Tetrahymenosis, Columnaris Disease and Motile Aeromonad Septicaemia in Golden Perch, *Macquaria ambigua* (Richardson), from Australia

BRETT HERBERT [1,2] and P. GRAHAM[2]

[1]*James Cook University of North Queensland, Australia*
[2]*Freshwater Fisheries and Aquaculture Centre, Kennedy Highway Walkamin 4872, Australia*

ABSTRACT

Golden perch cultured in earthen ponds in northern Australia suffered severe mortalities in two successive winters from infections with *Tetrahymena corlissi* and motile aeromonads. Skin lesions were initially detected as small red pinpoint lesions that rapidly increased in size to large round, deep skin ulcers with hyperaemic margins within 3 to 4 days. Laboratory trials of chemotherapeutics done *in vitro* on fish infected with *T. corlissi*, included potassium permanganate, malachite green, methylene blue and salt, formalin and salt, and copper sulphate, and were ineffective in killing *Tetrahymena corlissi.* The aetiology and progress of the disease suggests that golden perch was naïve to the local strain of *T. corlisii*, increasing its pathogenicity and speed of ulceration, as there was poor immune response to initial invasion by *T. corlissii* through dermal tissue. Infection with *T. corlissii* responded well to treatment with Emytryl (400 mg/g dimetridazole), a systemic protozooicide, administered orally at 30mg/kg/fish/day. This drug was effective within 3 days of treatment as mortalities ceased in ponds, and healing of ulcers was observed in fish in tanks.

Herbert, B. and Graham, P. 2008. Tetrahymenosis, columnaris disease and motile aeromonad septicaemia in golden perch, *Macquaria ambiqua* (Richardson), from Australia, pp. 179-192. *In* Bondad-Reantaso, M.G., Mohan, C.V., Crumlish, M. and Subasinghe, R.P. (eds.). Diseases in Asian Aquaculture VI. Fish Health Section, Asian Fisheries Society, Manila, Philippines. 505 pp.

Corresponding author: Brett Herbert, Brett.Herbert@biosecurity.gov.au

INTRODUCTION

The disease status of golden perch has been well documented (Rowland and Ingram, 1991), due to their widespread use in fishery enhancement programs. However, to date, there has been no report of *Tetrahymena sp*. infestation in this species. *Tetrahymena spp.* are ubiquitous free-living hymenostome ciliates, that cause disease in a wide variety of fish, crustaceans, amphibians and turbellarians (Hoffman *et al.*, 1975; Wright, 1981; Lom, 1984; Ferguson *et al.*, 1987; Lom and Dyková, 1992; Edgerton *et al.*, 1996; Ponpornpisit *et al.*, 2000). *Tetrahymena corlissi* recorded primarily as a parasite of freshwater tropical fish and amphibians, is histophagous, and can destroy surface tissue, invading the skin, skeletal muscle and internal organs, causing mass mortalities in aquarium fishes (Lom and Dyková, 1992; Imai *et al.*, 2000; Wakita *et al.*, 2002). *T. corlissi* has been associated with gross clinical signs of epidermal sloughing, raised scales, skin ulceration and lesions, and skeletal muscle inflammation and necrosis. Clinical disease is rapid in onset and often fatal (Lom and Dyková, 1992; Imai *et al.*, 2000; Wakita *et al.*, 2002).

Motile aermonads are gram negative bacteria, ubiquitous in aquatic ecosystems. These bacteria are routinely isolated as both normal flora and as primary or secondary pathogens from sick or moribund fish. Motile aeromonad septicaemia may cause acute or chronic infections in many different species of freshwater fish. The bacteria responsible (*Aeromonas caviae*, *A. hydrophila* and *A. sobria*) all produce extracellular enzymes and toxins causing cell lysis and necrosis (Roberts, 1993). The disease is strongly correlated with stress, overcrowding, poor hygiene and stress-mediated immunosuppression (Toranzo *et al.*, 1987; Roberts, 1993; Thune *et al.*, 1993). Previous motile aeromonad septicaemia (*A. sobria*) of golden perch at the Freshwater Fisheries and Aquaculture Centre (FFAC) had symptoms of petechial haemorrhage on the ventral surfaces of the fish, disoriented swimming behaviour, and septicaemia leading to death in 4-5 days (unpublished observations).

Columnaris disease caused by *Flavobacterium columnare* has been documented worldwide in over 36 species of freshwater fishes including cultured species, barramundi *Lates calcarifer* (Bloch), channel catfish *Ictalurus punctatus* (Rafinesque), tilapia *Oreochromis* sp. and ornamental fish including goldfish *Carassius auratus* (L.) [Carson *et al.*, 1993; Soltani *et al.*, 1996; Plumb, 1999]. Gross lesions typically include gill necrosis, skin ulceration, jaw erosion and fin and tail rot to varying degrees (Ullrich, 1992; Clayton *et al.*, 1998). Columnaris disease at FFAC is usually manifested as a saddleback lesion across the body, usually posterior to the second dorsal fin (Mosig, 2002). Outbreaks of columnaris disease are associated with environmental factors including low water temperatures, crowding, high organic loads, handling, poor nutrition and stress (Chowdhury and Wakabayashi, 1991; Wakabayashi, 1991; Carson *et al.*, 1993; Soltani and Burke, 1994; Altinok and Grizzle, 2001; Shoemaker *et al.*, 2003).

