Health problems of the Western Australian dhufish

F.J. Stephens, J.J.Cleary, G. Jenkins, B. Jones, S.R. Raidal and J.B. Thomas

FRDC Project 98/328

Fisheries Research and Development Corporation Report
FRDC Project No. 1998/328

Health problems of the Western Australian dhufish

F.J. Stephens, J.J. Cleary, G. Jenkins, B. Jones, S.R. Raidal and J.B. Thomas

Division of Veterinary and Biomedical Sciences
Murdoch University
Murdoch
Western Australia

April 2002
ISBN: 0-86905-817-7

Table of Contents

List of Figures

List of Tables

1998/328 Health problems of the Western Australian dhufish

Principal Investigator: Dr Shane Raidal
Address: Division of Veterinary and Biomedical Sciences
Murdoch University
Murdoch Drive
Murdoch
W.A. 6051

Telephone: (08) 9360 2418
Fax: (08) 9310 4144
email: raidal@numbat.murdoch.edu.au

Objectives

1. To understand the pathogenesis of exophthalmos in captive, otherwise healthy West Australian dhufish.

2. Control of significant health problems occurring in the West Australian dhufish.

Non-technical summary

OUTCOMES ACHIEVED: Captive dhufish are very susceptible to multiple health problems that result in ill thrift or death. Successful aquaculture of dhufish requires the maintenance of robust fish by the provision of an environment that is optimal for the species. The provision of suitable stocking density, physico-chemical characteristics of water and diet appear to be key components of a management strategy to reduce dhufish health problems.

The purpose of this project was to describe and investigate health problems in captive West Australian dhufish, *Glaucosoma hebraicum*. The dhufish is a potential aquaculture species due to its popularity as an edible species and fishing pressure on wild fisheries. The two most significant health problems apparent in captive dhufish were exophthalmos in otherwise apparently normal fish and infestation of gills with a monogenean parasite, *Haliotrema abaddon*. Several other health problems were also described and investigated during the project.

Exophthalmic lesions were described, followed by investigations into the aetiology and pathogenesis of the condition. Epidemiological data were gathered to identify risk factors that may increase the pre-disposition of dhufish to the development of exophthalmos. The anatomical arrangement of vasculature supplying the eye was described and the

haemoglobin-oxygen transport properties of dhufish blood that pre-dispose dhufish to exophthalmos were studied. Oxygen partial pressure in the normal retina and oxygen content of gas bubbles in exophthalmic eyes were recorded. Risk factors for the development of exophthalmos were investigated in an experiment using unaffected fish, variable water temperature, fright-induction and exercise regimes.

Gas and haemorrhage was present in the choroid of exophthalmic eyes, with haemorrhage in retrobulbar tissues resulting from perforation of the sclera in some eyes. Oxygen content of gas in eyes with recently developed exophthalmos was high (up to 73%). In some eyes with retrobulbar haemorrhage, oxygen tension approached zero, indicating severe disruption of blood supply to the eye. Oxygen tension at the retinal-vitreal junction of normal dhufish eyes was high (344 ± 26 mm Hg), with oxygenated blood supplied to the choroid body from the gills via the pseudobranch. The finding of a single haemoglobin with pronounced Root and Bohr effects in dhufish was significant and may contribute to the susceptibility of the species to exophthalmos.

Investigations suggest that exophthalmos is physiological in origin and is related to the environmental differences between the natural habitat of the fish and the conditions that are experienced in aquaculture. Dhufish appear to be highly adapted to a relatively inactive life-style with relatively constant environmental conditions at high hydrostatic pressure. Rapid changes of temperature or blood acid-base characteristics may precipitate the development of exophthalmos.

The monogenean parasite, *Haliotrema abaddon*, was described and stages of its life-cycle identified. As the parasite was troublesome in captive fish, potential treatments were investigated using *in vitro* and *in vivo* studies. Praziquantel was identified as the most effective 'in water' treatment of fish infested with *H. abaddon*. Other useful but less effective and safe treatments were low salinity baths (<1.5 ppt for ninety minutes) and 0.5 mg L^{-1} trichlorphon for 36 hours.

Life in tanks appears stressful for many dhufish, resulting in health problems such as exophthalmos and disease outbreaks, including severe *H. abaddon* infestation and bacterial and fungal diseases. Multiple risk factors appear to pre-dispose the fish to these conditions. They include environmental factors such as water temperature, depth and physico-chemical composition, diet and stocking density; host factors such as physiological and social adaptation to a relatively solitary, sedentary lifestyle in a deep-water habitat and pathogen factors such as increased fecundity and decreased generation time in warmer water temperatures. Decreasing fish stress and maintaining environmental conditions close to those in the natural habitat, including increasing tank depth and decreasing light intensity are expected to improve the overall health of captive dhufish.

Keywords: dhufish, *Glaucosoma hebraicum*, health, exophthalmos, parasite, Monogenea, aquaculture.

Acknowledgements

This project would not have been possible without the financial support and interest of the Aquaculture Development Fund of Western Australia, Department of Fisheries in Western Australia and the Aquaculture Development Unit of Challenger Tertiary and Further Education.

Background

Interest in culture of the West Australian dhufish, *Glaucasoma hebraicum* Richardson, 1845 (Family: Glaucosomatidae) resulted from declining catches in the wild fishery and its high value (Fisheries WA 2000). The high commercial value of the fish is the result of its large size and thick, yellowish-white fillets of excellent eating quality (CSIRO 1999). A small-scale project by a private enthusiast indicated its suitability for culture as it was docile and without aggressive or cannibalistic tendencies. The development of dhufish aquaculture at the Aquaculture Development Unit (ADU) of Challenger Tertiary and Further Education (TAFE), commenced in 1995. The project was funded by Challenger TAFE and the Fisheries Research and Development Corporation (FRDC), with supplemental income provided by Fisheries WA and Recfishwest.

Recent research of the biology of dhufish in the wild (Hesp and Potter 2000), confirmed that the species is long lived. The oldest fish recorded was 42 years of age. Fifty percent of male dhufish attained sexual maturity at 2.6 years of age or 320 mm in length. Female dhufish grew more slowly and 50% of fish reached sexual maturity at 2.5 years or 301 mm in length. Fish attained 50cm in length (the legal length for capture) at a mean of 6.9 and 5.8 years for females and males, respectively. Spawning is seasonal with multiple spawnings in the season, between November and April.

Dhufish inhabit coastal waters in the southwest of Western Australia from Shark Bay (114° 30' E; 25° 30' S) to the Recherche Archipelago (121° 54 E; 33° 52' S). It is a relatively solitary, sedentary, demersal species that is known to feed at night (Kailola et al., 1993; McKay, 1997).

Hesp and Potter (2000) reported the growth rate and changes in habitat of dhufish during various stages of their life. Juveniles grew to a mean of 108 mm in length in the first year of life and inhabited regions of hard substrate adjacent to reefs. At about fourteen months of age and 150 mm in length they moved to low reefs with rock ledges of up to 30 cm. Once the fish were greater than 300 mm in length they were found on the edges of limestone and coral reefs where reef edges were greater than two metres in height and at a depth of 10 to 150 m.

The major component of the diet of wild dhufish is various species of reef dwelling fish (Marr 1980; Robinson 1987) included wrasse and other prey including octopus, various crustacea, kelp (Marr 1980), eels and cuttlefish (Robinson 1987).

Significant health problems occurred in dhufish in the initial stages of the project. The fish were more susceptible to health problems than other species held at the same facility, including snapper, *Pagrus auratus* (Bloch and Schneider), King George whiting, *Sillanodes punctata* (Cuvier), and black bream, *Acanthopagrus butcheri* (Munro). One troublesome problem was clinical exophthalmos unrelated to ill health of the fish. The prevalence of exophthalmos in captive dhufish was extremely high in the first year of dhufish aquaculture (Pironet and Jones 2000). As haemorrhage was evident in the affected eyes it was thought that the condition was induced by trauma to the relatively large protruding eye. As a result several changes were made to handling and management of the fish, including decreasing light intensity to the tanks (Dr Brian Jones, personal communication) and handling fish on soft, compressible surfaces. The prevalence of exophthalmos was lower in subsequent years, but continued to occur at an unacceptable rate. There were several other possible causes of the exophthalmos, including infectious agents, mechanical trauma, gas bubble disease, and increased pO_2 and/or decreased gas solubility resulting in gas bubble formation when gas micronuclei were present. The possible causes required further investigation to enable the development of control measures to reduce its prevalence in captive dhufish.

Another prevalent and poorly understood problem was severe infestation with a dactylogyrid gill parasite. Treatment of this parasite was difficult, resulting in significant mortality. Several other parasites had also been reported in captive and wild dhufish (Hesp and Potter, 2000; Pironet and Jones 2000) Commercial viability of dhufish aquaculture depended on improved understanding of the pathogenesis and control of these and other health problems of captive dhufish.

Need

Various health issues were identified in earlier FRDC supported projects (95/095, 96/308, 96/103). Some of these health issues have implications for wild dhufish stocks and others have the potential to compromise the ability to commercialise aquaculture of the species.

Although some health problems in captive dhufish can be partially controlled, the cause of exophthalmos remains poorly understood as does the life cycle, host-parasite relationship and methods of controlling dactylogyrids.

There are insufficient resources available within the ADU, Fisheries Western Australia and Murdoch University to adequately investigate the causes, prevention and control of health problems in captive dhufish. In addition, this project will address the shortage of aquatic health specialists in Australia and the need for additional veterinary training in fish health.

The overall aim of the project is to investigate the health problems of dhufish so as to improve the viability of future commercial aquaculture of the species, whilst at the same time, providing training in the investigation and management of fish health problems.

Objectives

1. To understand the pathogenesis of exophthalmos in captive West Australian dhufish.

2. Control of significant health problems occurring in captive West Australian dhufish.

Methods

Fish

Wild dhufish for use as broodstock and for research purposes were caught between Fremantle and Lancelin on handlines using baited barb-less hooks in less than 20 m of water so as to reduce the effects of tissue damage caused by decompression. A permit from Fisheries WA enabled collection of fish that were smaller than the legal capture size of 50 cm. A sterile hypodermic needle was used to pierce the swim bladder immediately following capture to vent excess gas formed during rapid ascent at capture. Fish were transported on the day of capture to the ADU in an insulated 200 L plastic container containing seawater with oxygen supplemented by an air stone and cylinder of compressed oxygen. At the ADU the fish were transferred to a 4000 L fibreglass tank. During the next fortnight, fish were uniquely identified using the Trovan Passive Transponder System[1]. Tags were inserted into the peritoneal cavity. During the quarantine period fish received two ninety-minute freshwater baths to remove external protozoan and metazoan parasites (Stoskopf, 1993) before being allocated to other tanks of wild-caught dhufish.

The fish were stocked into conico-cylindrical fibreglass or concrete tanks at a stocking density of up to 1 kg 1000 L^{-1}. The initial tank used to hold dhufish was approximately 3 m deep, however, as handling, cleaning and observing fish in this tank was difficult, broodstock fish were later held in 20 000 to 30 000 L fibreglass tanks approximately 6.5 m long, 3 m wide and 1.5 m deep with rounded tapering bottoms for ease of cleaning. Some deeper tanks of similar volume were available, including a 1.8 m deep fibreglass tank for wild-caught fish used in the current project and circular concrete tanks.

Tanks were covered with a firmly fixed net to prevent fish jumping from tanks after being startled by sudden movements, changes in light intensity or loud noises. At least 30% of each tank was also covered with black plastic to decrease light intensity.

Fish greater than 2 kg in weight were used as potential broodstock, with six to eight fish, including two male fish, allocated to each broodstock tank. Handling of wild-caught broodstock in the spawning season was minimal to reduce stress and increase the success

[1] AEG and Telefunken Electronic, Germany. Obtained from Central Animal Records, Keysborough, Victoria

of spawning (Pickering 1998). Fish observation and tank cleaning were also minimised. Natural photoperiod was maintained.

Two groups of F1 fish from the same artificial spawning, (groups 5 and 6, Table 5) were held in two tanks for the first eighteen months of this project. Four tanks of juvenile dhufish from three artificial spawnings (groups 1 to 4, Table 5) were available for study until they reached eighteen months of age, together with juveniles from the following spawning season.

Water supply

Two water sources were available for use in dhufish culture: bore water and seawater. Bore water was pumped from two bores in calcareous limestone adjacent to the ADU. The bores were 11 and 17 m deep. Seawater was pumped from coastal waters approximately 1 m below the low tide mark near the mouth of the Swan River estuary. Significant amounts of organic and inorganic matter were present in this water following storms and periods of high rainfall, and occasional toxic algal blooms occurred in the river.

Water from both supplies entered fish tanks after passing through a PVC column approximately 1 m high packed with netting to aid the removal of any supersaturated gases and to increase oxygenation should these problems arise.

