Diseases of Opakapaka *Pristipomoides filamentosus*

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ABSTRACT

Opakapaka (*Pristipomoides filamentosus*), a highly valued commercial deep-water snapper, is under investigation at the University of Hawai'i through support of the Department of Land and Natural Resources. One endeavor is to develop aquaculture methods for enhancement of wild stocks of this fish and related species, as well as for commercial culture of opakapaka. We conducted a proactive study to elucidate potential diseases that could cause problems in opakapaka held in captivity. A total of 143 moribund and healthy opakapaka were examined in July-August 2001. Fish were wild-caught and then held in seawater netpens or in flow-through circular aquaria. The most common and problematic infection was *Cryptocaryon irritans.* Other external parasites included *Diplectanum opakapaka* (Monogenea) and a Caligus sp. (Copepoda). Common internal parasites were Metanematobothrioides opakapaka (Didymozoidae; Digenea) and a new Goussia sp. (Coccidia). Ichthyophonus sp. and epitheliocytsis were observed in a few fish. Bacterial infections of the swim bladder were detected in several fish, due most likely to degassing of this organ at capture (i.e., deliberate puncture). Exophthalmia, most likely due to barometric imbalances as opakapaka are captured in deep waters, was frequently observed. We concluded that the external monoxenous parasites, especially Cryptocaryon, and possibly the coccidian, pose the greatest potential health threats for this species for aquaculture.

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INTRODUCTION

Culture of opakapaka (*Pristipomoides filamentosus*), a highly valued commercial deep-water snapper, is under investigation at the University of Hawai'i through support of the Department of Land and Natural Resources. The purpose of this endeavor is to develop aquaculture methods for enhancement of wild stocks of this fish and related species as well as the development of this species for commercial aquaculture.

The usual scenario for investigations or identification of serious diseases in aquaculture is that as the culture of a fish species expands, devastating economic and biological losses due to "new" or "unknown" diseases follow, and then research is conducted to identify their cause and develop methods for their control. In contrast, the purpose of this study was to provide a proactive approach to identify potential health problems that may be encountered in opakapaka before large-scale culture of this species is underway.

Before this study, we observed infections by Cryptocaryon irritans, a common parasitic ciliate that is well recognized as a serious pathogen in warm water mariculture. An idiopathic eye disease presenting as gas emboli around the globe was also recognized as a common problem in captive opakapaka at this facility. Certainly these represent only two of many other health problems that will be encountered as successful breeding and rearing of this species is achieved, and subsequent captive production is increased. We, therefore, conducted a more thorough investigation to elucidate present and potential disease problems in opakapaka held in captivity.

MATERIALS AND METHODS

A total of 143 moribund and healthy opakapaka were examined in July-August 2001. Fish represented those held in seawater netpens, and flow-through circular aquaria kept at the Hawai'i Institute of Marine Biology (HIMB) at Coconut Island, Oahu. Fish in the tanks or netpen categories had been previously captured by hook and line, and were held in captivity for up to about 45 days. Six of the fish held in tanks came from a diet study. Eleven fresh wild caught fish included in the study were examined immediately after capture or after being held in captivity for a maximum of 3 days. Fifteen of the fish from tanks were collected as moribund fish early in July 2001 and tissues were preserved without macroscopic examinations. Twelve of the fish examined from tanks represented specimens from a diet study. The fork length of the fish held in netpens and tanks was 16.5- 42.5 cm (x = 19.4 cm), while wild fish were much larger (40-65 cm, x = 57.5 cm).

Fish were euthanized with an overdose of MS-222. Wet mount preparations of the gills and skin scrapings were examined by light microscopy. Then the fish were dissected and examined. Representative organs - eyes, gills, skin with muscle, heart, liver, kidney, spleen, and intestine were preserved in Davidson's solution and processed for histology.

RESULTS

Results from necropsies and histological evaluations are summarized in Table 1. Color images of the lesions and pathogens described here can be found at http://www.soest.Hawai'i.edu/SEAGRANT/opakapaka/opakapaka.html

Table 1. Prevalence of major diseases or infections in opakapaka *Pristipomoides* filamentosus held at Coconut Island. Number infected or affected/number examined (%).