In two successive winters of 2001 and 2002, golden perch grow out trials at FFAC were devastated from mass mortalities of fish from infections with *Tetrahymena*, motile aeromonad septicaemia and columnaris disease. This paper describes the gross, histology, microbiology and parasitology findings from golden perch affected by these diseases.

Results from chemicals used in ponds and *in vitro* trials in tanks against *Tetrahymena corlissi* are also presented.

METHODS AND MATERIALS

Growth Trials

Growth trials were conducted on golden perch, in 2000/2001 and 2001/2002. Two groups (A and B) were grown in 2000/2001, and one group (C) in 2001/2002. All ponds used in the growth trials were 350 m^2, with a volume of 230 m^3, lined with polyethylene, and fully netted and fenced to exclude predators. Ponds were aerated with submerged perforated polyethylene pipes. Aspirators provided supplementary aeration and mixing, as required during pond chemotherapy treatments. Pond preparation prior to stocking included removal of organic matter, liming with agricultural lime at 1.2 tonnes/ha and dry-out for one month. Incoming water was filtered with 500µm screens. Water quality parameters in ponds and in tanks measured daily included pH, pond water temperature, and dissolved oxygen. All dead fish and sick fish were removed from ponds daily and recorded. All fish were fed daily to satiation with a sinking barramundi grower diet (Nutreco Pty. Ltd., Rosny Park, Tasmania) (43% protein, 15% lipid, 22% carbohydrate, 11% ash). Feed trays were used to monitor feed consumption.

Group A: In March 2000 two different strains of fingerling golden perch (Murray-Darling strain and Fitzroy River strain) were purchased from a commercial hatchery (30-50 mm TL). The two strains were weaned in tanks before transfer to ponds in May. Water lettuce (*Pistia stratiotes*) was introduced from local ponds in December 2000 for algal control and to provide fish with shelter. In April 2001, fish were graded and moved into 4 new ponds (Table 1). Fish were again graded in April 2001 and moved into newly prepared ponds, along with the water lettuce. Water was sourced from a ground water well. Maximum biomass in each pond was 323 g/m^3, and density was 1.3 $fish/m^2$.

Group B: Fingerlings of *Macquaria ambigua* arrived at Walkamin on 24 Nov 2000, and

Table 1. In plastic chemotherapeutic trials. As all fish had severe lesions, not all survived for the full length of the trial. At least two fish from every trial were alive at the end. None of these trials were effective in stopping division of T corlissi or in killing them'.

Chemical	Concentrations	Duration	Method
$KMnO_4$	1, 5, or 15 mg/L	1 and 20 hrs	20L bucket
Malachite green	0.1 or 1 mg/L	1 and 20 hrs	20L bucket
Methylene blue and salt	1 or 3 mg/L MB 10‰ salt	1 and 20 hrs	20L bucket
Formalin and salt	70 mg/l formalin salt (12‰)	1 and 5 hrs	20L bucket
$CuSO_4$	25 mg/l	1 hr	20L bucket
Salt	10‰	5 to 7 days	100L tank

were weaned and stocked into nursery ponds on 22 Dec 2000. These were harvested, graded and stocked into new ponds on 14 May 2001. Fish were sampled with a seine net for measurements once every two weeks during nursery. During grading fish were treated with a salt bath (12‰) salt for 60 to 90 minutes. They were then sedated, caught and transported back to new ponds in 10‰ salt water, on 15-17 May. Fish from all ponds were mixed for statistical purposes, to have a complete mix of fish from a previous experiment. Ten ponds were stocked, with maximum biomass of 244 g/m^3 and maximum density of 2 fish/m^2.

Group C: Fingerlings of *Macquaria ambigua* arrived at Walkamin on 1 Nov 2001, and were weaned, and 1266 were stocked into each of 6 ponds on 19 Dec 2001. These ponds had been treated with 20mg/L chlorine and then sun dried for 6 months. Fish were sampled once every four weeks. These ponds were treated with Platypus Probiotic (International Animal Health products Pty. Ltd., Huntingwood, Australia)[1], a program of weekly treatments administered over a four-week cycle to introduce large numbers of beneficial *Bacillus sp*. bacteria into the water. The probiotics were administered according to the manufacturer's instructions. Maximum biomass achieved was 1.1 kg/m^3, and density was 3.95 fish/m^2.

Gross pathology, microbiology and histology

From the outset and throughout all infections, gross observations and fresh scrapes and smears were taken from sick and undamaged fish at least weekly to assess progress of infections, check efficacy of therapeutic treatments, and to ensure that the same infection was present throughout the course of the epizootics. In May 2001, January and July 2002 samples (each sample n=5-10) moribund golden perch from these epizootics were submitted to Oonoonba Veterinary Laboratory (OVL), for gross, histological, parasitological and microscopical examination. In July 2002, further samples of golden perch with 3 differing types of skin lesion were submitted for histological examination to determine the aetiology of each type of lesion.