Analysis of the bore water indicated salinity levels similar to seawater but with a pH of 7.6 to 7.7 and an elevated free carbon dioxide level assessed by various NATA laboratories as being between 5.4 and 99 mg L^{-1}. This variation was more likely to be the result of variation in the analysis method and storage of samples prior to analysis than real variation in the water source. Bore water quality was more consistent than seawater and had the advantage of a requirement for minimal filtration and minimal potential to harbour disease organisms. Its temperature ranged between 18 and 22 °C.

The chemical composition of seawater was similar to that of the natural habitat of dhufish, with the exception of changes resulting from hydrostatic pressure and runoff from the Swan estuary during periods of high rainfall. The disadvantage of seawater was the necessity for filtration (to 10 µm), particularly important in the removal of organic and inorganic sediment during certain natural events. Other disadvantages were the likelihood of fish pathogens entering the culture system in the seawater and more variable water temperatures, ranging from 15 to 26°C.

The natural habitat of dhufish is influenced by the Leeuwin current where water temperature ranges from 19 to 22°C (Pearce 1991). There is little diurnal variation in water temperatures in their natural habitat as it is not adjacent to a land-mass, unlike the seawater intake point for aquaculture tanks at the ADU (Pearce 1986).

Spawning and nutrition

Techniques and equipment used for production of fertile eggs and larvae were described in detail by Cleary and Jenkins (2000). As natural spawning of wild caught fish was only seen on one occasion (Pironet and Neira 1998), artificial spawning techniques were used to produce larvae (Cleary and Jenkins 2000). Human chorionic gonadotrophin (HCG), 1000 I.U. kg^{1}, and an analogue of gonadotrophin releasing hormone (LHRHa) prepared as a slow release pellet (50 $\mu g\ kg^{-1}$) were used to stimulate maturation and ovulation of oocytes. Spermiation of males was maintained by a 10 $\mu g\ kg^{-1}$ slow release LHRHa pellet and facilitated stripping of males over several consecutive days (Cleary and Jenkins 2000). Eggs were stripped and collected into a clean, dry cylinder prior to gentle mixing with sperm that had not contacted water. Seawater was added prior to further mixing, followed by transfer to a 100 L polypropylene cone with a flow of fresh seawater (1 L min^{-1}, 23°C) and vigorous aeration. After two to three days, larvae were released into a 40 L cylindro-conical tank with a screened outlet. Surface skimmers were used to remove oil from the surface of the water.

Dhufish larvae were raised using a variety of methods. The most successful method, resulting in a larval survival rate at day 28 of 37%, was 50% copepod nauplii, *Gladioferens imparipes*, grown in a culture of brown and green microalgae *Isochrysis galbana* (T-iso) and *Nannochloropsis oculata*, respectively and 50% artificially enriched rotifers, *Brachionus plicatis* and *Brachionus rotundiformis* (Payne *et al.* 2001). When larvae were 6 mm in length *Artemia* nauplii supplemented with Super Selco[2] were added to the culture water. The success of this diet was thought to be the result of the 3.6 ratio of docosahexaenoic acid (DHA) to eicosapentanoic acid (EPA), considered to be important in larval fish diets (Sargent *et al.* 1997). In contrast, dhufish larvae fed enriched rotifers had a DHA/EPA ratio of only 0.6.

A semi-intensive larval rearing green or brown-water system based on the methods of (Palmer *et al.* 1992), incorporating *Nannochloropsis oculata*, *Isochrysis galbana*, copepods, *Gladioferens imparipes*, and rotifers, *Brachionus plicatis* and *Brachionus rotundiformis* was the most successful method of relatively large-scale rearing of dhufish larvae. Adult copepods and *Artemia* nauplii were fed after larvae reached 5 mm in length. This regime was similar to that described for rearing brown spotted grouper larvae *Epinephelus tauvina* (Hussain and Higuchi 1980).

Marine fish larvae are generally fed rotifers, *Brachionus plicatis*, grown in microalgae cultures to increase their content of omega-3 fatty acids, followed by *Artemia* nauplii (Treece 1995). *Isochrysis* sp. is a preferred species of microalgae as it is rich in DHA (Renaud and Parry 1994). Calanoid copepods have a lipid content closely resembling marine fish eggs and larvae and are thought to be superior to rotifers as a larval feed (Sargent *et al.* 1999), however large-scale production of copepods is difficult (Stottrup 1999).

[2] Inve Aquaculture NV, Belgium

After metamorphosis at approximately 40 to 45 days post-hatch or 8 mm in length, dhufish are gradually weaned from a diet largely composed of *Artemia* nauplii to a diet of finely chopped, mixed seafood. Some juveniles were fed pellets such as a commercial snapper pellet or a home-made moist pellet containing fish oil, fish meal, wheat gluten, fish, squid and prawn flesh supplemented with minerals and vitamins. Juveniles are fed three times daily, gradually decreasing to once a day or every second day. They are held in cylindro-conical fibreglass tanks at a stocking rate of approximately 4 kg 1000 L^{-1}.

Freshly thawed whole garfish, mullet, whiting, pilchards, octopus, squid and prawns of appropriate size were fed three times a week to fish of over 1 kg liveweight. An immunostimulant based on β1,2 glucan, (Fingard® or Macrogard®, Vetafarm, NSW) was fed with a vitamin and mineral supplement in a gelatin capsule. The capsules were fed for the first two weeks in every four week period.

Fish handling and anaesthesia

Fish were handled with single use polyethylene disposable gloves to prevent abrasive and toxic damage to the mucus and epithelial layers of fish. Prior to handling, fish were generally sedated or anaesthetised as this decreased the risk of trauma and the possible adverse effects of severe lactic acidosis following prolonged swimming activity or struggling. Initially 2-phenoxyethanol was used and found to cause rapid onset and recovery from anaesthesia, however, fish often ceased ventilation during anaesthesia and the chemical is not approved for use in fish for human consumption. It was replaced by AQUI-S (AQUI-S New Zealand Ltd) for most fish handling purposes.

Fish were not fed on the day of handling as regurgitation of food was a common problem during sedation and anaesthesia. Fish weighing less than 500g were sedated in approximately 0.02 mL L^{-1} AQUI-S added direct to tank water following a reduction in the volume of water in the holding tank. When the fish no longer responded to sudden movement they were caught in a handheld net.

The water level of tanks containing fish larger than 500g was lowered until it was knee-deep and fish were herded into partially submerged containers made from polyethylene. Approximately ten 2 kg fish could be held in a 425 L container. A transparent sheet of polyethylene was attached to the rim of the container with plastic clips to prevent fish from jumping from the container during induction of anaesthesia, and oxygenation was maintained by an airstone supplied from a cylinder of compressed oxygen.

Fish were anaesthetised with AQUI-S at the rate of 0.07 mL L^{-1}. Anaesthetised fish were transported in plastic 'slings' that were sealed at one end and were revived in fresh, clean water. If necessary, they were artificially ventilated by opening the mouth and slowly moving the fish and allowing water to pass over its gills.

Blood sampling

Plastic-covered high density moistened foam moulds with cut out sections in the region of eye were used to firmly hold fish during procedures such as caudal vessel bleeding and

implanting of pellets and tags. The moulds were of different sizes to suit the fish. Plastic measuring cradles suspended from a spring balance were used to weigh and measure fish over 1 kg in weight.

Blood was usually obtained from the caudal vessels as described by Noga (1996), however the needle was inserted slightly laterally. Occasionally the sinus venosus (duct of Cuvier) was used for obtaining venous samples (Lied *et al.* 1975). Blood was drawn into plastic syringes containing 30 μL to 70 μL of 1000 I.U. mL^{-1} sodium heparin. The amount of heparin used was just sufficient to prevent clotting of the sample volume thereby reducing the effects of heparin on the blood parameters to be studied (Hamilton *et al.* 1978; Turton 1983). Air was immediately expressed from samples destined for blood gas analysis and syringes were stored in ice slurry prior to analysis.

Gill biopsy
Biopsies for determining the number of metazoan and protozoan parasites were obtained using the method described by Nowak and Lucas (1997). They were obtained from anaesthetised or heavily sedated dhufish and immediately examined on a compound microscope using a glass microscope slide and coverslip.

The area of gill that was removed did not appear to re-grow and bleeding occurred if the proximal 30% of primary lamellae were removed. The accuracy of the method was validated by comparing the number of *H. abaddon* in biopsies compared with the number on the entire gill arch on the opposite side of the fish.

Necropsy procedure
The post mortem technique described by Ferguson (1989) was used. Eyes were fixed in Bouins fluid and other tissues in 10% formalin buffered with filtered seawater. Heart blood collected with a sterile disposable hypodermic syringe and needle, sterile swabs or tissues masses were cultured on tryptose soy agar to which blood and salt was added. Isolates were characterised using specific media and biochemical tests by Agriculture WA.

Skin/gill smears
The blunt side of a sterile scalpel blade was used to obtain tissue samples from skin lesions and gills. Tissue was examined in a few drops of culture water as for biopsies.

When health problems and disease occurred, a definitive diagnosis of the cause was made wherever possible, followed by treatment. Detailed histories of the wild-caught fish allocated to this study and two groups of F1 fish enabled analysis of the potential effect of various environmental and nutritional factors. Epidemiological information was used to assess the risk factors contributing to each problem.

Investigation of exophthalmos
Exophthalmic eyes and other tissues from normal and affected fish were examined to identify and describe lesions. Dissolved oxygen and temperature of culture water were recorded to evaluate the possibility of supersaturation of water and fish tissues with air.

The anatomical structure of organs and physiological processes of possible relevance to oxygen supply to the eye were studied, including investigation of the vasculature of the gills, pseudobranch and choroid body, and haemoglobin and oxygen transport properties of blood from dhufish and other marine teleosts. Finally, pO_2 was measured in normal and exophthalmic dhufish eyes and experiments were designed with the aim of reproducing exophthalmos by exposing dhufish to risk factors identified during epidemiological studies of the condition.

A comparison of haematology and acid-base parameters in dhufish in summer and winter was undertaken to examine the effects of seasonal water temperature on dhufish. Haematology, assays of blood ATP content and blood gas and acid-base analysis were performed in summer and winter. Oxygen dissociation curves for dhufish were used to demonstrate haemoglobin-oxygen affinity and the potential effect of some allosteric effectors such as ATP, CO_2 and H^+. Similar investigations of dhufish held in seawater and bore water were used to compare the properties of blood from the two pCO_2 environments.

Much of the basic investigation of dhufish haemoglobin was performed on fish randomly selected from the culture tanks. Water source and temperature and details of the fishes history were obtained in each instance. At times it was possible to design scientific experiments using randomly allocated, similar fish and controlled conditions. In other experiments, involving the collection of blood and the use of larger fish, facilities were not available to allow replication of treatments and controls in identical culture conditions. In these instances pseudo replication was provided by using the same fish in the same tank at different times following different water conditions (summer versus winter) or on similar fish that had been undergone similar culture management techniques with the exception of water source (bore water versus seawater).

The effects of temperature on dhufish blood oxygen transport were investigated using pseudo-replication following long term temperature differences. The same fish were held continuously in seawater in the same tank and sampled at the end of summer and winter. A limitation of this experimental design was that other factors may affect fish at different times. For example, external parasite burdens were frequently higher in summer. Blood sampling was done following a period of good health when fish had been actively feeding.

The effect of high pCO_2 bore water on dhufish blood oxygen transport and acid-base balance were investigated using pseudo-replication of two groups of fish in similar tanks but with different water supplies. One group of fish was held in bore water and the other group from the same spawning was held in seawater continuously for eighteen months. The tanks were of similar construction, depth and diameter, however the tank containing bore water was longer, had a slightly lower water velocity and was in a more secluded area of the facility. Management of the tanks was identical. Fish were not handled or

treated during the preceding eighteen months. Sampling was performed on two occasions when water temperatures had been within the same range for several weeks.

In another experiment to reproduce risk factors thought to be important in the generation of exophthalmos, one hundred and forty four F1 dhufish, 85.2 $\pm$ 17.7g and 151.7 $\pm$ 12.2 mm with no evidence of exophthalmos were lightly sedated with AQUI-S and randomly allocated to eighteen circular plastic 180 L tanks, eight fish per tank. The water exchange rate was 30 L hour^{-1}, the tanks were illuminated for 10 hours daily and dissolved oxygen (DO) was held at between 95 and 100%. Fish were observed for exophthalmos and fed daily. Tanks were vacuumed and DO and water temperature were recorded daily.