Disease or Infection	**Total**	**Tanks**	**Netpens**	*Fresh caught*
Exophthalmia*	35/128(27)	10/47(21)	25/70(36)	0/11
Swim bladder inflammation*	23/128 (18)	13/47(28)	10/70(14)	0/11
Cyst of unknown origin	2/143 (1)	0/62	1/70 (1)	1/11(9)
Epitheliocystis	2/143 (1)	1/62 (2)	0/70	0/11
Goussia sp. (surface type)	6/143 (5)	3/62(5)	3/70(4)	0/11
Cryptocaryon irritans	35/143 (24)	35/62(56)	0/70	0/11
Ichthyophonus†	4/143(3)	3/62(5)	1/70(1)	0/11
Didymozoid digenean	31/143 (22)	9/62(14)	15/70(21)	7/11(64)
Monogenea (e.g., *Diplectanum*)*	11/128(9)	8/47(17)	3/70 (4)	0/11
Caligid Copepods*	34/128(27)	34/47(72)	0/70	0/11
Encysted helminths ‡	44/143(31)	21/62(34)	16/70(23)	7/11(64)

* 15 fish were not included in analysis as samples were evaluated only by histology, and swim bladder or eyes were often absent in collection.

† One fish exhibited intact *Ichthyophonus* parasites, while three had degenerative structures consistent with that organism.

‡ Based on both macroscopic observations of white nodules in viscera and histology. Note that presence of macroscopic "white nodules" was not always reported, and thus prevalence is probably higher.

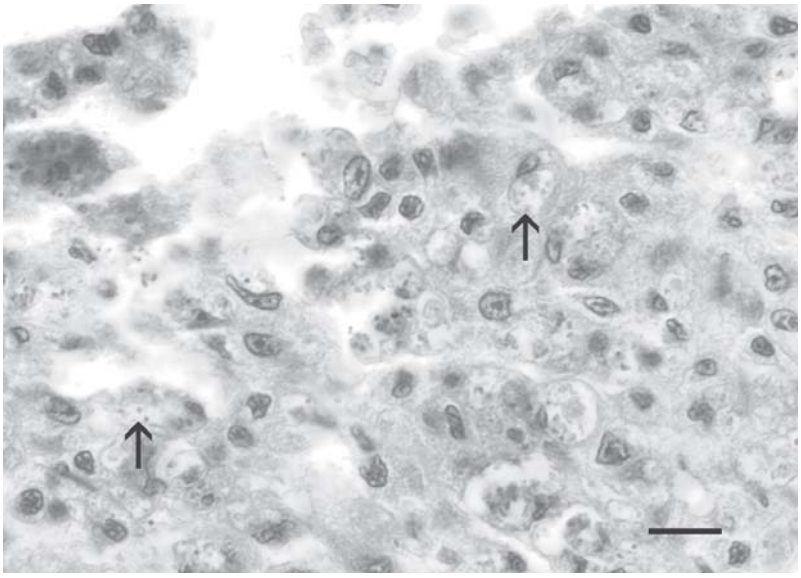

Figure 1. Intracellular piscirickettsia-like organisms (arrows) in macrophages. Bar = 10 µm.

Swim bladder infections and inflammation

Several moribund fish exhibited septic swim bladders. At necropsy, the swim bladder wall was opaque, and the swim bladder often contained an opaque, viscous to caseous, whitish or yellow exudate. Histologically, the lesions were characterized by severe, chronic inflammation. Masses of presumptive Gram-negative bacilli were observed in most lesions, and one fish exhibited a fungal infection of the swim bladder. The most interesting finding regarding swim bladder infections was the presence of piscirickettsia-like organisms within macrophages in the swim bladder of two affected fish (Fig. 1).

Eye lesions

Many fish exhibited eye lesions, generally characterized by exophthalmia (pop-eye), with gas bubbles in or around the eye. Representative samples were evaluated by histology. In general, the eyes exhibited gas bubbles (emphysema) and hemorrhage in the retrobulbar vascular plexus. Many of these eyes subsequently developed chronic inflammatory lesions.