In January 2002, a sample of dead tadpoles of the native frog *Litoria microbelos* found in a pond containing several dead golden perch were also submitted for gross and histological examination, as it was suspected the amphibians may be carriers of *Tetrahymena.*

In June 2001, fish with grossly visible small red 'pinpoint' skin lesions, 1 to 2 mm diameter, were observed to monitor progression of the skin lesions over time.

Fish and tadpoles were sacrificed, organs and tissues including skin, gills, brain, heart, liver, head and caudal kidney, spleen, pancreas, eye, stomach, intestine, abdominal fat, swim bladder, bladder, skin and skeletal muscle were fixed in Bouin's fixative for 24 hours then processed routinely for histology (Bancroft and Stevens, 1990).

[1] Use of this product does not indicate or imply endorsement

Wet mount preparations, parasitology and microbiology

Movement and morphological characteristics of live protozoan parasites observed on wet mount preparations were studied for species identification at both FFAC and OVL. Air dried skin smears were either stained with Klein's dry silver impregnation method (Lom and Dykova, 1992) or preserved in Bouin's fixative, and sent to Dr. Peter O'Donoghue (University of Queensland Department of Parasitology) for species confirmation. Skin scrapes were taken from the leading edge and from deep within the centre of skin ulcers and plated onto Sabourads dextrose agar (SDA) with added chloramphenicol and gentamycin, and marine agar with added thiamine (MAT) for fungal isolation and culture. Swabs were taken from the skin ulcers, heart and caudal kidney of several fish with skin ulcers, and plated onto sheep blood agar (SBA) and marine agar with added vitamins (MAV) for bacterial isolation and culture.

Pond treatments and *in vitro* chemical trials

Chemicals recommended by Boyd (1982), Kabata (1985) and Noga (1996) were added to ponds containing affected fish for the treatment of the *Tetrahymena* infection. Four ponds were treated with 30mg/L formalin, then 8 days later with 7 mg/L potassium permanganate ($KMnO_4$), then 3 days later $KMnO_4$ at 5 mg/L. $KMnO_4$ demand was calculated according to Boyd (1982). Two ponds were treated with 20ml/L formalin, four days later 30ml/L formalin, then five days later 7 mg/L $KMnO_{4,}$ the 3 days later 5 mg/L $KMnO_4$. Samples were taken approximately six hours and 24 hours after each treatment from each pond and wet smears from scrapes examined. Prior to chemotherapy application the water level was dropped and an aspirator placed in each pond, to increase mixing and dissolved oxygen levels. Inflow water was added to refill the pond, and the chemical administered to the inflow water to ensure mixing of the chemical in the pond water.

Several chemicals recommended by Boyd (1982), Kabata (1985) and Noga (1996) for the treatment of ectoparasites were tested on infected fish from the 2001 outbreak as small volume treatments. For each chemical tested, 5 small (<50g) fish with skin ulcers were placed in an aerated 20 l plastic bucket containing the chemical. At the end of each chemical treatment, skin scrapes were taken from the edges and at the centre of skin ulcers of each fish, wet mounts were prepared and observed for the presence and activity of *Tetrahymena sp.* under a light microscope.

In June 2002, fish in ponds affected with tetrahymenosis were administered Emtryl Soluble (400 g /kg dimetridazole soluble powder, Rhône-Poulenc/ Aventis Animal Nutrition Pty Ltd) at a dose rate of 30 mg dimetridazole/kg/fish for 10 days. Emtryl was dissolved and sprayed onto the food with gelatine. Gelatine was used to help the drug stick to the pellets and increase retention time on pellets while in the water. Feed consumption was monitored daily using feed trays. Several fish affected with tetrahymenosis were taken out of ponds and placed in 200L tanks and similarly administered with Emtryl for observational purposes to assess healing of skin ulcers and health in response to administration of the antiprotozoal. Skin scrapings were then done from the edge and centre of skin ulcers to determine whether *T. corlissi* was still present.

RESULTS

Mortality

The first mortalities occurred in April 2001, one day before fish were handled, graded and transported to new ponds. Mortality rates of affected fish in the 14 ponds in 2001 varied from 32% to 86%, with an average of 40% ± 18.7%. In the winter of 2002 mortalities was 92% in the single pond affected. In both years, mass mortalities coincided with cool water temperatures (Figures 1 and 2).