In experiment 1, treatments were randomly allocated to the tanks. Six tanks had bore water continuously held at 25.0 $\pm$ 0.5°C, six had temperatures that cycled between 25.5 and 20.8 $\pm$ 0.5 °C daily and six tanks were held at 20.8 $\pm$ 0.5°C. The fish in three tanks from each temperature regime were exercised daily from day two for six days. Exercise was provided by water flowing in an anticlockwise direction from a 240 L min^{-1} pump in the upper region of the water column. The exercise period was increased daily from ten minutes on day one to thirty-five minutes on the final day. The pump was placed in tanks of fish that were not subjected to exercise. Fish developing exophthalmos were observed and removed for necropsy at the completion of the trial.

In experiment 2, fish from the cold water and fluctuating water supplies in experiment 1 were pooled in a large container and re- allocated randomly to twelve tanks of cold water. After 24 h, water temperatures were increased to 25.0 $\pm$ 0.5°C in six randomly allocated tanks. The following day a fluctuating temperature regime was commenced in three of these tanks.

Control tanks were covered in black plastic and the other tanks suddenly illuminated each morning in an attempt to cause a 'fright' response. The response was less than anticipated and less than that caused by daily vacuuming of tanks. All fish were individually handled and weighed during the experiment. Several fish struggled and fell from about 1 metre onto the concrete floor during weighing. These fish were noted, together with the side of their body that received the impact. Fish management and water quality parameters were the same as in experiment one.

Haematocrit and haemoglobin analysis was performed using standard microhaematocrit[3] and spectrophotometric cyanomethaemoglobin analysis[4] with blood being centrifuged prior to spectrophotometric analysis. Red blood cell counts were performed manually (Dacie and Lewis 1975). Mean corpuscular haemoglobin concentration (MCHC), mean corpuscular volume (MCV) and mean corpuscular haemoglobin (MCH) were derived from the results.

[3] Phoenix Clements Medical, Sydney, Australia

[4] Coulter Electronics Ltd, Luton, England, UK

Blood gases, pH, oxygen dissociation curve, Bohr effect and Hill coefficient of dhufish whole blood were investigated and compared with those of snapper, a species that was markedly less susceptible to the development of exophthalmos. Blood gases and pH were determined approximately 90 min post collection using an ABL 5 blood gas machine[5] situated at the Murdoch University Veterinary Hospital, ten kilometres from the ADU. Fish handling and transport resulted in a mean delay of 90 min between sampling and analysis. The effects of this delay were assessed by transporting two fish to Murdoch University, followed by anaesthesia and bleeding using the standard method. Blood samples were analysed immediately and then every thirty minutes.

The effect of heating dhufish blood to 37°C and the use of algorithms for human blood by the ABL 5 was validated by equilibrating blood in known concentrations of CO_2 and O_2 and comparing pH measurements with those obtained on a calibrated Shindengen pH meter. Eight samples of dhufish blood from a fish held at 23°C were equilibrated at 23°C in air with 0.21% (1.60 mm Hg) and 1.05% CO_2 (7.98 mm Hg). Blood was immediately analysed on the ABL 5.

The Bohr factor was calculated for blood collected from the caudal vessels or sinus venosus into syringes containing sodium heparin was immediately transferred to 2 mL containers containing sodium fluoride (used to arrest glycolysis) and the anticoagulant, potassium oxalate. Blood was pooled following storage in ice slurry and inspected for coagulation. The Bohr effect of snapper and dhufish whole blood was investigated using pH modification methods outlined by Bridges and Morris (1989). In the first study, the CO_2 Bohr factor was calculated by equilibrating 0.7-1.0 mL aliquots of whole blood in the following pairs of gas mixtures: 1% CO_2 in air and 1% CO_2 in nitrogen; 0.2% CO_2 in air and 0.2% CO_2 in nitrogen; 0.03% CO_2 in air and 0% CO_2 in nitrogen. Approximately equal parts of blood from each pair equilibration gases of similar pCO_2 were weighed into syringes and mixed with the aid of stainless steel washers inside syringes using the mixing technique described by Edwards and Martin (1966) and modified by Dhindsa *et al.* (1971) and Scheid and Meyer (1978). The mixed blood was immediately analysed on the ABL 5 blood gas analyser. P_{50} was estimated from a linear regression of pO_2 values of blood with O_2 saturation values estimated at between 40 and 60%. The Bohr factor was calculated from the slope of the linear regression of log P_{50} versus blood pH for the three levels of pCO_2.

A second technique was used to estimate the Bohr factor of dhufish blood. A Radiometer ABL 520 blood gas analyser was used to calculate P_{50} from a single blood sample using an algorithm for human blood. The fixed acid Bohr factor was estimated by modifying the pH of whole blood by the addition of small amounts of lactic acid or Tris to 1 mL of blood. P_{50} of each blood sample was estimated on the ABL 520 following equilibration in air at the same temperature as the culture water of the fish. The CO_2 Bohr factor was estimated from P_{50} obtained on the ABL 520 following equilibration of whole blood in air containing 0.03% CO_2, 0.2% CO_2 and 1% CO_2. The Bohr factor ($\Delta \log P_{50}/\Delta pH$) was calculated from the slope of a regression line obtained from plotting log P_{50} and blood pH

[5] Radiometer A/S, Copenhagen NV, Denmark

from samples equilibrated in the three CO_2 mixtures and following pH modification using fixed acid (Bridges and Morris 1989).

An oxygen dissociation curve was displayed for snapper and dhufish blood obtained from pooled blood obtained from the caudal vessels of several fish using the mixing technique described above, with the modification that samples of approximately 8 mL were equilibrated in humidified air or nitrogen. Whole blood O_2 saturation was plotted between 0 and 100%.

The effect of seasonal water temperatures and pCO_2 on the oxygen binding affinity of whole blood from dhufish was studied using an oxygen dissociation curve constructed using the mixing technique and equilibration in 1% CO_2 in air and nitrogen. Fish were held in seawater and sampled at the end of summer and winter.

The Hill coefficient, a measure of cooperativity of oxygen binding sites of haemoglobin was estimated by plotting log (% saturation/100 - % saturation) and log pO_2 obtained from the oxygen dissociation curve data at 0.015% CO_2 (Riggs 1970).

Centrifuged, lysed erythrocytes for pH_i estimation were sometimes unable to processed in the ABL 5 blood gas analyser due to their high viscosity. Therefore, pH_i was measured on a calibrated hand held Shindengen ISFET KS723 pH meter[6] that had been validated by comparing pH results with those on the ABL 5.

Electrolytes in whole blood in sodium heparin were determined concurrently with blood gas analysis. Blood was agitated and immediately analysed on a Vetlyte[7] electrolyte analyser. Only Cl^- was recorded as Na^+ was not expected to alter greatly and blood had been collected in sodium heparin and K^+ measurements were highly variable using this technique.

The presence of haemoglobin isomorphs in several species of fish was determined using electrophoresis. Haemoglobin was prepared from erythrocytes that were washed and centrifuged three times in 1.7% NaCl in 1mM Tris HCl, pH 8.0 and lysed in 1mM Tris HCl, pH 8 using the method of Riggs (1981). Haemoglobin was used immediately to avoid problems associated with instability of fish haemoglobins (De Young *et al.* 1994). Haemoglobins were separated on a cellulose acetate membrane using a Gelman Haemoglobin Electrophoresis System[8] in a semi-micro chamber and stained with Ponceau S. Freshly prepared cattle haemoglobin was used as a control (Ingermann and Terwilliger 1982).

[6] ShindengenElectric Mfg.Co. Ltd., Tokyo, Japan

[7] Idexx Laboratories, Australia

[8] Gelman Instrument Company, Ann Arbor, Michigan,USA

The Root effect of haemoglobin was assessed by comparing the degree of oxygenation of haemoglobin in buffers of varying pH. Haemolysate was prepared, as above, and diluted in pH 8.0 buffer to obtain an absorbance of close to 1 at wavelength 576 nm on a Beckman DU 650 spectrophotometer[9]. The same dilution was used to prepare haemoglobin haemolysate in two series of buffers. In the first series of experiments, a 0.05 M citrate buffer was used to prepare haemoglobin solutions of pH 5.5, 6.8, 6.9, and 7.0 and a 1mM Tris HCl buffer of pH 7.1, 7.3 and 8.0. In the second series of experiments haemoglobin was diluted in 0.1M HEPES 0.1M NaCl pH 8 buffer and in the same buffer of pH 7.3, 6.9 and 6.0 (Weber 1992; Wells *et al.* 1997).

A wavelength scan of triplicate samples of haemoglobin in each buffer was produced between 500 and 630 nm. The scan at pH 7.3 was used to calculate the % methaemoglobin in the sample using the method of Benesch *et al.* (1973). Haemolysates with methaemoglobin levels of greater than 5% (Tris buffer system) or 10% (HEPES buffer system) were discarded. Sodium dithionite was added to haemoglobin in 1mM Tris HCl, pH 8.0 to obtain a wavelength scan of totally deoxygenated haemoglobin.

The magnitude of the Root effect was estimated from the absorbance of haemoglobin in each buffer at wavelengths 540, 560 and 576 nm by comparing absorbance at the three wavelengths at pH 8.0, pH 6.9 and following deoxygenation with sodium dithionite in pH 8.0 buffer (Farmer *et al.* 1979; Wilhelm and Reischl 1981; Dafré and Wilhelm 1989; Wells and Baldwin 1990). Cattle haemoglobin that does not have a Root effect (Ingermann and Terwilliger 1982), was used to validate the method.

Approximately 34 μmol ATP and GTP per gram of haemoglobin was added to haemolysates in the pH 6.9 buffer to compare oxygenation with and without nucleoside triphosphates. Wavelength scans were used to calculate the % oxygenation of haemoglobin as above.

Freshly collected blood in sodium heparin held in ice slurry was used to assay ATP using a Sigma[10] ATP enzymatic kit. The method was validated using an ATP solution of known concentration.

A corrosion cast of vasculature of the dhufish gill, pseudobranch and choroid body was prepared using prepolymerised methyl methacrylate, Mercox[11]. Fish were anaesthetised with AQUI-S followed by MS-222[12] until ventilation ceased. The ductus arteriosus was cannulated with polyethylene tubing with the end flared by gentle heating in a naked flame. Tubing was selected to suit the size of the ventral aorta of the fish and firmly

[9] Beckman instruments Inc., Fullerton, California, USA

[10] Sigma Diagnostics, Inc. Sigma, Australia

[11] Okenshoji Co Ltd, Tokyo, Japan

[12] Western Chemical inc., Ferndale, WA, USA

attached to a blunt 16-20 gauge disposable hypodermic needle. Vasculature was perfused with normal saline containing sodium heparin until gills and pseudobranch were visually pale and perfusate draining from the perforated ventricle lacked erythrocytes. Air was excluded from the cannula by submersion of the fish in water and careful preparation of the Mercox perfusate to prevent the inclusion of gas bubbles.

A low concentration of Mercox catalyst, approximately 0.1 g per 10 mL of Mercox, was mixed and perfused through the cannula using hand-generated pressure. The low concentration of catalyst reduced viscosity and over-distension and rupture of capillaries in the gill, pseudobranch and choroid body. The perfused fish was left undisturbed at 4°C overnight to allow polymerisation of the Mercox resin prior to being placed in 15% NaOH for dissolution of fish tissues. Several rinses of 15% NaOH were applied over several days, followed by rinsing in clean water and submersion in 15% hydrochloric acid to remove fatty residues. Finally the cast was rinsed in water and stored in 100% ethanol prior to dissection, critical drying, mounting on a stub and coating with an alloy of gold palladium in a Polaron sputter-coater. The cast was observed with a Phillips Scanning Electron Microscope.

A silicon-coated fibre optic oxygen sensor and ultra violet spectrometer, FOXY system[13], was used to measure the oxygen content within gas bubbles in exophthalmic eyes and partial pressure of oxygen adjacent to the retina of normal eyes. Calibration and measurements were performed in gas (% O_2) or in water and vitreous humour (pO_2 in mm Hg) following the manufacturers instructions.

Fish were sedated in AQUI-S and transferred to a 20-L plastic container of seawater containing MS-222 and a pump for water circulation and aeration. A 16-g disposable hypodermic needle was used to perforate the caudal aspect of the cornea at the limbus. The oxygen sensor was then passed behind the lens, through the vitreous humour to the retina. The position of the sensor could be seen through the cornea. The sensor was blunt-ended and could be positioned adjacent to, but not penetrating the retina.

Drift during use was checked by periodical placement of the probe either in air or seawater saturated with oxygen. During measurement of pO2 in vitreous humour adjacent to the retina it was important that the probe was not actively pushed against the retina as this compressed the capillaries in the choroid. Fish were actively ventilating, but anaesthetised during the procedure to avoid fluctuations in respiratory gas exchange at the gills and its effects on pO2 and acid-base balance of blood entering the eye.