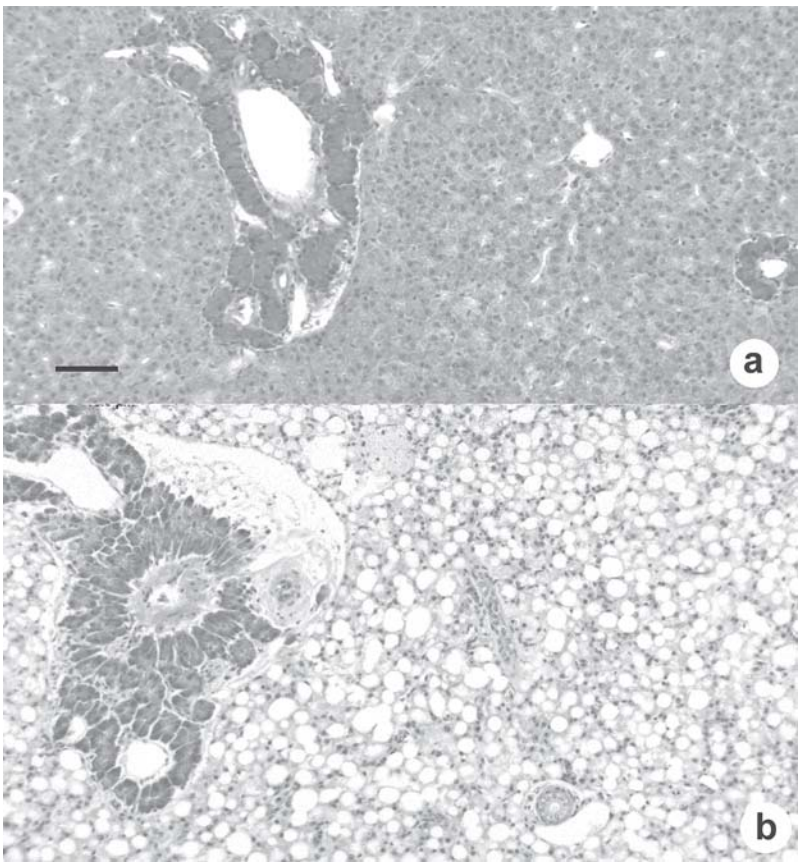

Figure 2. Opakapaka livers. **a.** normal liver. **b.** fatty liver, note hepatocytes filled with clear vacuoles representing fat. Bar = 50 µm.

Fatty liver disease

Hepatic lipodosis (fatty livers) was observed in several of the fish from diet studies. Fish with this condition exhibited diffuse lipid accumulation in hepatocytes, while these cells were otherwise normal. Fig. 2 contrasts normal livers with affected livers. This condition was observed in fish from an experimental diet study.

Epitheliocystis

Two fish exhibited light epitheliocystis infections of the gills.

