floated vertically at the surface of the lake.

Based upon the appearance of moribund fish it was suspected that the cause of death was a bacterial septicemia. Such a septicemia could be caused by infection by various bacteria including Edwardsiella, Aeromonas, Pseudomonas, or Vibrio species.

Bacteriology

The TSA slants which were inoculated with samples taken from the four specimens of O. mossambicus collected on April 13 all showed profuse growth after 16 hours at 23°C with little or no growth at 37°C. The same species of bacterium was isolated from specimens #2, #3, and #4. Based upon the characteristics listed in Table I and Table II, this bacterium was identified as a species of Pseudomonas (Cowan et al, 1975). Pseudomonas was the only species of bacterium isolated from specimens #2 and #3. A second bacterium Edwardsiella tarda was also isolated from specimen #4. E. tarda has been implicated as a causative agent in hemorrhagic septicemia, but this organism was not isolated from specimens #2 or #3. Furthermore, the physical symptoms exhibited by the afflicted fish were not similar to the common symptoms of edwardsiellosis (Winton, et al., 1983). We suspect this bacterium to be a contaminant picked up during culturing. Multiple bacterial species were isolated from specimen #1 indicating that overgrowth of saprophytic bacteria had occurred before the samples were inoculated. For this reason characterization of the bacteria from specimen #1 has been omitted.

The isolation of Pseudomonas, together with the physical symptoms of the afflicted fish, indicates that the fish suffered from hemorrhagic septicemia brought about by Pseudomonas infection (Bullock, 1965). Due to the fairly rapid passing of the epizootic only four specimens showing the typical physical symptoms of the disease were sampled for bacterial infection.

An inspection trip to the lake on April 20 found only a single lethargic O. mossambicus near the Imong River inlet. No other O. mossambicus were observed in the lake on this trip despite a three hour search. Unlike the afflicted fish found earlier, this specimen lacked cutaneous hemorrhages and its digestive system was full. No bacteria grew on TSA slants inoculated with samples of kidney material or body fluids.

Later on May 10, numerous O. mossambicus, as well as T. zilli and C. ocellaris were observed near the Imong River inlet. During a 10-minute dive an estimated 50 healthy specimens of O. mossambicus were counted. A single abnormal specimen was observed and collected with a hand net. The abnormal fish had difficulty swimming and its body was contorted, however

Table I

Characterization of Isolated Bacteria

	Bacterium A isolated from fish #2, 3, and 4	Bacterium B isolated from fish #4 only
Gram stain	-	-
morphology	short rod	short rod
motility	+ by flagella	+ by flagella
cytochrome oxidase reaction	+	-
acid from glucose	+ (slow)	+
gas from glucose	-	+
hydrolyses starch	-	-
H ₂ S production	-	+
indole production	+	+
growth at 37°	- or very slow	+
lysine decarboxylase	-	+
ornithine decarboxylase	-	+
adonitol fermentation	-	-
lactose fermentation	-	-
arabinose fermentation	-	-
sorbitol fermentation	-	-
dulcitol fermentation	-	-
phenylalanine deaminase	-	-
urease	-	-
citrate utilization	-	-
pigment production	red in old cultures	-

Table II
Antibiotic Sensitivity

Antibiotic test disk used	Bacterium A from # 2, 3, 4	Bacterium B from # 4
streptomycin	+	+
polymyxin B	+	-
chlortetracycline	+	+
chloramphenicol	+	+
erythromycin	+	+
sulfadiazine	-	-
penicillin G	+	-

it lacked the other characteristic symptoms of the disease described above. Again no bacteria grew on TSA slants that were inoculated with samples from this fish. We speculate that this fish may have recovered from the bacterial infection but sustained some neurological damage.

No deaths were reported for T. zilli or C. ocellaris during the epizootic. Only one agar slant, out of the 12 inoculated with samples from four healthy T. zilli showed growth, and that was only a single colony. The organism was neither Pseudomonas nor Edwardsiella and was probably a contaminant. Agar slants inoculated with samples from two C. ocellaris failed to show any growth of bacteria. One thus can conclude that T. zilli and C. ocellaris were free from any overt infection by the organisms afflicting the O. mossambicus.