Water analysis was performed to document the differences in composition between bore water and seawater in dhufish tanks. As a membrane diffusion instrument for recording total gas pressure (Colt 1986), was not available to detect supersaturation caused by increases in total gas pressure in culture water, dissolved oxygen (DO) was used as an indication of supersaturation in culture water using an OxyGuard portable oxygen meter

[13] Ocean Optics, Inc. Dunedin, Florida USA.

and probe[14]. The OxyGuard meter was calibrated regularly using the Winkler titration, referred to as the azide modification method 4500-O C (APHA 1995).

Water from fish holding tanks was analysed on site for salinity using a hydrometer compensated for temperature; pH using a calibrated pH meter; free CO_2 using titration method 4500-CO2 C (APHA 1995); calcium using the EDTA titrametric method 3500-Ca D (APHA 1995) and alkalinity, method 2320B (APHA 1995).

CO_2 and O_2 content in mg L^{-1} was converted to pCO_2 and pO_2 in mm Hg using solubility coefficients and conversion tables corrected for salinity and temperature (Colt 1984).

Water temperature in dhufish holding tanks containing seawater constantly being replenished from the ocean was recorded hourly in later summer and early autumn using a Hastings datalogger[15]. The effect of air temperature on water temperatures in culture tanks were assessed by comparing changes in water temperature with air temperatures recorded half hourly by the Bureau of Meteorology in Perth.

Gill dactylogyrid investigations

Gills for histopathological examination of lesions and the prevalence of parasites were fixed in 10% formalin in seawater prior to direct examination and embedding in paraffin, sectioning and staining with Haematoxylin and Eosin. Pieces of gill used for description of the parasite were placed in 1:4000 formalin solution and after approximately one hour were fixed in 3% formalin, then transferred to ethanol/formalin/acetic acid (AFA) and finally shipped to Idaho State University in 70% ethanol (Kritsky and Stephens 2001).

A survey of dactylogyrid parasite numbers on wild dhufish was undertaken. Dhufish above the legal size for capture (50 cm) were surveyed during late summer to ascertain the prevalence and intensity of *H. abaddon* in the wild. Fish were caught between 31°40'S, 115°30'E and 32°S, 115°40'E and held on ice until gills were excised within 6 hours of capture and placed in 10% formalin.. Gills were examined for parasites before and after standard paraffin embedding, sectioning and staining

Life cycle stages of *H. abaddon* were investigated during the project. Live *H. abaddon* from fish held in water of 22-25ºC were obtained by biopsy and post mortem and egg production monitored using the technique described by Roubal (1994). Eggs produced *in vitro* and some that were removed directly from fish gills were held in glass petri dishes at room temperature (20-25ºC) in sterile seawater containing 100 I.U. penicillin mL^{-1} and 100 µg streptomycin mL^{-1} as described by Roubal (1994) and examined daily using an inverted microscope.

The effect of various antiparasitic agents on *H. abaddon* was assessed using *in vitro* trials. Gill arches were removed from heavily infested captive fish and placed in seawater

[14] Technolab Marketing Pty Ltd, Kingston, Tasmania, Australia

[15] Hastings Dataloggers, Port Macquarie, NSW, Australia.

in a plastic petri dish containing 15 mL of a test medium. Three replicates of each treatment were used and seawater was a control in each trial (Table 1). Samples were incubated at ambient temperature, approximately 25°C, and each petri dish was observed every 30 or 60 minutes for 3 hours using a light microscope to assess the motility, shape and attachment to gill lamellae of *H. abaddon*.

Promising agents were then trialled. In the first experiment, dhufish weighing approximately 20g in 20L buckets containing water from their culture tank and aerated with oxygen. A treatment was allocated to each bucket and fish were observed during and after treatment (Table 2).

In the second experiment, three fish weighing 93 ± 9g were placed into each of twelve buckets containing 18 L of 19.5°C bore water aerated with oxygen. Treatment chemicals were added to the water in the buckets, with three replicates of each treatment (Table 3). At the completion of treatment gill biopsies were performed on two of the three fish in each treatment. Each fish was marked with a small fin clip specific for each treatment and returned to the main culture tank and mortalities following treatment were recorded. The number of parasites removed during treatment was estimated by passing water from the treatment buckets through a 20 μm screen. The screen was rinsed into a 500 mL glass beaker, sediment allowed to settle for 15 minutes, followed by removal of the supernatant. 1 mL of sediment was examined in a Sedgewick-Rafter counting chamber using an inverted microscope.

Following these preliminary trials, relatively safe, potentially useful 'in water' treatments were more thoroughly investigated using controlled trials. Dhufish spawned in captivity and weighing 116 ± 31g were used in the series of four treatment trials. The fish had variable *H. abaddon* infestations, with most of a sample of randomly selected fish having moderate (2-10 parasites per 0.5 cm primary lamellae) to heavy (>10 parasites per 0.5 cm lamellae) infestation.

Twenty four glass aquaria holding 120 L of water were darkened with black painted polystyrene foam paneling on the sides and bottom of the tank and black paint on part of the lids. Water was obtained from a saline underground reserve and passed through a heat exchanger (19.5 ± 0.5°C, flow rate 1.2 L min^{-1}). Flow was discontinued during treatments. During treatments of over ten hours duration water temperatures in tanks fell to 17.0 ± 1.0°C as insulation on aquaria was insufficient to maintain water temperatures, which fell toward ambient air temperature. Dissolved oxygen and water temperatures were monitored daily using an OxyGuard Gamma portable dissolved oxygen meter and mercury and glass thermometer, respectively. Dissolved oxygen was maintained at 85-105% by adjustment of air supply to air stones. Ammonia levels of water were assayed every six to twelve hours during trials of more than 12 hour duration using the spectrophotometric method outlined by Spotte (1992).

Table 1. Treatments tested *in vitro* for activity against *H. abaddon.*

Trial 1	Trial 2	Trial 3
Control	Control	Control
toltrazuril 50 mg L^{-1}	4 ‰ salinity	moxidectin 2 mg L^{-1}
toltrazuril 30 mg L^{-1}	8 ‰ salinity	moxidectin 1 mg L^{-1}
triclabendazole 120 mg L^{-1}	netobimin 140 mg L^{-1}	flubendazole 10 mg L^{-1}
moxidectin 2 mg L^{-1}	netobimin 70 mg L^{-1}	flubendazole 5 mg L^{-1}
moxidectin 1 mgL^{-1}	toltrazuril 50 mg L^{-1}	garlic 50 g L^{-1}
	toltrazuril 25 mg L^{-1}	garlic 25 g L^{-1}
	AQUI-S 10 mL L^{-1}	closantel 37.5 mg L^{-1}
	AQUI-S 5 mL L^{-1}	praziquantel 25 mg L^{-1}
	closantel 75 mg L^{-1}	toltrazuril 25 mg L^{-1}
	closantel 37.5 mg L^{-1}	
	ivermectin 2 mg L^{-1}	
	ivermectin 1 mg L^{-1}	
	isopropanolol 3 mL L^{-1}	
	praziquantel 50 mg L^{-1}	
	praziquantel 25 mg L^{-1}	

Table 2. Treatments applied to single fish in 20 L buckets to assess the potential toxicity of treatments.

Treatment time (mins)	Treatment
10	closantel 18.8 mg L^{-1}
40	praziquantel 25 mg L^{-1}
65	toltrazuril 12.5 mg L^{-1}
120	trichlorphon 30 mg L^{-1}

Table 3. Experimental design of a small-scale trial of efficacy and safety of some potential treatments for *H. abaddon.* The toxicity and efficacy of each treatment was assessed by observing fish behaviour and mortality, the number of parasites recovery from the bucket contents and the number of parasites remaining on fish following treatment.

Treatment	Duration (hours)
control	3
formalin 250 ppm	1
fenbendazole 25 mg L^{-1}	3
praziquantel 5 mg L^{-1}	3

Treatments used in the four experiments are summarised in Table 4. In each experiment three fish were randomly allocated to each tank and acclimated for twenty four hours. There were six randomly allocated replicates of each treatment and six control tanks in each trial.

Water supply to control tanks was interrupted for the duration of the longest treatment and a seawater sample was added to each tank to reproduce the effects of treatment addition. At the completion of treatment the base of each tank was vacuumed using a siphon to remove one third of the water volume. Tanks were rapidly re-filled, followed by return to a flow rate of 1.2 L min^{-1}, except following freshwater baths and 15 mg L^{-1} trichlorphon treatments where a higher flow rate was used as fish appeared to be distressed.

Salinity in freshwater treatments was reduced from 35 ‰ to less than 3 ‰ within 30 minutes using freshwater from the reticulated town supply, filtered through a carbon filter. The temperature of the freshwater was within 2ºC of the culture water. Final salinity was measured using a calibrated Eutech TD Scan 2 salinometer and maintained for 90 minutes following salinity reduction. At the completion of treatment salinity was increased by the rapid addition of a large volume of saline water.

Praziquantel was mixed in a small amount of isopropyl alcohol and added to seawater. The effect of isopropyl alcohol on dhufish and *H. abaddon* had earlier been validated and found to not significantly affect either species following *in vitro* trials on infested gills and holding fish in small containers of treated water. Trichlorphon was dissolved in a small amount of water prior to addition to aquaria.

Treatments were assessed by two methods. The first method involved water being siphoned from the base of aquaria at the completion of the treatment time was passed through a 30 μm screen, followed by flushing with approximately 500 mL water. Material flushed from the screen was allowed to settle and 2 mL samples of the sediment was examined *H. abaddon* using an inverted microscope and Sedgewick-Rafter counting chamber.

In the second assessment, gill biopsies were performed on fish on the day following completion of treatment. Fish were sedated with 0.008 mL L^{-1} of AQUI-S™ (AQUI-S New Zealand Ltd.) prior to removal of the tips of ten to fifteen primary lamellae from the ventral region of the second gill arch and immediately examined on a light microscope. AQUI-S, at the dose rate used, was validated as not significantly affecting parasite numbers on gills in the time required to sedate and biopsy fish in each aquarium. The number of *H. abaddon* on ten randomly selected primary lamellae measuring between 0.5 and 0.7 cm in length was recorded per fish.

Fish mortalities were recorded and dead fish dissected without delay. Gills and internal organs were placed in 10% buffered formalin and standard histological methods were used to examine key organs. Gill biopsies obtained immediately after death were used in biopsy results for the treatment.

Biopsy and sieve results were analysed individually from each trial using SigmaPlot Version 5 and SigmaStat Version 2 (SPSS Inc). Biopsy results were normally distributed and treatment groups in each trial were compared using one-way ANOVA. Parasite counts in sediment were not normally distributed and were analysed using box plots and Kruskal-Wallis one-way ANOVA on ranks. Biopsy results and sieve counts represented only a proportion of total parasite numbers that remained on fish gills or were removed during treatment, respectively. Graphical representation of the comparison between similar classes of treatments was made following pooling of information from all four trials as no significant differences, using one-way ANOVA, was found in results from control groups from each trial.

Table 4. Experimental design showing treatment times and duration in each of four experiments using dhufish infested with *H. abaddon*.

Trial Number	Treatment	Dose	Duration
1	control		5 hours
	praziquantel	5mg L^{-1}	5 hours
	trichlorphon	15 mg L^{-1}	2 hours
	freshwater	0.6 to 3.9 ‰	1.5 hours
2	control		24 hours
	formalin	25 ppm	10.5 hours
	trichlorphon	0.5 mg L^{-1}	24 hours
3	control		44 hours
	toltrazuril	12.5 mg L^{-1}	6 hours
	trichlorphon	0.5 mg L^{-1}	44 hours
	praziquantel	2 mg L^{-1}	44 hours
4	control		24 hours
	freshwater	0.7 to 1.3 ‰	1.5 hours
	praziquantel	2 mg L^{-1}	24 hours
	formalin	25 ppm	24 hours

Results and discussion

Fish mortality

Post weaning mortalities in F1 fish from spawnings prior to the commencement of the present project were low (groups 5, 6, Table 5), with the exception of nine of the ten fish from group 7 which died during and shortly after a freshwater bath when they were 18 months of age. Another fish, from group 5, that had a distended abdomen for several weeks prior to its death, had large, vitellogenic ovaries and histological evidence of ischaemia and pressure necrosis of abdominal organs.

Several disease outbreaks in F1 fish spawned during the tenure of this project resulted in significant mortalities. The number of mortalities in two groups, (groups 2 and 3 of Table 5) together with some epidemiological information on growth rate, water source and water temperature are displayed in Figure 1. The major causes of mortality are outlined in Table 5 for these and other groups of F1 fish.