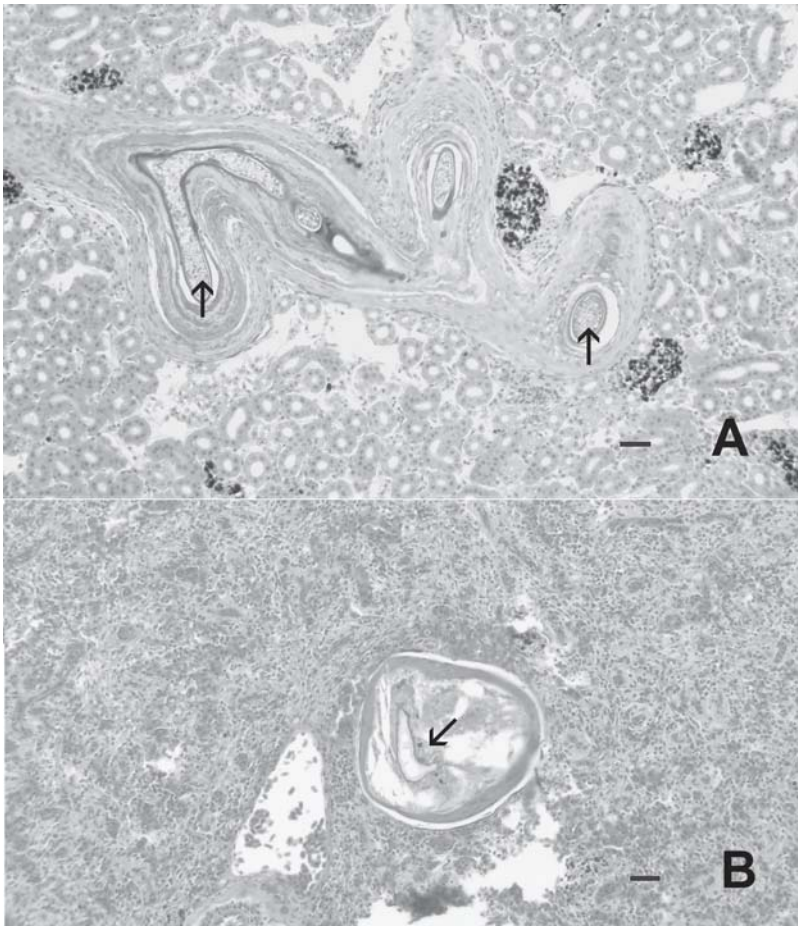

Figure 3. *Ichthyophonus* in opakapaka. **A.** Inflammation and fibroplasia around intact organism (arrows). Note fungus-like appearance of parasite. **B.** Possible degenerated organism (arrow) in lesion. Bar = 50 mm.

Ichthyophonus

One fish exhibited infection by *Ichthyophonus* sp. (Fig. 3). In addition, three other fish exhibited degenerative lesions that we suspect were resolved *Ichthyophonus* infections, in which remnants of presumptive hyphal walls were seen in the lesions.

Cryptocaryon irritans

Gill infections associated with clinical disease were observed in fish held in tanks, but not in those held in netpens at the HIMB (Table 1). Many fish from tanks exhibited early *Cryptocaryon* infections in which the parasite was present but was not associated with significant pathological changes. However, fish that were moribund showed severe lesions consistent with the disease - e.g., severe, diffuse epithelial hyperplasia of the gills.

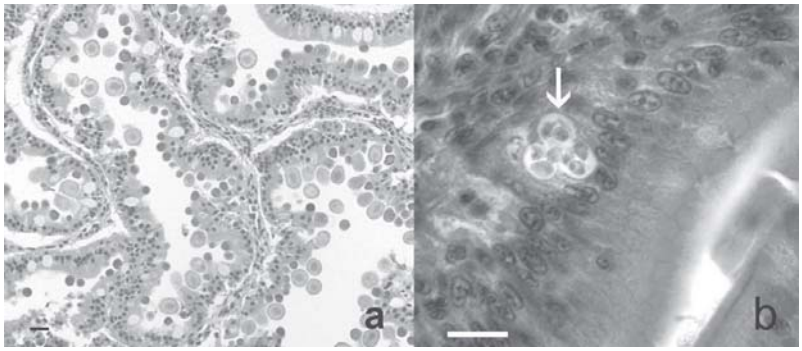

Figure 4. Coccidians in opakapaka. **a.** Intestinal epithelium-surface type found in several captive fish. Note numerous developmental stages, including macro- and microgamonts. No sporulated oocytes were observed. **b.** A different species occurring deep within the epithelium and lamina propria. Arrow = sporulated oocysts in the epithelium. Bar = 10 mm.