Frogs and toads often serve as a reservoir of Pseudomonas infection which can afflict fish (Warren, 1981). The common "redleg disease" observed in frogs is a hemorrhagic septicemia caused by a Pseudomonas spp. Fena Lake harbors a large population of Bufo marinus. Many of these toads appeared lethargic and emaciated. However, none of the TSA slants inoculated with samples from five toads showed any bacterial growth, indicating that these toads lacked any overt Pseudomonas infection.

Surveys of fish populations and epizootic progression

Anecdotal reports from Navy and Fena Laboratory personnel indicate that the first signs of abnormality in the lake were observed about April 1, 1984 when sport fishermen reported that "the fish stopped biting". Starting about April 6 dead adult O. mossambicus were observed floating upon the lake in increasing numbers. When we visited the lake during the week of April 8 the remaining fish appeared to be concentrated in the bays near the stream inlets. Large numbers of afflicted fish were floating on the surface of the lake near the southern end. Almost all of the moribund or dead fish were adults of more than 13 cm in length. By April 13 informal fish population surveys found a decreasing number of O. mossambicus and the numbers of dead or moribund fish collected by Navy personnel began to decrease. A further informal survey on April 20 found only a single live O. mossambicus and, as explained above, it did not appear to be affected by the disease. The results of the formal visual surveys of the fish population in the shallow, Hydrilla-choked, area of the lake are shown in Table III. On May 4 no O. mossambicus were seen in the area, but individuals of the other three species were seen, all of which appeared healthy. The data obtained from the visual surveys are useful for noting changes in the relative abundances of fishes in this section of the lake. However, the schools of C. ocellaris appeared to be attracted to the diver and may, therefore, have been over represented in the counts.

By May 25 healthy O. mossambicus were again common in the survey area. It is suspected that the fish must have migrated back into this region from the deeper portions of the lake since the individuals seen were all large adults. It is unlikely that such numbers of large adults could have moved into this region from the streams which were, by now, only a few centimeters deep in many areas. At the date of the last survey, the fish populations appeared to have returned to normal except that no juvenile O. mossambicus were seen. Over 2,300 dead or dying O. mossambicus were removed from the lake by Navy personnel before April 21 when removal efforts were suspended due to lack of further mortalities.

Table III

Counts of four species of fish in one area of Fena Lake. Counts were made by divers equipped with mask and snorkel. Numbers in parenthesis represent the percentage of the total counts for that date.

Date	<u>O. mossambicus</u>	<u>C. ocellaris</u>	<u>T. zilli</u>	<u>A. marmorata</u>
May 4	0 (0)	45 (80)	9 (16)	2 (4)
May 25	34 (27)	50 (39)	23 (18)	1 (1)
June 1	27 (30)	50 (56)	11 (12)	2 (2)
June 8	14 (20)	49 (70)	7 (10)	0 (0)

Lake conditions and sources of possible stress

Disease and subsequent mortality of warm water fishes are frequently attributed to infection with gram negative bacteria. For example, Roberts and Sommerville (1982) report that septicemias caused by gram-negative bacteria are the most common bacterial causes of mortality in Tilapia culture. They emphasize that these diseases are actually a response to stressful conditions, but note that severe outbreaks may occur even in the absence of any identifiable predisposing factors.

We can only speculate on likely forms of stress that could have contributed to the disease outbreak at Fena Lake. We suspect that dissolved oxygen concentrations in and near the dense stands of Hydrilla verticillata may reach very low levels at night and early morning. This is due to respiratory activity of aquatic plants at night and lack of photosynthetic oxygen production. This is commonly known to deplete the oxygen content of ponds to the point where mass mortalities of fish occur. Also, the report on Fena Lake by Kennedy Engineers Inc. (1974) noted low oxygen levels in several areas of the lake.

At the time of the initial fish deaths, the water level in the Fena Lake was low, and the upper littoral regions of the extensive Hydrilla beds were exposed. These beds occupy much of the shallow regions at the southern end of the lake. Water flow into the lake through the various rivers or streams was greatly reduced at this time. Essentially, only the Imong River at the southern end continued to feed water into the lake. Judging from the number of dead fish collected during the die off, the lake contained a large, and possibly excessive, population of O. mossambicus. Fish surveys conducted later indicated that O. mossambicus congregate in the southern half of the lake near the stream inlets. As mentioned above, the oxygen levels within this region of the lake may have decreased at night as a result of the high rates of respiration. This, plus the overcrowding of fish, could have stressed the fish population.