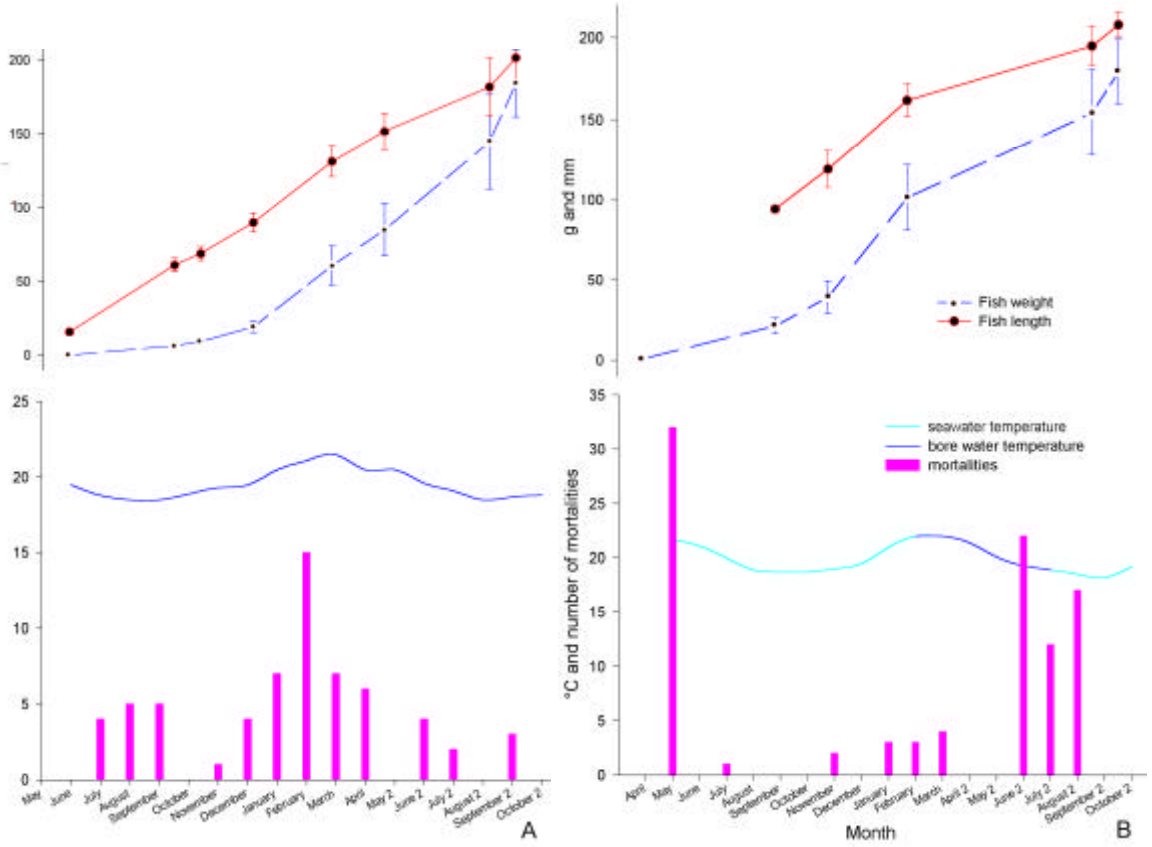

Figure 1. Growth rate, mortality and incidence of exophthalmos of two groups of captive dhufish. A. Hatchery reared juvenile dhufish reared by traditional rotifer feeding, followed by a commercial pelleted diet designed for snapper (group 2, Table 5). B. Hatchery reared juvenile dhufish fed a diet of copepods, followed by flesh (group 3, Table 5).

Some juvenile dhufish were obtained from several spawnings in 2000. The fish from all spawnings failed to thrive and there were several peaks of mortality. In an early outbreak gastritis was evident, with scattered necrosis of cells in the gastric glands and marginated chromatin in hepatocytes of fish at eighty days of age. *Vibrio harveyii* and *Photobacterium damselae* were cultured during two periods of high mortality when fish were nine and twelve months old, respectively. High mortalities occurred overnight following a probable interruption of incoming water flow to tanks in February 2001. There was a high prevalence of exophthalmos with gas visible in the eyes of many surviving fish following this incident. In May/June 2001 many fish again had severe exophthalmos, were anorexic, dark in colour and there was a persistent moderate rate of mortality. The fish had been fed a commercial snapper pellet since February.

Five of forty-five wild-caught fish (11%), died in early summer shortly after the start of the current project. Necropsy and histological sections indicated that several infectious agents may have contributed to mortality. Three of the four fish that were necropsied were severely infested with *H. abaddon,* with a mean of more than thirty parasites on each primary gill lamella. Other findings were epicarditis, an atrophied muscular layer of the stomach, lack of zymogen granules in the pancreas and severe, diffuse multifocal necrosis of the liver consistent with septicaemia caused by organisms such as *Vibrio* sp. or unidentified toxins.

In another peak of mortality, twenty-eight of sixty-one wild-caught fish (46%) died between July and December 1999 and twelve of thirty-three remaining fish (36%) died between January and March 2000. These mortalities were associated with long-term listlessness and anorexia that commenced several weeks after bore water replaced seawater as the water source (Fig. 2). Other species at the same facility maintained excellent health in the same water over the corresponding time period. The dhufish lost weight, and although the supply of seawater was resumed in mid November the fish did not regain good health, evidenced by appetite, body condition, colour and sheen until late March 2000. A severe infestation of *Caligus* sp. in all tanks of wild-caught fish occurred in February requiring urgent treatment and was associated with fish mortality (Fig. 2). Mortalities often occurred during or immediately after handling and anaesthesia.

Exophthalmos

Spontaneous exophthalmos occurred in all groups of otherwise apparently normal, captive dhufish. The condition was usually unilateral, and gas bubbles were often visible within the eye (Fig. 3) or in periorbital tissue. Lesions varied in severity from a slight, almost imperceptible protrusion of the eye to severe protrusion followed by rupture of the cornea or dorsal periorbital tissue.

Five F1 fish in group 3 developed exophthalmos within a two day period at 260 days of age and 21.47 $\pm$ 4.89 g in weight. The plastic tank covering had been removed just prior to onset of exophthalmos and there was a full moon at the time of onset of exophthalmos.

Increased activity and possibly startling may have occurred at night as large skylights were situated directly above the tank. The plastic was replaced and no further problems occurred.

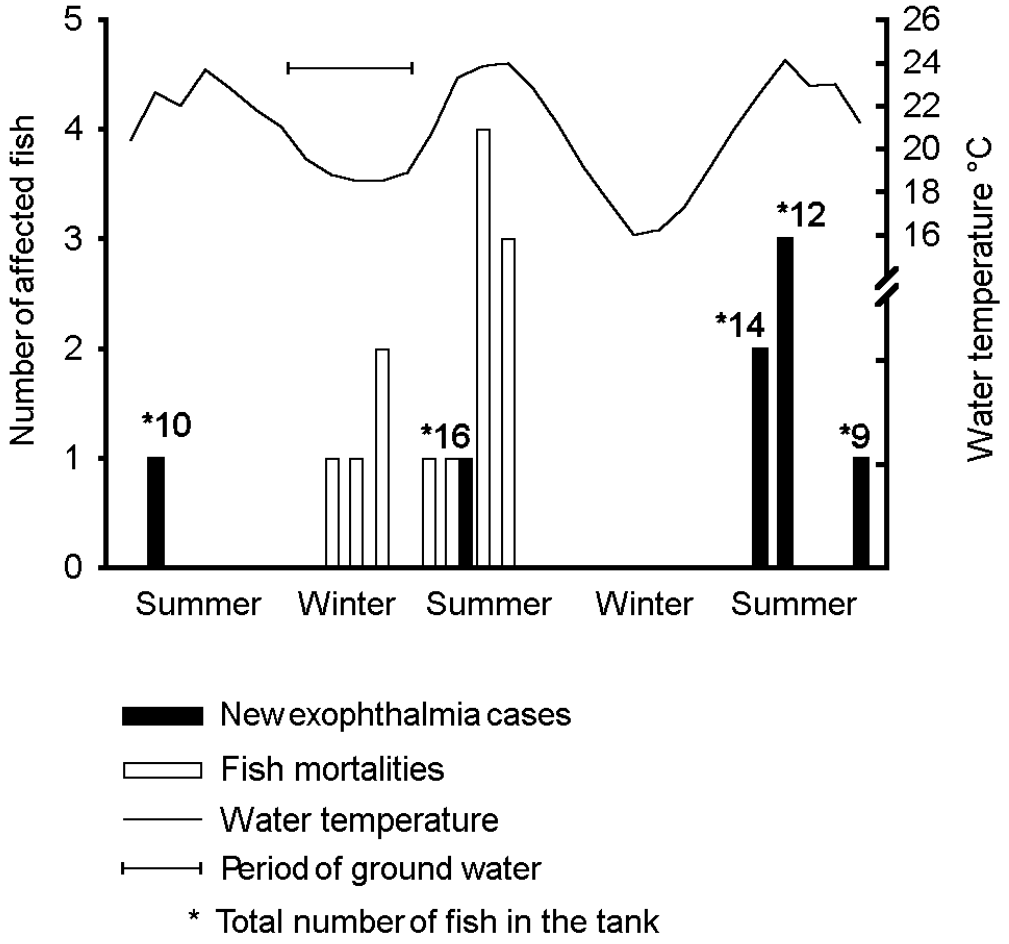

Figure 2. Mortality and exophthalmos in one tank of wild-caught, captive dhufish.

F1 fish aged 12 months (Table 5, group 3) were caught at random in a scoop net at the peak of a spate of exophthalmos. Only eleven of thirty-eight (28.9%) randomly selected fish had no evidence of exophthalmos, although sampling bias was probably present as exophthalmic fish may be more easily caught. Three of the exophthalmic fish had bilateral lesions, the left eye of ten fish was affected and the right eye of fourteen fish. The fish were moved to a slightly smaller tank supplied with bore water of lower water temperature (21°C versus 24°C for seawater) and within one week the exophthalmic lesions in many fish had dramatically reduced in size, especially in eyes that were only slightly affected.

Exophthalmic fish had a smaller weight to length ratio than fish with no exophthalmos and both weight and length were significantly different between exophthalmic and normal fish, $P<0.001$ and $P=0.002$, respectively, student t-test (Table 6). This correlated with the observation that exophthalmic fish did not eat in the weeks following the development of severe exophthalmos. They became dark, frequenting the bottom of the tank often close to the central standpipe, had a head-down orientation, often with the affected eye tilted toward the tank floor.

Figure 3. Exophthalmos in a dhufish. Many small gas bubbles are visible within the aqueous humour.

Exophthalmos was more common in both wild-caught large fish and F1 fish during the summer months when fish were held in seawater (Fig. 2). The prevalence of exophthalmos in F1 fish held exclusively in bore water was low (group 2, Table 5), and contrasts with the prevalence in the other three groups of fish from the same spawning season. Factors that may have contributed to the low prevalence of exophthalmos in these fish were lower activity due to lower water temperatures in summer or listlessness caused by poor diet and growth rate in the first 10 months of life.

In wild-caught fish the majority of exophthalmic lesions developed in the period from November to March each year (Fig. 2) in fish that were otherwise apparently healthy. Exophthalmos was most prevalent during periods of high water temperature following several days of high air temperatures in summer (Figs. 2 and 4). Water temperatures in tanks followed the air temperatures of the adjacent land mass (Fig. 4).

Table 5. The prevalence of exophthalmos in six groups of captive dhufish. Groups one to four were from three spawnings in the same breeding season. Management of larval and juvenile nutrition and water quality varied between the four groups. Groups five and six were from a spawning two years earlier and were held as one group in the first year of life in constant water temperature. Fish were examined for exophthalmos prior to amalgamation of groups and commencement of alternative management strategies. S indicates fish held in seawater. B indicates fish held in bore water. Where both water sources were used the most frequently used source is shown first.

Tank	Initial number juveniles stocked	Water source	Mortalities (quarterly for previous 15 months)					Age of fish at final examination (months)	Exophthalmos prevalence
1	383	S / B	15	21	78*	84**	40**	20	20 / 35 (57%)
2	301	B	14	5	29*	10	5	18	12 / 180 (7%)
3	231	S / B	1	3	10	22†	29†	20	61 / 90 (68%)
4	443	S / B	-	48	24	113‡	103‡	14	30/115 (26%)
5	52	S / B	1	0	0	0	1	37	18 / 37 (49%)
6	40	B / S	0	0	0	0	1	37	20 / 36 (55%)

Major necropsy findings: * bacterial septicaemia, ** *H. abaddon* and epitheliocystis, † *H. abaddon* and *Caligus* sp., ‡ *Exophiala* sp. Fish removed from the tanks for other reasons have not been included.

Table 6. Length and weight of juvenile dhufish: exophthalmic and non-exophthalmic. Fish were anaesthetised, measured and weighed in February following a period when many new cases of exophthalmos developed.