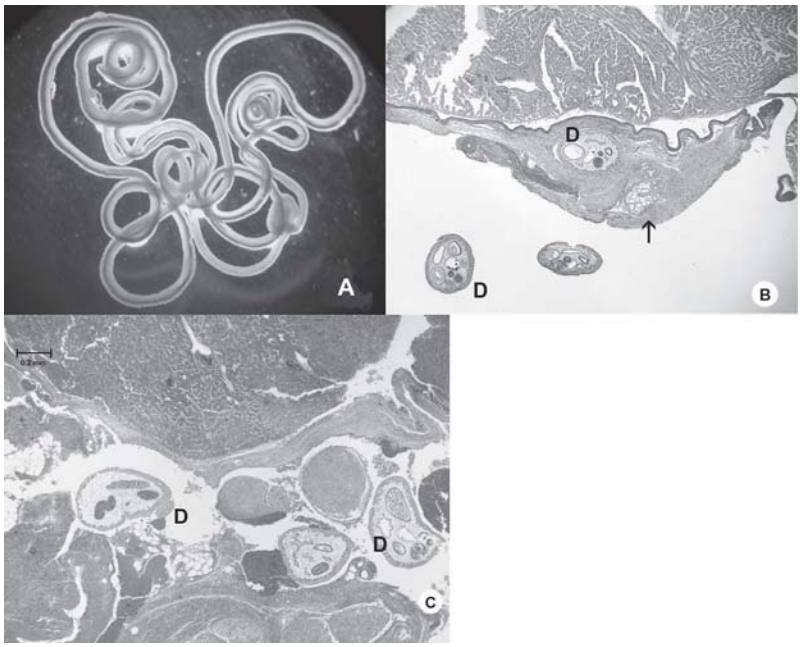

Figure 5. Didymozoid digenetic trematode *Metanematobothrioides opakapaka* from visceral organs. **A.** Whole worm dissected from tissues. **B.** Histological sections of worms in pericardium of heart. Arrow = inflammation surrounding worms (D). **C.** Cross sections of worms (D) in viscera near kidney.

Goussia spp. (Coccidia)

We observed a coccidian species, most likely a *Goussia* sp., infecting the surface region (brush boarder) of the intestinal epithelium. Many fish were infected, and two moribund fish showed severe infections (Fig. 4). Fish with heavy infections exhibited enteritis and atrophy of the infected epithelium. A second Goussia sp. was observed in one fish from the tanks (Fig. 4). In this case, the organism developed deep within the epithelium and occasionally in the lamina propria of the skin. In addition, in contrast to the other Goussia species from opakapaka, sporulated oocysts were frequently observed.

Helminths

Two species of Monogenea were observed on the gills; *Diplectanum opakapaka* and *Pseudodiscocotyla opakapaka.* Infections were light to moderate and were not associated with significant gill lesions or disease.

One didymozoid trematode *(Metanematobothrioides opakapaka)* was frequently observed. Trematodes of the family Didymozoidae are unusual digeneans in that the adult stages are extraintestinal in marine fishes (whereas most other adult digeneans occur in the gut lumen). The worms were large and elongate (up to about 100 mm), and thus superficially resembled nematodes. They were often observed in the gill chamber, heart, or near the anterior kidney. Histological sections of the infected tissue revealed little tissue reaction. The most severe lesion was moderate, chronic epicarditis of the heart in one fish (Fig. 5).

Larval cestodes were observed in the intestinal lumen of some fish, and were not associated with severe pathological changes. Encysted metacercariae of trematodes were occasionally observed in tissue sections. In addition, many granulomas were observed in tissue sections that contained debris, which may represent degenerated parasites. As the contents of the cysts were severely degenerated (encapsulated spheres with necrotic centers), specific identifications were not possible.

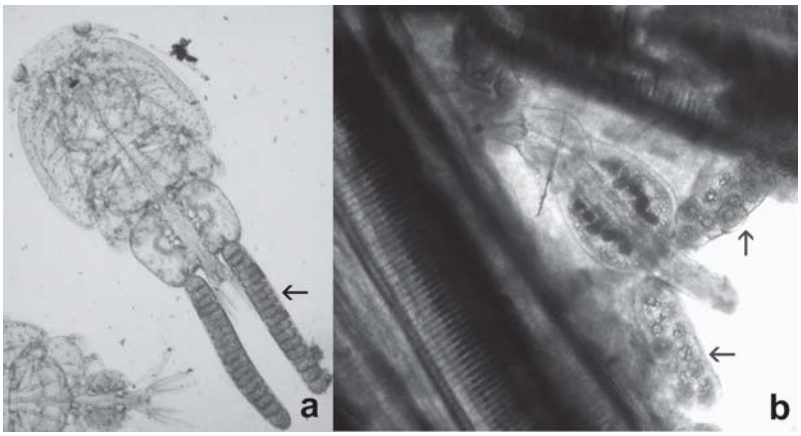

Figure 6. Copepods from opakapaka. **a.** Caligus sp., a common parasite of captive fish. **b.** Lerneopodid copepod on gills of wild-caught opakapaka. Arrows = egg sacs at posterior end of female copepods.