In addition, the passage of a cloud of volcanic haze from the eruption of Mauna Loa in Hawaii greatly decreased sunlight intensity for several days. The reduced light for photosynthesis during the day could also have contributed to lowered oxygen concentrations. Under these unfavorable conditions, Pseudomonas spp., which are normally present in the water and sediments, could have initiated an epizootic infection. If the fish were overcrowded, the disease could rapidly spread through the population. The disease affected only the O. mossambicus population. Although more than 2300 dead O. mossambicus were observed, there were no reports of deaths in the large populations of T. zillii and C. ocellaris that inhabit similar regions of the lake. It appears that these other species were either more resistant to this opportunistic pathogen or that they were under less stress than the O. mossambicus.

Another likely form of stress is also related to the crowding of the fish. Since male O. mossambicus are highly territorial, the falling water level could have resulted in higher densities in the littoral zone and increased territorial aggression among the breeding males.

Some authors (e.g. Bullock and McLaughlin, 1970) have proposed that, although stress is an ultimate cause of bacterial septicemia, there may be an intermediate viral step in the progression of the disease. They propose that stressful conditions initiate a viral infection which leaves the fish susceptible to attack by the normally harmless Pseudomonas which, in turn, may kill the virally infected fish. Although this hypothesis has gained

some support in the literature, no virus has been directly implicated in the disease. Therefore, this hypothesis remains speculative.

Members of Pseudomonas are ubiquitous inhabitants of water and soil. Although some strains are pathogenic to man, they are generally opportunistic pathogens which require reduced resistance, such as might be caused by a burn or wound, before they attack humans. Since these bacteria are normally found in water and soil, their presence in the lake is not viewed as any threat to public health. However, we support the decision of the Navy to close the lake to fishing during the epizootic, and would not recommend that anyone eat fish which appear to be abnormal. Since the epizootic subsided by April 20, fish taken after the first of May are expected to be free from bacterial infection by this organism. However another outbreak could occur under similar conditions if the population of O. mossambicus returns to its previous levels.

Recommendations

Suggested Methods for Preservation of Specimens from Further Outbreaks

In the event of future occurrences of fish mortality, proper collection and preservation of specimens will facilitate identification of the cause of death. Choice of specimens for preservation is important. If possible, moribund specimens should be collected. If this is not possible, the specimens should be collected immediately upon death. A few minutes in the tropical sun can result in damage to the tissues of dead fish, encourage the growth of saprophytic bacteria, and confuse diagnosis. Specimens should be caught with a net and not speared. One should avoid handling the fish as much as possible. The methods of preservation will depend upon the cause of death, which can not be easily determined in the field. Therefore, multiple specimens should be preserved with the use of a variety of methods (Post, 1983).

Chilling: If the specimen is to be sent to a local (Guam) laboratory for diagnosis, it may first be chilled for up to three or four hours. Fish are best chilled by placing them in a waterproof container (such as a plastic bag) and placed in ice or rapidly refrigerated. If death was caused by bacterial infection, the laboratory may be able to isolate the bacterium from the chilled specimen provided that chilling was rapid and that isolation is carried out within 3 to 4 hours.

Freezing: Freezing of fish causes extensive damage to tissues, and often obscures the cause of death. Therefore, freezing is only useful for preserving specimens which are thought to have died of a bacterial infection. Bacteria can usually be isolated from a frozen specimen although cold sensitive pathogens may not survive. Specimens should be placed in a clean, waterproof container and rapidly frozen, preferably with dry ice, or place them directly on the coils of a good freezer unit. A delay in freezing can allow overgrowth of saprophytic organisms which will confuse diagnosis. Specimens must not be allowed to thaw before reaching their destination for diagnosis.

Chemical preservation: Fixation of specimens in a chemical preservative such as 10% formalin (formaldehyde) will preserve the characteristic appearance of the tissues. A fish disease expert should be able to tell much about the cause of death from correctly fixed and preserved specimens. Whole fish less than 5 centimeters (two inches) long can be preserved by immersing them in a 10% solution of formalin. Larger fish should have

their body cavity opened up before immersion in the formalin. To open the body cavity, carefully slit the abdomen open with a clean blade starting near the anus and cutting forward almost to the gills. This must be done carefully to avoid piercing the intestines. Formalin can be poured into the body cavity before the specimen is immersed in the remaining solution. The container must be sealed tightly as formalin solutions have a tendency to leak.