	Weight (g ± S.D.)	Length (mm ± S.D.)	Length to weight ratio
Exophthalmic fish (n=16)	87.0 ± 15.3	154.8 ± 10.2	1.81 ± 0.22
Normal fish (n=15)	113.2 ± 20.8	166.1 ± 9.2	1.50 ± 0.22

Water temperatures of up to 26°C were recorded in the culture tank with a diurnal variation of 0.6 to 1.4°C. Temperatures peaked at approximately 7pm daily and were at a minimum at approximately 9 am, lagging behind water temperatures by three to five hours.

Exophthalmos was evident within twelve hours of the initiating physical or physiological factor and generally increased in severity over a 48-h period, with haemorrhage becoming visible in periorbital tissues in severe cases. The presence of gas in the eye was indicated by an alteration in refractive index within the eye or the presence of grossly visible gas bubbles (Fig. 5). In some exophthalmic eyes the eyeball maintained a superficially normal appearance, however, periorbital tissues became distended with gas and/or haemorrhage (Fig. 5). After several days haemoglobin breakdown resulted in haemorrhagic areas changing from bright red to green.

Exophthalmos was more common in summer when water temperature reached peaks of up to 26°C from a base of approximately 23°C (Fig. 3). Seven of the eight initial lesions occurred in December and January and the lesion in April followed a short period of increased water temperature. Exophthalmos did not appear to be correlated with the duration of time spent in captivity, dissolved oxygen in culture water, previous exophthalmos in fish with prior unilateral lesions, supersaturation of culture water or other disease processes. This pattern was similar in other tanks of wild-caught and F1 fish but the large number of fish in tanks precluded the recording of each new case of exophthalmos. Exophthalmos sometimes developed, but less frequently, in fish held in cooler water temperatures. Exophthalmos was not seen in cultured dhufish until after 4 months of age and was not correlated with peaks in fish mortality.

Gas bubbles were consistently present within the choroid of exophthalmic eyes (Fig. 6). In severe exophthalmos gas was present in the aqueous and vitreous humours or in retrobulbar tissue associated with haemorrhage. Dissection of fish with resolved lesions revealed protrusions of exuberant dark red tissue from the sclera several millimetres in diameter (Fig. 7), presumably the result of perforation and haemorrhage during the exophthalmic episode. Cataracts and increased buoyancy were occasionally seen in fish with exophthalmos. Bacterial cultures of affected eyes were negative.

Histologically, the choroidal layer of exophthalmic eyes was thickened and contained gas bubbles surrounded by haemorrhage. After several days, haemorrhagic areas in choroidal and retrobulbar tissue were infiltrated with diffuse populations of macrophages. Ischaemic necrosis and dystrophic mineralisation were evident in conjunctiva adjacent to the cornea in some cases and after 14 days regenerating capillaries were present within the necrotic tissue that was also infiltrated with large numbers of macrophages. In one exophthalmic eye, fibrinous degeneration and necrosis of muscle fibres was present in the tunica media of the ophthalmic artery at its entry point to the choroid. In many eyes the sclera was perforated adjacent to or within several millimetres of the optic disc where there was no scleral cartilage.

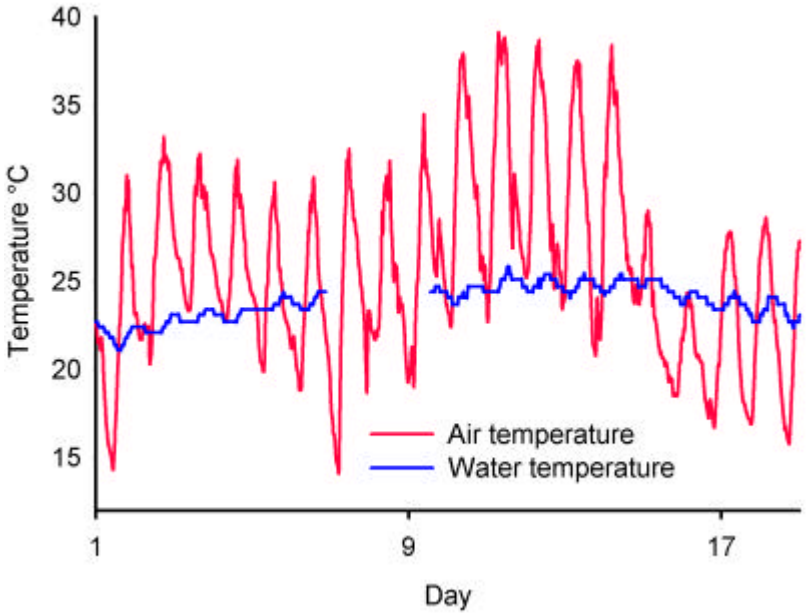

Figure 4. Water temperatures in a dhufish culture tank compared with air temperatures for the corresponding time period. Maximum and minimum daily water temperatures gradually increased following several days of high air temperatures, lagging several hours behind maximum and minimum air temperatures.

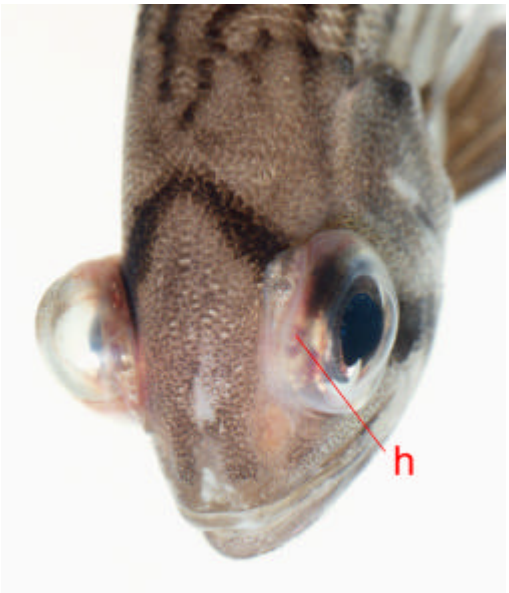

Figure 5. A juvenile dhufish with bilateral exophthalmos. A large gas bubble is demonstrated in the right eye. Smaller gas bubbles and haemorrhage (h) can be seen in periorbital tissue of the left eye, typical of exophthalmos associated with rupture of the sclera and retrobulbar gas and haemorrhage.

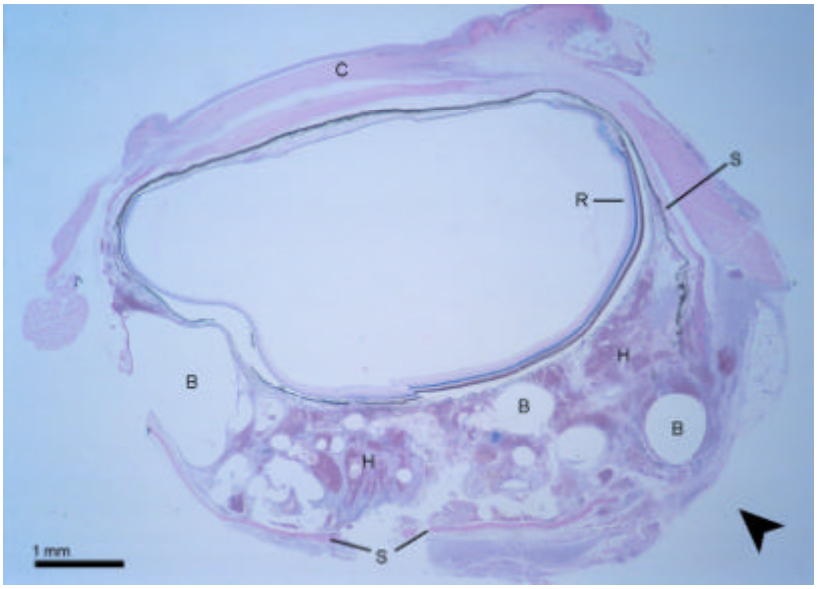

Figure 6. Histological appearance of a typical exophthalmic eye. Grossly, gas and haemorrhage were visible in periorbital tissue (as in the left eye in Fig. 5). Large gas bubbles (B) and haemorrhage (H) are visible in the choroid and have entering the retrobulbar area through a rupture (arrowhead) of the sclera (S). C, cornea. R, retina.

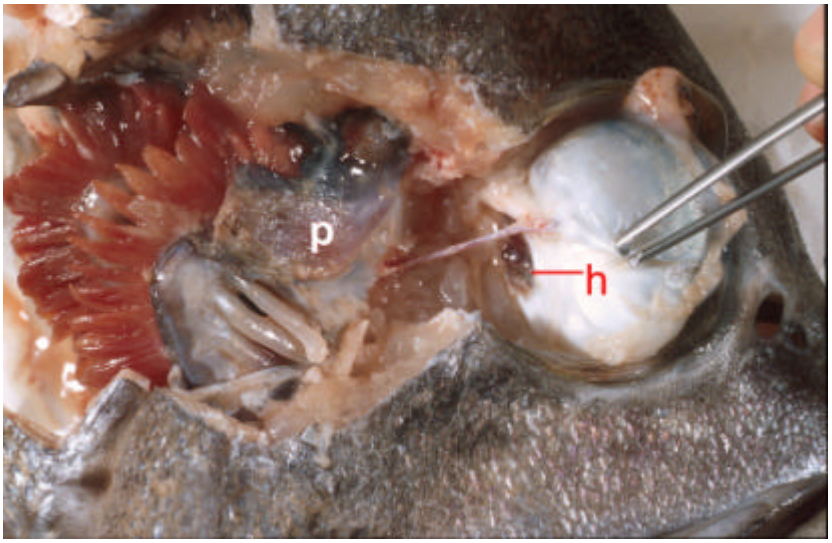

Figure 7. Dissection of a dhufish eye with a previous exophthalmos. The ophthalmic artery passes from the pseudobranch (p) to the sclera. An area of resolved haemorrhage (h) can be seen, indicating rupture of the sclera during exophthalmos.

Retinal degeneration was noted in the exophthalmic eyes of two fish with pronounced reduction in retinal thickness and necrosis of the photoreceptor cell layer. In the absence of repeated stimuli for gas formation in the eye, lesions resolved after several weeks either with a gradual decrease in protrusion of the eye leaving a fold of tissue in the dorsal periorbital area, or phthisis bulbi following perforation of the cornea and loss of the lens.

The oxygen content of gas bubbles within the anterior chamber of exophthalmic eyes varied from 21 to 73% and was higher in acute lesions. The highest recording was 2 days after onset of exophthalmos (Fig. 8). Gas bubbles in two eyes with exophthalmos of less than one week duration contained 53 and 58% oxygen. The oxygen content of bubbles in eyes of five fish with more chronic lesions ranged from 21 to 41%.

In contrast, the oxygen tension at the retinal-vitreal junction was below 100 mm Hg in four exophthalmic eyes. These eyes either had chronic lesions or no gas bubbles were visible in aqueous humour. Ablation of eyes with severe exophthalmos but no visible gas within the globe resulted in the release of relatively large amounts of gas when performed under water. Histologically, most eyes with low retinal pO_2 had a perforated sclera and retrobulbar haemorrhage originating from the choroid.

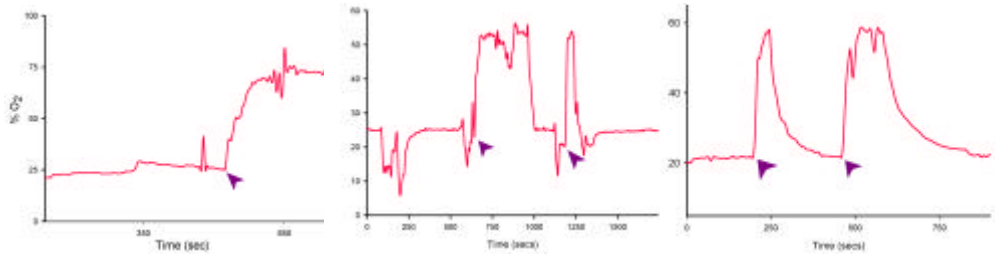

Figure 8. Traces from a fibre optic O_2 sensor, displaying the O_2 content of gas bubbles in eyes of three dhufish with recent exophthalmic lesions. Arrowheads indicate the time of entry of the O_2 sensor into the gas bubble.

Mean pO_2 recorded at the vitreal-retinal interface of eyes of normal dhufish was 344 ± 26 mm Hg, and ranged from 306 to 365 mm Hg, n=4. In comparison pO_2 at the vitreal-retinal interface of normal snapper eyes was 246 ± 93 mm Hg, range 198 to 386 mm Hg, n=4.