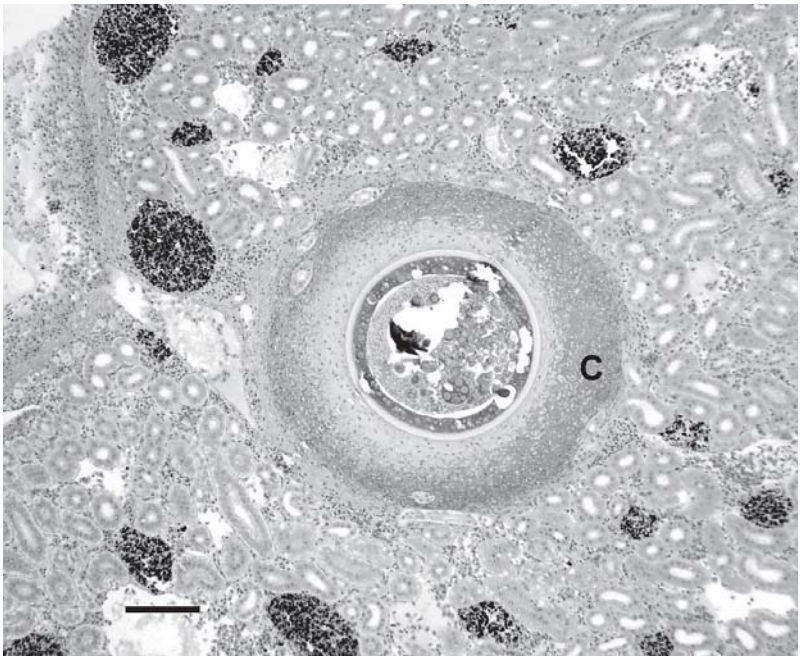

Figure 7. Cyst of unknown origin surrounded by metaplastic cartilage (C) in kidney.

Copepods

Two types of copepods were observed. A *Caligus* sp. was frequently found on the skin of captive fish and an attached lernaeopodid (a member of the family Lernaeopodidae) was found on the gills of wild caught fish. (Fig. 6). Dr. Z. Kabata, (Fisheries and Oceans Canada, Pacific Biological Station, Nanaimo, British Columbia, Canada) kindly examined photographs of the latter and stated that they likely represent a new genus. Dr Z. Kabata also examined the preserved specimens of the caligids and these may represent a new species.

Cysts of unknown origin

These enigmatic structures, also referred to as "unidentified fish objects" or "UFOs" were observed in the kidney of one wild-caught fish and in the gills of one fish from the netpens. They were associated with only local tissue reactions, including prominent cartilage metaplasia (Fig. 7).

DISCUSSION

Infections of the swim bladder were likely due to iatrogenic causes, i.e., associated with introduction of contaminated hypodermic needles during de-gassing of swim bladders immediately after capture, or due to a tract from the skin surface being created by the needle. Both would allow bacteria to infect the swim bladder. Wada *et al*. (1993) described mixed infections by an acid-fast bacterium and an imperfect fungus in a marine tropical fish, the Napoleon fish (*Cheilinus undulatus*).

A similar situation resulting in fungal infections has been reported in captive red snappers *(Lutjanus campechanu)* by Blaylock *et al*. (2001). Considering the severity of the swim bladder bacterial infections, it was remarkable that this did not progress to septicemia. Based on our experiences, such progression would be expected with similar infections in other fish species. This suggests that opakapaka held in captivity have a very competent immune system that is capable of containing severe infections. Most infections of the swim bladder were caused by Gram-negative bacilli. However, two fish showed intracellular bacteria suggestive of *Piscirickettsia. Piscirickettsia salmonis* and related organisms have caused severe disease in aquaculture species, including pen-reared salmon, white sea bass, and tilapia (Fryer and Lannan, 1996). Such infections have been observed in tilapia reared at HIMB, but their relationships to the organism in opakapaka is unknown.