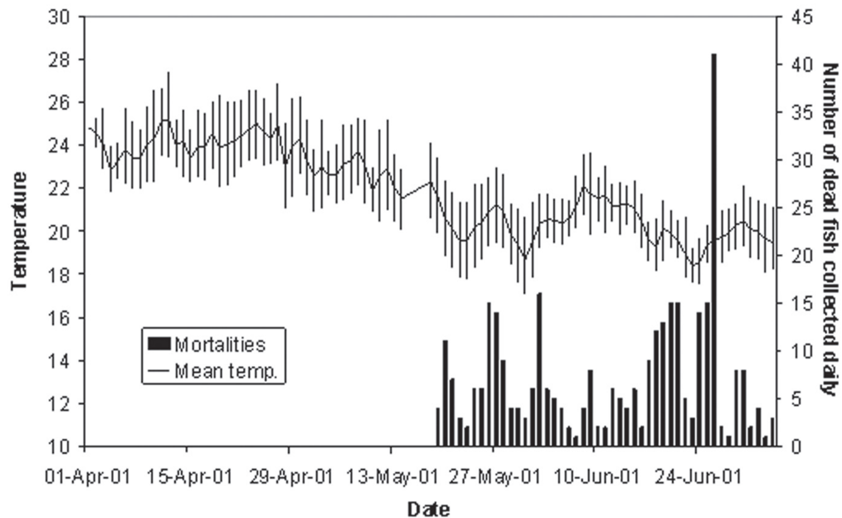

Figure 1. Mortalities caused by *Tetrahymena* in a typical pond, and temperature, in the 2001 epizootic (Group B).

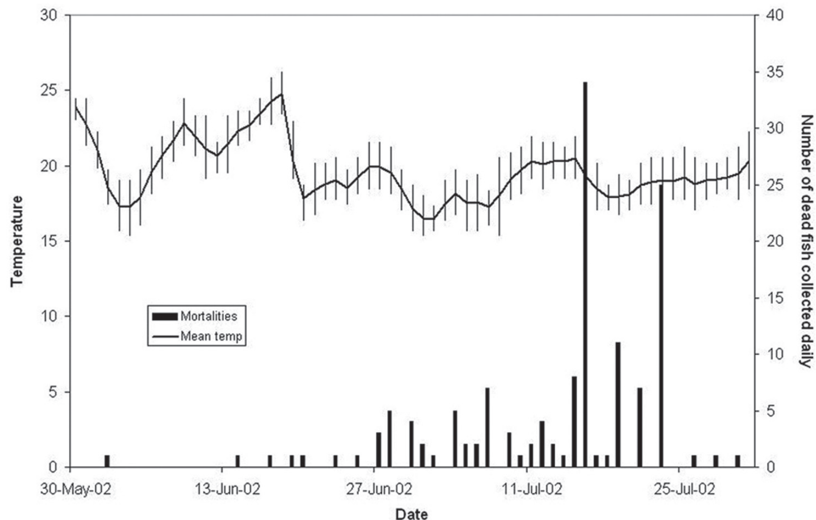

Figure 2. Mortalities in the single pond affected in 2002.

Water quality

Water quality parameters were within normal range for golden perch. pH varied from measured 7 to 9 during the entire period of the growth trials. Dissolved oxygen was maintained at above 90% saturation. Pond water temperature varied during 2001 from a minimum of 17.6°C in winter to a maximum of 26.6°C in summer (Figure 1).

Gross pathology

Figure 3. Early lesion on side of perch, about 10 mm across. This is a shallow lesion with haemorrhage around margins.

Fish submitted in May 2001 showed reddening of the pectoral, dorsal and anal fins, and ulceration of the skin (Figure 3) with 'saddleback' lesions under the dorsal fins typical of infection with columnaris disease caused by *Flavobacterium* sp. The gills of all infected fish were anaemic. Fish had large, round deep skin ulcers with hyperaemic margins exposing the skeletal muscle (Figure 4). The skin ulcers were located variously on the body of the fish but were most common laterally or the caudal peduncle. Several fish had white, fuzzy growths attached to the skin ulcers, resembling that of fungal infection. Gross internal examination of several infected fish with deep skin ulcers showed many had peritonitis with a red-brown exudate typical of motile aeromonad septicaemia.

Figure 4. Lesion of freshly dead fish. Haemorrhagic margin of lesion with skeletal muscle necrosis and secondary infection with fungi and bacteria.

Fish submitted in July 2002 had 4 different types of grossly visible skin lesions; including small 1-2 mm dark marks on intact skin on the caudal peduncle; deep round skin ulcers with hyperaemic margins varying in size from 0.5 to 8 cm; areas of shallow skin erosion beneath the dorsal fin with reddening of the caudal fins; and areas of skin that were pale in colour but with no erosion, rostral to the dorsal fin.

Observations made on ulcer development on fish held in tanks in 2001 with visible small 'pinpoint' red skin lesions, 1 to 2 mm diameter, showed the lesions grew rapidly

into skin ulcers that doubled in size daily, until the ulcer reached 5 to 7 cm diameter within 2 to 4 days, sometimes occupying 10% surface area of the fish. Small fish (30-120 g) died within 1-2 days whereas larger fish (>120 g) died within 2 to 5 days.

Wet mount preparations

Examination of wet mount preparations of skin smears from the leading edge of areas of skin ulceration in fish with "saddleback' lesions under the dorsal fins submitted in May 2001 showed numerous filamentous, gliding bacteria forming 'haystacks' typical of *Flavobacterium columnare*.