Recommendations for reducing the potential of reoccurrence

The major suspected stress on the fish populations in Fena Lake is the regional depletion of dissolved oxygen in the littoral areas of the lake as a result of respiration and decay of the dense stands of the aquatic weed Hydrilla verticillata. It is recommended that the aquatic vegetation be mechanically harvested and disposed of elsewhere. This should, partially, alleviate the problem of oxygen depletion. Additional changes which may result from the mechanical harvesting of the submerged vegetation are discussed at length by Carpenter and Adams (1977). Among these are a reduction of nutrients in the water column and sediments, a reduction in the rate of sediment accretion, and an increase in water clarity.

The dense beds of submerged vegetation have been noted by previous workers as a major problem in the lake. In fact, the fish were introduced to the lake expressly for the control of these weeds (Brock and Yamaguchi, 1955; Brock and Takata, 1961). As this biological control program has not been effective, a mechanical harvesting program seems prudent.

Recommended Ecological Monitoring Program

If the aquatic vegetation is routinely harvested from Fena Lake, monitoring should be carried out in order to assess the impact of the harvesting program on the lake ecosystem. The monitoring program should include an examination of the nutrient concentrations in the water column and sediments, an assessment of the fish populations in the lake, determination of the dissolved oxygen levels throughout the lake during the early morning hours, examination of sediment cores from the bottom of the lake, and determination of the rate of sediment accretion. The methods and rationale for these measurements are outlined below.

Nutrient determinations: There is some controversy over whether submerged aquatic vegetation acts as a source or a sink for nutrients. Of course, the plants tie up a considerable amount of nutrients in biomass, and this would be removed from the lake ecosystem during the harvesting program. Aquatic plants such as these have been shown to take up nutrients via their roots and some of these nutrients may then be released into the water column via the leaves, and thus contribute to the dissolved nutrient pools in the water column. In addition, decaying plant biomass will release nutrients to the water column through the processes of remineralization. Therefore, after the plants are harvested one may expect to see some changes in the nutrient concentrations in both the water column and the sediments. Nutrients that should be monitored are nitrate, ammonium, total nitrogen, orthophosphate, and total phosphate. The techniques for measuring these nutrients in water and sediments are standard methods now in use at the Fena Laboratory.

Assessments of the Fish Populations: The relative abundances of the fish populations can be determined by visual surveys conducted either by divers or with an underwater video system. There may be some changes in the relative abundances of the fishes of the lake as a result of the mechanical harvesting of the aquatic vegetation. For example, one of the common species in the lake, T. zilli, feeds primarily on aquatic vegetation. Another, O. mossambicus, depends on the organic matter in the sediments as its major source of nutrition. Therefore, the populations of these fishes could be reduced as a direct result of the harvesting program.

The use of a underwater video system for these surveys is suggested since it would minimize the influence of the investigator on the behavior of the fish. The University of Guam Marine Laboratory has recently obtained funds from the Department of the Navy to acquire an advanced underwater video system which would be suitable for this purpose.

Sediment accretion and sediment cores: We suspect that the proposed harvesting program would reduce the rate of sediment accretion in the lake. This rate can be determined with the use of sediment traps constructed from PVC plastic pipes. The UOG Marine Laboratory has used such methods successfully in marine ecosystems. The traps should be placed in a number of locations throughout the lake. This will provide an indication of the present sedimentation rates.

Sediment cores can provide evidence on the nature and degree of the processes of sedimentation throughout the lake's history. By examination of sediment cores one can determine whether sedimentation occurs primarily as a steady rain, has a strong seasonal component resulting from river discharge during heavy rain, or is affected by episodic events such as large storms.

Dissolved oxygen determination: The concentration of dissolved oxygen in the lake is important to the health of the fish populations. The critical period, with regard to the problem of reduced dissolved oxygen, is just before sunrise. The reason for this is that during the night the aquatic vegetation consumes oxygen but does not produce oxygen from photosynthesis. Thus, during the night, both animals and plants consume oxygen. Because the plant beds are so dense the dissolved oxygen concentrations may be driven below the levels required for the survival of fish. The techniques for monitoring dissolved oxygen are quite routine. The most convenient method requires dissolved oxygen probe and meter.

Sampling schedule: The samples should be taken at least once per month. The nutrient and the dissolved oxygen samples should be followed through diel cycles several times during the year. This will be especially important during periods immediately following heavy rains and during periods when the lake level is rapidly dropping. It is also recommended that samples be taken both before and after any mechanical harvesting program is initiated.

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