Eye lesions were also common. As with the swim bladder lesions, the underlying cause is probably iatrogenic i.e., due to gas bubble disease induced by transporting the fish to the surface after capture in deep waters. However, spontaneous exophthalmia associated with gas bubbles in the eyes was recently reported in West Australian dhufish (*Glaucosoma hebraicum*) that were reared from hatching in captivity as well as in wild-caught fish (Stephens *et al*., 2001). Moreover, we have seen an identical condition in ling cod (Ophiodon elongatus) that were hatched in captivity. Stephens *et al*. (2001) concluded that the likely cause of the lesions in captive-reared dhufish (and some other deep-water marine fishes) was a reduced ability to adapt to rapid changes in activity patterns or water quality differences, even if hatched in captivity. Stephens *et al*. (2002) suggested that susceptibility of captive dhufish to exophthalmos may be related to oxygen transport problems in the retina due to intracellular acidosis in erythrocytes. For opakapaka, rearing fish in surface-water netpens or tanks would represent a situation with lower water pressure and warmer water temperature than their normal environment.

We observed several other pathogens that were probably not related directly to captivity. Epitheliocystis was observed in only two fish, and both had light infections. This infection is common in wild marine fishes, and has occasionally caused disease in aquaculture (Lannan *et al*., 1999). The disease is particularly problematic in cultured bream (*Sparus aurata*) (cf. Paperna, 1977) and sea bass (*Dicentrachus labrax*) (cf. Crespo *et al*., 2001).

Ichthyophonus is common in wild marine fishes and occasionally is problematic in fish culture (McVicar, 1999), and we detected the organism in a few fish. Previously the parasite has been considered a fungus, but molecular systematics suggest that it is related to the choanoflagellates (including *Dermocystidium* and the rosette agent of salmon). The infection is cosmopolitan, and has been reported from cold and warm-water marine fishes. We recently used small subunit rDNA sequences to study the phylogenetic relationships among Ichthyophonus hoferi isolates from various hosts in the northeastern Pacific Ocean (Criscione *et al*., 2002). This study suggests that *Ichthyophonus hoferi* may actually represent an assemblage of morphologically similar species, and perhaps those infecting tropical and subtropical fishes are different from those of herring and other cold-water species. Although *Ichthyophonus* lesions in opakapaka were mild and only a few fish were infected, this infection should be monitored, as it has the potential to spread in culture situations and can be highly pathogenic.

Crytocaryon was the most significant pathogen. This ciliate is a well-recognized serious pathogen in warm-water marine aquaculture and aquaria (Dickerson and Dawe, 1999). It is difficult to treat with routine bath chemotherapeutics as it has an off-host stage and the on-host stage (trophont) embeds under the skin and gill epithelium. Moreover, the use of external baths to treat parasites in large, extensive aquaculture operations, such as netpens, is usually impractical due to costs and problems associated with environmental contamination. This ciliate already is recognized as a potential cause of high mortalities in tank-held opakapaka at HIMB. Interestingly, infections in netpen-held opakapaka appear to be absent (Table 1), possibly due to flushing of the off-host stages of the parasite from the pens and relatively low density of fish in netpens compared to tanks. One of us (A. Moriwake) conducted treatment experiments and found that transferring fish to a new tank every 3 days for 3 cycles was effective for controlling infections (see (http://www.soest.Hawai'i.edu/SEAGRANT/opakapaka/opakapaka.html for details of this study).

Many species of *Goussia* and other coccidians infect fishes, and some are pathogenic in aquaculture (Molnár, 1999). The gut epithelium is a primary site of infection by Coccidia, in both fishes and other vertebrates. Infections at the surface of epithelial cells, as seen here, are similar to *Cryptosporidium* infections of mammals, and this type of *Goussia* infection in fish may likewise be pathogenic. For example, Goussia (= *Epieimeria*) *anguillae* is a pathogen of eels (Lacey and Williams, 1983), and we described chronic mortality associated with a wasting syndrome in opaleye (*Girella nigricans*) due to *G. girellae* held at the Scripps Aquarium, San Diego (Kent *et al*., 1988). Therefore, this infection in opakapaka should be considered as a potential problem and should be monitored.