Examination of wet mount preparations from skin scrapes done at the leading edge and centre of deep skin ulcers from fish in 2001 revealed thousands of active hymenostome ciliated protozoan parasites, pyriform in shape with somatic cilia covering their entire surface. Scrapes prepared from the centre of the skin ulcers had fewer parasites, and presence of fungal hyphae. Skin scrapes from normal areas of skin were negative for ectoparasites. Examination of wet mount preparations from gill smears showed low numbers of the flagellated protozoan parasite *Ichthyobodo necator*, the ciliated protozoan parasite, *Trichodina* sp. and *Ichthyophthirius multifiliis*.

Air dried smears from ulcer margins, stained with Klein's dry silver impregnation method at OVL, were sent to Queensland University Microbiology Department for confirmation of identification by Dr. Peter O'Donoghue and identified as *Tetrahymena corlissi* Thompson, 1955 on the basis of their characteristic morphological features.

Table 2. Morphometric characterization of *Tetrahymena corlissi* (X = mean, SE = standard error, min = minimum, max = maximum, n = number of observations). Provided by Peter O'Donoghue, University of Queensland. All length measurements in µm.

Character	X	SE	min	max	n
Trophozoite - length (range)	55.4 (32-70)	3.8	32	70	10
Trophozoite - width (range)	38.0 (26-50)	2.2	26	50	10
Buccal cavity – length (µm)	8.0 (6-10)	0.4	6	10	10
Buccal cavity – width (µm)	4.0 (3-5)	0.2	3	5	10
Number of meridional kineties	28.1	0.5	25	30	10
Number of post-oral kineties	2	0	2	2	10
Macronucleus – length (µm)	11.8	0.6	8	14	10
Macronucleus – width (µm)	8.0	0.4	6	10	10
Micronucleus – length (µm)	3.0	0.2	2	4	8
Micronucleus – width (µm)	2.0	0.2	1	3	8

Microbiology

Fungi isolated from deep skin ulcers in May 2001 included *Curvularia* sp., *Fusarium* sp., *Paecilomyces* sp. and *Scopulariopsis* sp. These were considered secondary pathogens and

will not be considered further. *Aeromonas sobria* was isolated from the skin ulcers, heart and caudal kidney of fish submitted in May 2001. *Aeromonas sobria* and *A. hydrophila* were isolated from the heart and from deep skin ulcers of fish submitted in July 2002.

Histopathology

Histological examination of deep skin ulcers from fish submitted in May 2001 and July 2002 showed the skin was ulcerated to the level of the skeletal muscle, and occasionally to the abdominal cavity. Numerous colonies of gram-negative bacilli were seen among necrotic dermis and necrotic skeletal muscle. Numerous *Tetrahymena corlissi* were detected in the scale pockets, epidermis, dermis and between necrotic bundles of skeletal muscle. In some fish, *T. corlissi* were in the in the omental tissues of the peritoneal cavity, and in the meninges of the brain. Granulomas and an increased number of melanomacrophage centres were in the liver of infected fish.

Histological examination of the three different types of skin lesion from fish submitted in January 2002 revealed differing pathology. The deep skin ulcers had similar pathology as described above, except that the ulcers extended only down to the level of the stratum compactum. Colonies of Gram negative bacteria covered the ulcerated surface and microcolonies were close to the edge of the ulcers. There were dilated blood vessels in the dermis, a generalised inflammatory infiltrate of mononuclear cells in the dermis and among the skeletal muscle and necrosis of the dermis and skeletal muscle fibres. *T. corlissi* were present in the loose connective tissues of the dermis. The paler areas of skin had epidermal hyperplasia with the epidermal layer 2 to 4 cells thicker than normal. The small dark marks visible on the caudal peduncle consisted of intact layer of epidermis. *T. corlissi* were within epidermis and dermis and were associated with necrotic cells, infiltrates of mononuclear cells in the hypodermis and a few dilated blood vessels with haemorrhage. No colonies of bacteria were detected.

Pond treatments and *in vitro* chemical trials

Chemicals added to ponds during 2001 were ineffective in killing *T. corlissi.* $KMnO_4$ demand was 4 mg/L. All fish with skin lesions in ponds treated with $KMnO_4$ died the following day and fish in ponds continued to die after both $KMnO_4$ and formalin treatments had been applied. *Tetrahymena* numbers on lesions of dead and dying fish appeared to be unchanged, and they were observed actively dividing and swimming during and after the treatment periods.

In vitro trials done in 2001 on fish infected with *T. corlissi*, were all ineffective. Examination of wet mount preparations from skin scrapes done from the centre and margin of skin ulcers of affected fish showed presence of actively swimming and dividing *T. corlissi.*

Prolonged 10‰ salt baths for 7 to 10 days were effective at killing exposed *T. corlissi*. After 3 days of treatment, the ulcer hyperaemia was absent and the skeletal muscle in the centre of the skin ulcers had turned white. Shallow skin scrapes done from ulcer margins showed no evidence of *T. corlissi,* however, deep scrapes taken from the ulcer margins showed small numbers of *T. corlissi.*

In the 2002 outbreak oral treatment with Emytryl at 30mg/kg fish/day for 10 days showed cessation of mortalities within 3 days of treatment. Visual observations of fish medicated with Emytryl held in tanks showed healing of ulcers within three days.