In experiment 1 where dhufish were exposed to varying temperature regimes with and without exercise, two of twenty-four fish held at 25.5°C developed unilateral exophthalmos overnight following exercise. The first exophthalmos developed after ten minutes of exercise on day one and the other after sixteen minutes of exercise on day four. On the morning following exercise there was enlargement of the globe and altered

refraction indicating the presence of gas. The next day exophthalmos was severe with a ring of haemorrhage and gas bubbles visible in the periorbital tissue. The fish were dark in colour and did not feed. At necropsy, gas bubbles were found in the choroid and retrobulbar tissue and the sclera had ruptured.

The startle response induced by sudden illumination was less than anticipated and less than that caused by daily tank cleaning procedures. One fish developed unilateral exophthalmos unassociated with fright induction when the water temperature was initially raised from 20.8 to 25.5°C. Six fish struggled and accidentally fell onto a concrete floor from about 1 m during handling and weighing for this experiment. These fish were noted, together with the side of their body that received the impact but none developed exophthalmos immediately following this fall or during the experiment. Exophthalmos did not occur following sudden increases in light intensity, fish handling or tank cleaning operations.

The vascular supply to the dhufish eye was similar to that reported in other species. Blood was oxygenated in the gills before flowing to the pseudobranch (Fig. 9) and then to the choroid body of the eye. The afferent pseudobranch artery arose from the ventral end the first gill arch and entered the bone of the operculum before passing dorsally to the pseudobranch (Fig. 10 B). Figure 11 is a schematic diagram illustrating the vascular arrangement of the heart, gills, pseudobranch and choroid body.

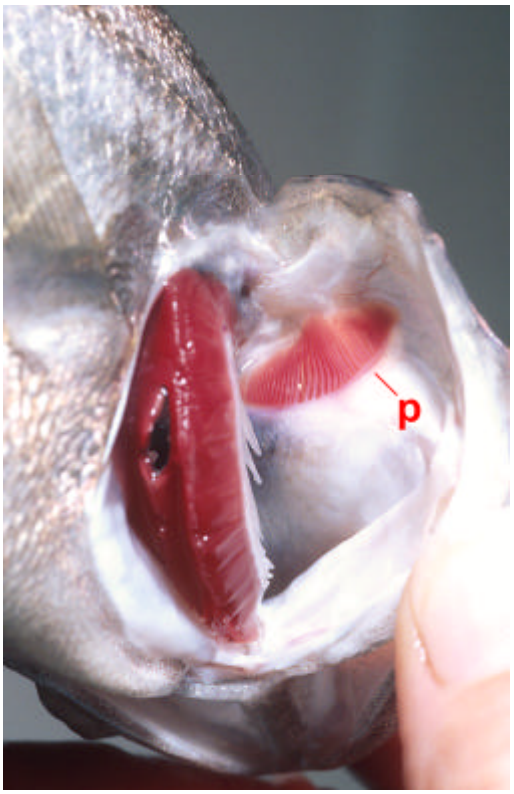

Figure 9. The position of the pseudobranch (p) and gills in a live dhufish.

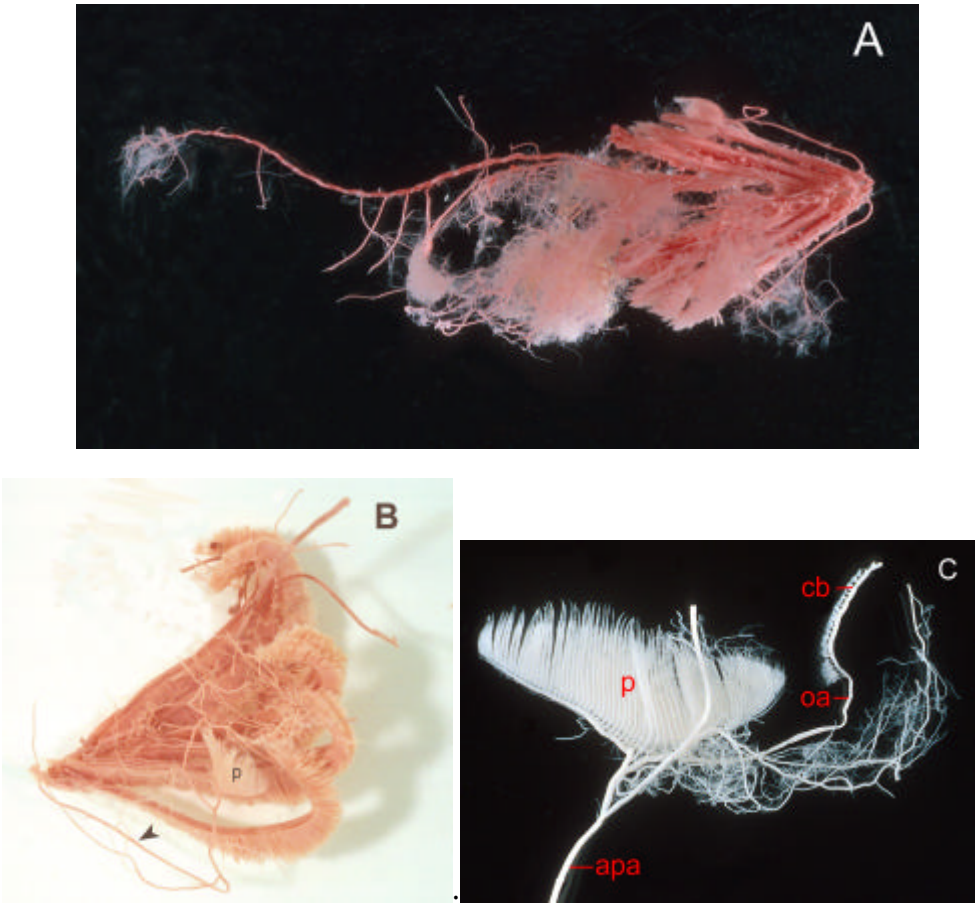

Figure 10. Corrosion casts of the vasculature of the gills, pseudobranch and choroid body of dhufish. A. Vasculature of a whole dhufish. B. Vasculature of the gills and pseudobranch. The afferent pseudobranchial artery (arrowhead) extends from the first branchial arch to the pseudobranch (p). C. Vasculature of the pseudobranch (p), afferent pseudobranchial artery (apa), ophthalmic artery (oa) and a partial cast of the choroid body (cb).

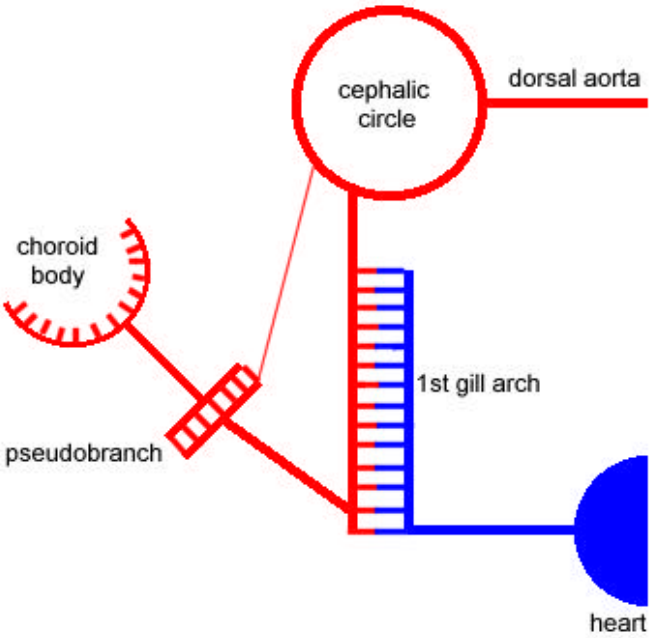

Figure 11. Schematic diagram depicting blood flow from the heart to the choroid body of dhufish. Black = deoxygenated blood. Grey = oxygenated blood.

The pseudobranch in dhufish was a hemibranch, similar in structure to a gill arch but having only one set of primary lamellae (Fig. 12C). As in gills, each primary lamella of the pseudobranch had two rows of secondary lamellae that alternatively left the primary lamella from different sides (Fig. 12E) and branched into a capillary network supported by pillar cells, seen as holes between the capillaries in secondary lamellae.

Histologically the secondary lamellae of the pseudobranch were fused and covered with epithelium (Fig. 13 A). Neurones were visible in the tunica media of the afferent pseudobranchial artery (Fig. 13 B).

In both dhufish and snapper, the choroid body was almost circular with an extensive network of parallel capillaries, the rete mirabile (Fig. 14). In the corrosion casts obtained during this study, only the afferent capillaries were present.

The thickness of the retina of dhufish ranged between 202 and 460 μm thick, mean 332 μm, S.D. 82 μm, n= 14.

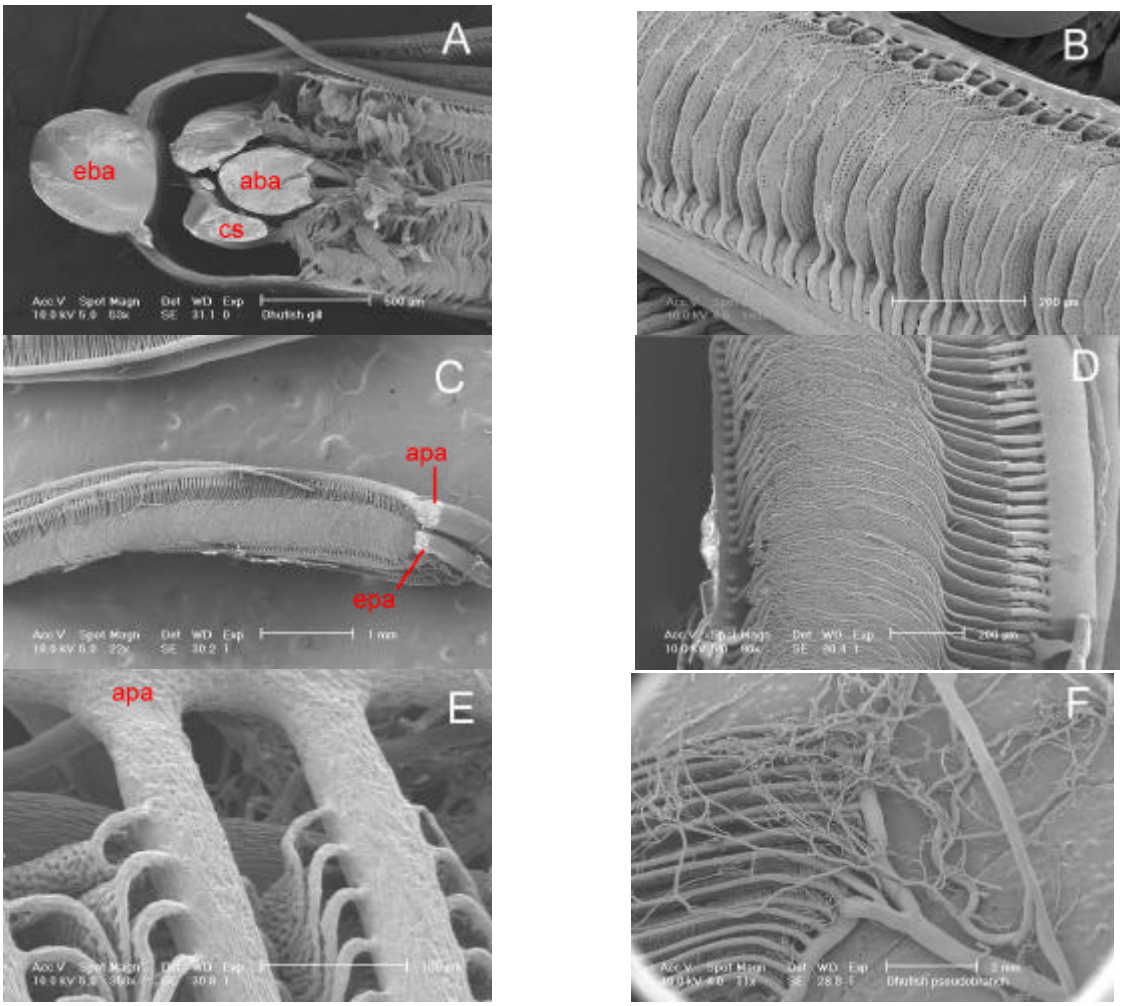

Figure 12. Scanning electron microscopy of corrosion casts of dhufish gills and pseudobranch. A. Gill arch in cross section. Afferent branchial artery (aba), efferent branchial artery (eba), central sinus (cs). B. Secondary gill lamellae within a primary lamella. C. Cross section of a primary lamella from the pseudobranch. Afferent pseudobranchial artery (apa), efferent pseudobranchial artery (epa). D. Secondary lamellae of the pseudobranch. E. Secondary pseudobranch lamellae branching from the primary lamella. F. Flattened sheet of anastomosing vessels situated on the lateral aspect of the pseudobranch. Vessels anastomosed with the pseudobranchial capillaries and the dorsal aorta.