Although probably a different species, the infection and pathogenic changes caused by the coccidian that occurred deep within the gut mucosa were suggestive of *Goussia carpelli* infections in carp and goldfish. In this infection, "yellow bodies" are formed around the oocysts, and the parasite causes atrophy of the gut and a wasting syndrome (Kent and Hedrick, 1985).

Gill and skin infections by monogenean worms are well-recognized problems in marine and freshwater fishes held in captivity. Two species of Monogenea were observed: *Diplectanum opakapaka* and *Pseudodiscocotyla opakapaka.* Infections by both monogeneans in the present study were light and not associated with significant gill lesions. Another *Diplectanum* species, *D. latesi,* has caused mortality in captive sea bass (*Lates calcarifer*) (cf. Rajendran *et al*., 2000). Monogeneans can usually be eradicated with baths containing formalin or organophosphates, but the use of these compounds in netpens or other large-scale operations may be impractical. Furthermore, we have found formalin ineffective for treating these infections on opakapaka. We found that the best way to control large monogeneans (presumably members of the family Capsalidae) on the skin of opakapaka was to pick them off individually using forceps. A treatment of 15 min in fresh water appears to be close to the maximum amount of time that opakapaka can tolerate.

Digenetic trematodes are usually less problematic in intensive aquaculture because they require an intermediate host and do not replicate in fish. However, the didymozoid infection was prominent and common as all the fish in the present study were wild caught. Therefore, the impact of the infection on the overall health of these opakapaka is unknown.

Caligid copepods can be extremely problematic in netpen culture i.e., *Lepeophtheirus salmonis* is one of the most important problems in the netpen farming of salmonids in Europe (Boxshall and DeFaye, 1993). However, to date, we have not associated significant disease with the caligids on captive opakapaka. The use of both formalin (25-100 ppm) and freshwater baths was effective in eliminating *Caligus*. However, it should be noted that opakapaka showed signs of distress (e.g., head popping out of the water) when treated with higher doses (75 and 100 ppm) of formalin. After one min in freshwater bath, no parasites were observed on the fish (see, e.g., http://www.soest.Hawai'i.edu/SEAGRANT/opakapaka/opakapaka.html).

Fatty livers are often seen in cultured fish fed inappropriate diets (e.g., excessive fat). The long-term effects of this condition are unknown, but this change is usually reversible. It is likely that these liver changes were due to inappropriate diets, as the fish with the condition were from an experimental diet study.

The enigmatic structures referred to as "unidentified fish objects" or "UFOs" are rather common in marine fishes (MacLean *et al*., 1987). We observed these in the gills of one fish and the kidney of another. They generally are associated only with local tissue reactions, including prominent cartilage metaplasia (Heidel *et al*., 2002). Some researchers have proposed that these might represent ectopic eggs, but they have also been observed in male fish. Therefore, at present, the consensus of most researchers is that they are degenerated microorganisms of unknown identity.

In conclusion, this survey confirmed previous observations of aquaculturists that *Cryptocaryon irritans* may be a serious pathogen of opakapaka. Other parasites such as the coccidians (*Goussia* spp.), monogeneans and copepods should be monitored, as related species are recognized causes of disease in marine aquaculture. These parasites are monoxenous (one host) and thus may proliferate in or on fish in captivity. Interestingly, the external parasites were much more prevalent in fish held in tanks, suggesting that the netpen environment was more favorable for avoiding proliferation of these potential pathogens in captive fish.

Bacterial infections and associated inflammatory changes in the swim bladder were very likely the result of de-gassing captured fish by insertion of a hypodermic needle into the swim bladder, and thus at this point the bacteria are considered as only opportunists. Likewise, exophthalmia was consistently associated with gas emboli in the eyes, again probably associated with collecting procedures. However, other causes should be considered if this condition arises in captive-reared fish.

ACKNOWLEDGEMENTS

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