DISCUSSION

This is the first report of *T. corlissi* causing disease in food fish. Both outbreaks of tetrahymenosis commenced during early winter when pond water temperatures dropped. The natural distribution of golden perch is 900km south of FFAC, in areas with lower temperatures. As the temperature tolerances of golden perch are 4-37°C, it is unlikely that temperature is the primary stressor. In the outbreak of 2001 handling fish may have caused skin abrasion, predisposing fish to infection with *Flavobacterium columnare*, *A. sobria* and *T. corlissi*. The fish were fed a diet for barramundi as no specific diet has been formulated for golden perch. Fish may have been immuno-compromised from low water temperatures or from a lack of particular essential nutrients. Fish may have had no effective immunity to local strains of *T. corlissi*, *Aeromonas* spp., and *F. columnare.* Jade perch (*Scortum barcoo*) kept in nearby ponds were also affected by tetrahymenosis (personal observation), whereas six endemic species of fish on site were not. The appearance of infections in winter suggests that possibly *Tetrahymena* is active in colder weather, but the absence of marked immune response in early infections in 2002, and the rapidity of death of golden perch, suggests that golden perch were naïve to the local *T. corlissi*. This suggests that, in this case, translocation of a fish beyond its native range has resulted in exposure of the fish to a virulent endemic pathogen.

In both the 2001 and 2002 outbreaks, *A. sobria* and *A. hydrophila* were identified as causing motile aeromonad septicaemia in the golden perch. Seasonal outbreaks of motile aeromonad septicaemia are often seen in stressed or immuno-compromised fish cultured in ponds (Roberts 1993). *A. sobria* produces a potent enterotoxin (Carson 1990), contributing to rapid necrosis of skin and muscle. Once motile aeromonads have invaded the integument of a compromised fish host, bacterial septicaemia can develop and result in rapid death. Lesions initiated by *T. corlissi* appeared to cause rapid sloughing of epidermis and destruction of dermal tissue. Secondary bacterial infection then ensued, and the resultant toxins, haemorrhage, osmotic stress and septicaemia resulted in death of the fish.

In this case, *T. corlissi* was differentiated from other *Tetrahymena* spp. previously described as opportunistic parasites of freshwater fish on the basis of morphological features. *T. pyriformis* is smaller in size (40 x 20 µm cf. 60 x 40 µm), possesses fewer meridional kineties (17-21 cf. 25-30) and does not possess a caudal cilium. *T. rostrata* is larger and has more meridional kineties (32-48 cf. 25-30) including up to 4 postoral kineties (cf. 2).

Only one case of tetrahymenosis in pond reared food fish has been reported, when *Tetrahymena* sp. infected Australian freshwater silver perch, *(Bidyanus bidyanus)* grown in earthen ponds, causing scale lifting, skin ulceration, muscle swelling and necrosis (Callinan and Rowland, 1994). That study did not report the haemorrhagic margins of the lesions, which were a feature of the epizootic at FFAC. Our findings were similar to these

and other studies (Ferguson *et al.*, 1987; Lom and Dyková, 1992; Imai *et al.*, 2000; Wakita *et al.*, 2002) in that *T. corlissi* invaded through the skin into the underlying skeletal muscle causing deep skin ulceration, muscle necrosis, inflammation and infection of internal organs and tissues.

We are uncertain whether the *A. sobria* or *T. corlissi* was the primary pathogen responsible for the deep skin ulcers in the 2001 outbreak, since both pathogens were isolated from the ulcers and both are capable of producing deep skin ulcers with red margins in fish (Roberts, 1993; Thune *et al.*, 1993; Imai *et al.*, 2000). In 2002 histological examination of fish with skin lesions consisting of paler than normal areas of intact skin, found *T. corlissi* in the epidermis and dermis, underneath the intact epithelium, and no bacterial colonies were discernable. *T. corlissi* was detected on areas of apparently healthy skin, and in the scale pockets and subcutaneous tissues, further suggesting that *T. corlissi* was the primary pathogen in the 2002 outbreak. *T. corlissi* is histophagous and invades the scale pockets in other species of fish (Imai *et al.*, 2000), so is likely to be the primary pathogen in these infections.

Several species of indigenous, native freshwater fishes (sleepy cod, *Oxyeleotris lineolatus*; barramundi, *Lates calcarifer*; long finned eels, *Anguilla reinhardtii*) cultured in earthen ponds at FFAC, using the same water supply, were unaffected. Species of native fish living in the settlement ponds where water drained from ponds containing golden perch affected *T. corlissi*, including rainbow fish, *Melanotaenia splendida*, sleepy cod and hardyheads, *Craterocephalus stercusmuscarum* were also unaffected. It is possible that native freshwater fish species had innate immunity to *T. corlissi.*

The *T. corlissi* infecting golden perch appeared to be resistant to all bath treatments of chemicals tested. Callinan and Rowland (year) recommended 10g/L salt for 60 mins or 25 mg/L formalin for treatment of tetrahymenosis in silver perch reared in tanks. However we found salt and formalin baths were ineffective against *T. corlissi*. Our experience indicated that only long term bath treatments were effective against the surface dwelling *Tetrahymena*, but not the deep tissue infection, indicating treatment with a systemic drug. Indeed, the only treatment effective in treating *T. corlissi* infection was orally administered systemic anti-protozoal drug Emytryl (400 mg/g dimetridazole) at 30mg/kg/fish/day. This drug was effective within 3 days of treatment as mortalities ceased in ponds, and healing of ulcers was observed in fish in tanks. Dimetridazole has been used effectively to control other motile internal protozoal infections such as *Hexamita spp*. (Hoffman and Meyer, 1974; Gratzek, 1993).

The 2002 outbreak of tetrahymenosis occurred in a pond in which probiotics were used. Although *A. sobria* was not isolated from established skin lesions, *A. hydrophila* was isolated, but only from deep skin lesions. The probiotics used in this case are used in crustacean aquaculture to reduce populations of *Vibrio* and viruses (Moriarty, 1998; Gatesoupe, 1999). The less acute nature of the 2002 outbreak may indicate that the probiotics were effective in reducing susceptibility to infection with *Aeromonas sobria* and *A. caviae*. In the previous six years, *A. sobria* has been isolated from all golden perch from ponds submitted for veterinary examination. This implies that the probiotic treatment impacted on pathogenic bacteria in the pond.

The challenge presented by *A. sobria* and *T. corlissi* is significant to golden perch culture. Both are ubiquitous, and as such present a potential threat to successful pond culture, particularly if fish are introduced and are naïve to a local strains of *Aeromonas* spp. or *Tetrahymena* spp. Our results showed that the *T. corlissi* present on golden perch was resistant to most commonly used ectoparasite chemotherapeutants. Preliminary results from the outbreak in 2002 showed that dimetridazole administered orally is effective against *T. corlissi*.

ACKNOWLEDGMENTS

Rachel Bowater and staff at Oonoonba Veterinary Laboratory provided veterinary advice and laboratory support services. Peter O'Donoghue confirmed identity of the specimens and provided measurements and details on morphology. Rachel Bowater and Ramesh Perera offered constructive criticisms of the manuscript and many helpful suggestions. This work was conducted as part of the DPI&F Aquaculture New Initiatives program. Dave Bull, Neil Harris and Bevan O'Grady provided valuable assistance with stock care and management.

REFERENCES

Altinok, I. and Grizzle, J.M. 2001. Effects of low salinities on *Flavobacterium columnare* infection of euryhaline and freshwater stenohaline fish. *J. Fish Dis*. 24:361-367.

Bancroft, J. D. and Stevens, A. 1990. *Theory and Practice of Histological Techniques,* 3rd ed. Churchill Livingstone. Edinburgh.

Boyd, C.E. 1982. Water Quality Management for Pond Fish Culture, Research and Development . Series No. 22. Elsevier: Amsterdam.

Callinan, R.B. and Rowland, S.J. 1994. Diseases of silver perch, pp.67-75. *In* Rowland, S.J. and Bryant, C. (eds.). Proceedings of Silver Perch Aquaculture Workshops. Austasia Aquaculture, Grafton and Narrandera.

Carson, J., Schmidtke, L.M. and Munday, B.L. 1993. *Cytophaga johnsonae*: a putative skin pathogen of juvenile farmed barramundi, *Lates calcarifer* Bloch. *J. Fish Dis*. 16:209-218..

Chowdhury, M.B.R. and Wakabayashi, H. 1991 A study on *Flexibacter columnaris* infection in loach, *Misgurnus anguillicaudatus* (Bleeker, Guenther). J. *Fish Dis.* 14: 389-394.

Clayton, R.D., Stevenson, T.L. and Summerfelt, R.C. 1998. Fin erosion in intensively cultured walleyes and hybrid walleyes. *Prog. Fish-Cult.* 60 (2):114-118.

Edgerton, B., O'Donoghue, P., Wingfield, M. and Owens, L. 1996. Systemic infection of freshwater crayfish *Cherax quadricarinatus* by hymenostome ciliates of the *Tetrahymena pyriformis* complex. *Dis. Aquat. Org.* 27:123-129.

Ferguson, H.W., Hicks, B.D., Lynn, D.H., Ostland, V.E. and Bailey, J. 1987. Cranial ulceration in Atlantic salmon *Salmo salar* associated with *Tetrahymena* sp. *Dis. Aqua. Org.* 2: 191-195.

Gatesoupe, F.J. 1999. The use of probiotics in aquaculture. *Aquaculture* 180:147-165.

Gratzek, J.B. 1993. Parasites associated with freshwater tropical fishes, pp. 573-590. *In* Stoskopf, M.K. (ed.). Fish Medicine. WB Saunders Company, Sydney.

Hoffman, G.L. and Meyer, F.P. 1974. Parasites of freshwater fishes. A review of their control and treatment. TFH Publications, Neptune City, New Jersey.

Hoffman, G.L., Landolt, M., Camper, J.E., Coats, D.W., Stookey, J.L. and Burek, J.D. 1975. A disease of freshwater fishes caused by *Tetrahymena corlissi* Thompson, 1955, and a key for identification of holotrich ciliates of freshwater fishes. *J. Parasitol*. 61:217-223.

Imai, S., Tsurimaki, S., Goto, E., Wakita, K. and Hatai, K. 2000. *Tetrahymena* infection in guppy, *Poecilia reticulata*. *Fish Pathol*. 35:67-72.

Kabata, Z. 1985 parasites and diseases of fish cultured in the tropics. Taylor and Francis, London.

Lom, J. 1984. Diseases caused by protistans, pp. 114-168. *In* Kinne, O. (ed.). Diseases of Marine Animals Volume IV, Part 1. Introduction Pisces. Biologische Anstalt Helgoland, Hamburg, Germany.

Lom, J. and Dyková, I. 1992. Ciliates (Phylum Ciliophora Dofelin, 1901), pp. 237-288. *In* Lom, J. and Dyková, I. (eds.). Protozoan Parasites of Fishes. Elsevier, Amsterdam.

Moriarty, D.J.W. 1998. Control of luminous *Vibrio* species in penaeid aquaculture ponds. *Aquaculture* 164:351-358.

Mosig, J. 2002. Research on sleepy cod in North Queensland, Australia. Hatchery International 3:4-8.

Noga, E. J. 1996. Fish diseases: diagnosis and treatment. Mosby, St. Louis.

Plumb, J.A. 1999. Overview of warmwater fish diseases. *J. Appl. Aquaculture* 9:1-10.

Ponpornpisit, A., Endo, A. and Murata, H. 2000. Experimental infections of a ciliate *Tetrahymena pyriformis* on ornamental fishes. *Fish. Sci.* 66:1026-1031.

Roberts, R.J. 1993. Motile aeromonad septicaemia, pp. 143-174. *In* Inglis, V., Roberts, R.J. and Bromage, N.R. (eds.), Bacterial disease of fish. Blackwell Science Ltd., Oxford.

Rowland, S.J. and Ingram, B.A. 1991. Diseases of Australian native freshwater fishes with particular emphasis on the ectoparasitic and fungal diseases of Murray Cod (*Macculochella peeli*), golden perch (*Macquaria ambigua*) and silver perch (*Bidyanus bidyanus*). Fisheries Bulletin 4. New South Wales Fisheries, Sydney.

Shoemaker, C.A., Klesius, P.H., Lim, C. and Yildirim, M. 2003 Feed deprivation of channel catfish, *Ictalurus punctatus* (Rafinesque), influences organosomatic indices, chemical composition and susceptibility to *Flavobacterium columnare*. *J. Fish Dis*. 26:553-561.

Soltani, M. and Burke, C.M. 1994. Responses of fish-pathogenic *Cytophaga/ Flexibacter*-like bacteria (CFLB) to environmental conditions. *Bull. Eur. Ass. Fish Pathol*. 14:185-187.

Soltani, M., Munday, B.L. and Burke, C.M. 1996. The relative susceptibility of fish to infections by *Flexibacter columnaris* and *Flexibacter maritimus*. *Aquaculture* 140:259-264.

Thune, R.L., Stanley, L.A. and Cooper, R.K. 1993. Pathogenesis of Gram-negative bacterial infections in warmwater fish. *Ann. Rev. Fish Dis.* 3:37-68.

Toranzo, A.E., Baya, A.M., Robertson, B.S., Barja, J.L., Grimes, D.J. and Hetrick, F.M. 1987. Specificity of slide agglutination test for detecting bacterial fish pathogens. *Aquaculture* 61:81-97.

Ullrich, S. 1992. Bakterielle Fischkrankheiten in Untereider und Unterelbe und ihre Beeinflussung durch Umweltfaktoren. Berichte aus dem Institut fuer Meereskunde an der Christian-Albrechts- Universitaet Kiel.

Wakabayashi, H. 1991. Effect of environmental conditions on the infectivity of *Flexibacter columnaris* to fish. *J. Fish Dis*. 14:279-290.

Wakita, K., Imai, S. and Hatai, K. 2005. Histopathological study on *Tetrahymena* infection in Dwarf Gourami (*Colisa lalia*). 5th Symposium on Diseases in Asian Aquaculture 24-28 Nov 2002., Gold Coast, Australia.

Wright, J.F. 1981. *Tetrahymena pyriformis* (Ehrenberg) and *T. corlissi* Thompson parasitic in stream dwelling triclads (Platyhelminthes: Turbellaria). *J. Parasitol*. 67:131-133.