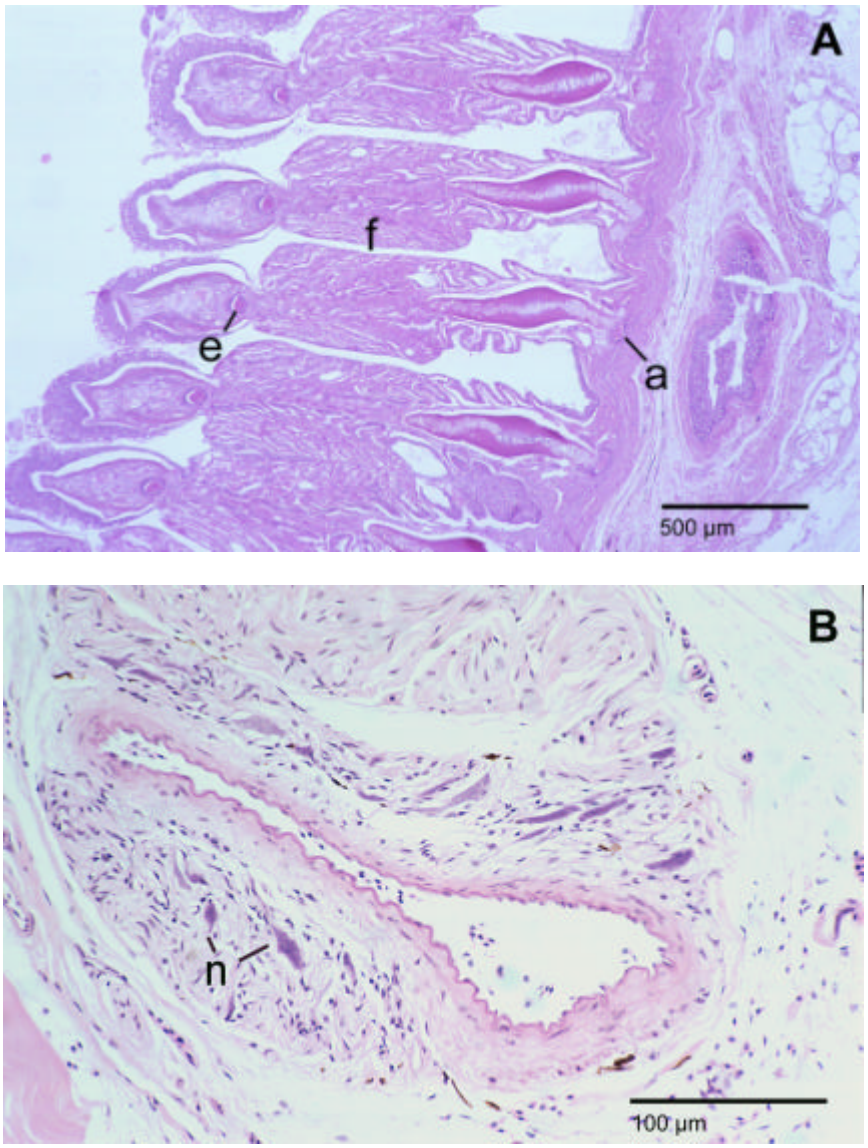

Figure 13. Histological structure of the pseudobranch. A. Histological structure of pseudobranchial lamellae showing fusion of secondary lamellae (f). Afferent (a) and efferent (e) pseudobranchial arterioles can be seen in cross section at the proximal and distal ends of primary lamellae. B. Cross section of the afferent pseudobranchial artery showing several neurones (n) within its wall.

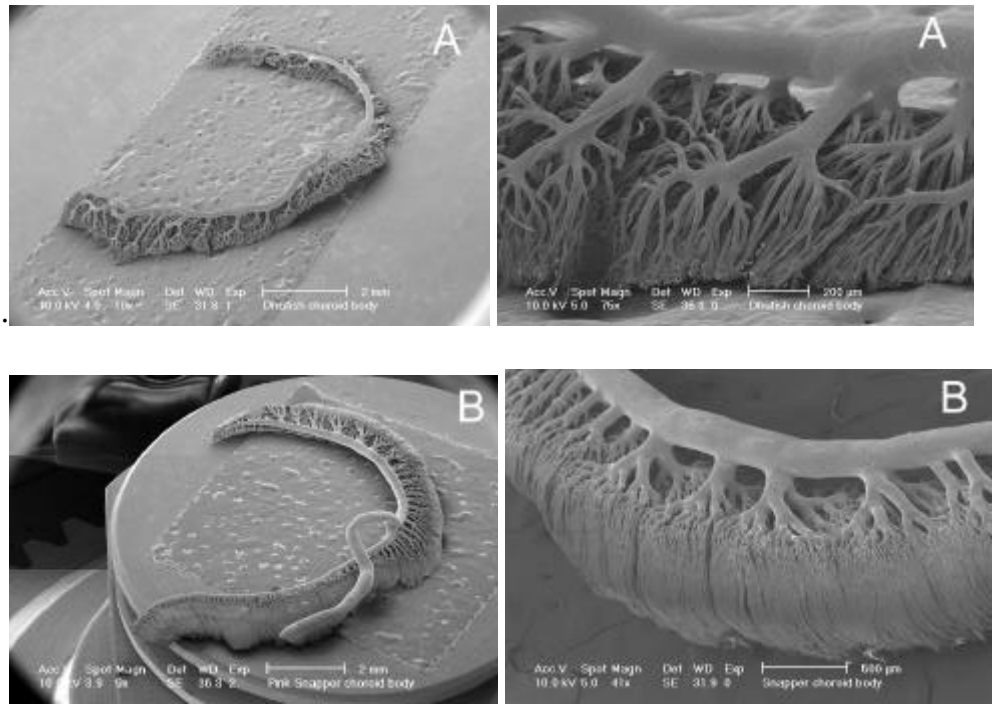

Figure 14. Anatomy of choroid body of dhufish and snapper. Scanning electron microscopy of Mercox cast. A.. Dhufish. Choroid body showing the parallel arterial capillaries of the rete mirabile. B. Snapper. Equivalent microphotographs demonstrating the similarities and differences between the choroid bodies of snapper and dhufish.

Electrophoresis revealed a single haemoglobin in both cattle and dhufish blood. Snapper and black bream blood contained six and five haemoglobins respectively (Fig. 15) and King George whiting blood contained three major haemoglobins.

The magnitude of the Root effect of dhufish haemoglobin was greater than that of snapper, King George whiting and black bream haemoglobin (Table 7). ATP and GTP increased the magnitude of the Root effect in black bream, dhufish and King George whiting haemoglobin. ATP was the more potent effector in black bream and dhufish and GTP in King George whiting haemoglobin.

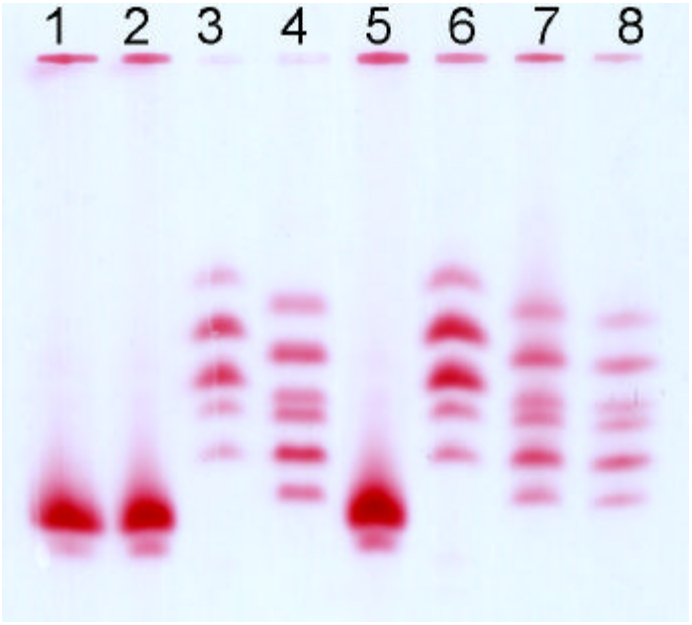

Figure 15. Cellulose acetate strip of haemoglobin isomorphs in dhufish, snapper and black bream. A single haemoglobin is shown for dhufish (lanes 1, 2, 5), five haemoglobin types in black bream (lanes 3, 6) and six haemoglobin types in snapper (lanes 4, 7, 8) following electrophoresis of the haemolysate from each species.

Table 7. The relative magnitude of the Root effect at pH 6.9 of haemoglobins (Hb) from dhufish, snapper, King George whiting and black bream. Dhufish haemoglobins are the least oxygenated at pH 6.9, with greater deoxygenation in the Tris buffer as a result of allosteric effectors (Cl^-).

Species	% Hb oxygenation	% Hb oxygenation, 20µl 1mM ATP	% Hb oxygenation, 20µl 1mM GTP
snapper (HEPES buffer)	96.5	96.7	99.6
snapper (Tris buffer)	91.0	-	-
black bream	97.2	90.1	95.3
King George whiting	89.1	73.6	70.7
dhufish (HEPES buffer)	80.2	62.0	66.0
dhufish (Tris buffer)	58.3	34.6	-

The wavelength scans of dhufish haemoglobin (Fig. 16) illustrate partial deoxygenation at pH 6.9 and results are compared for haemolysate in Tris and HEPES buffers. Table 8 indicates pH values obtained from dhufish plasma and lysed erythrocytes.

Hyperbolic oxygen dissociation curves were obtained for snapper and dhufish whole blood (Fig. 17). The oxygen binding affinity of snapper blood was higher dhufish blood. The presence of higher levels of CO_2 significantly reduced the oxygen binding affinity of dhufish whole blood.

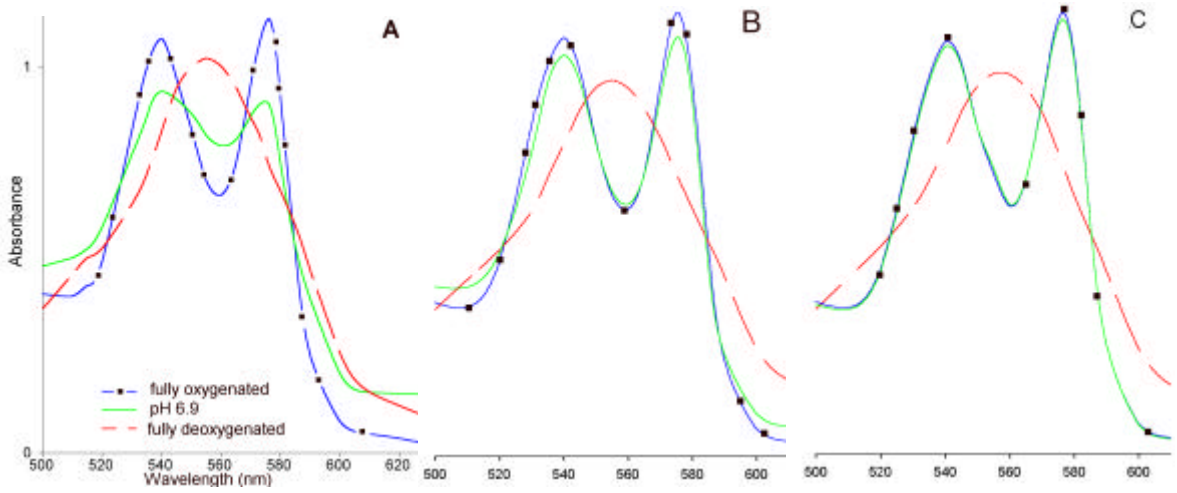

Figure 16. Wavelength scans of dhufish and snapper haemoglobin illustrating partial deoxygenation of haemoglobin at reduced pH. Fully oxygenated haemoglobin is at pH 8.0. Haemoglobin at pH 8.0 was deoxygenated by the addition of sodium dithionite and produced plots with maximal absorbance at 560 nm. The absorbance of partially deoxygenated haemoglobin at wavelengths of 540, 560 and 576 nm was intermediate between the absorbance of oxygenated and fully deoxygenated haemoglobin. Deoxygenation is more pronounced in dhufish haemoglobin seen by absorbance at 540, 560 and 576 nm being closer to deoxygenated than oxygenated haemoglobin. A. Dhufish haemolysate in Tris HCl buffer. B. Dhufish haemolysate in HEPES buffer. C. Snapper haemolysate in HEPES buffer.

Table 8. Comparison of plasma pH (pH_e) and intracellular pH (pH_i) of dhufish blood. pH_i was approximately 0.4 lower than pH_e in the same blood sample, and was in the region where the Root effect may alter haemoglobin oxygenation.

	pH_e	pH_I
Low initial pH(n=3)	7.36 ± 0.08	6.89 ± 0.06
High initial pH (n=5)	7.63 ± 0.04	7.20 ± 0.07

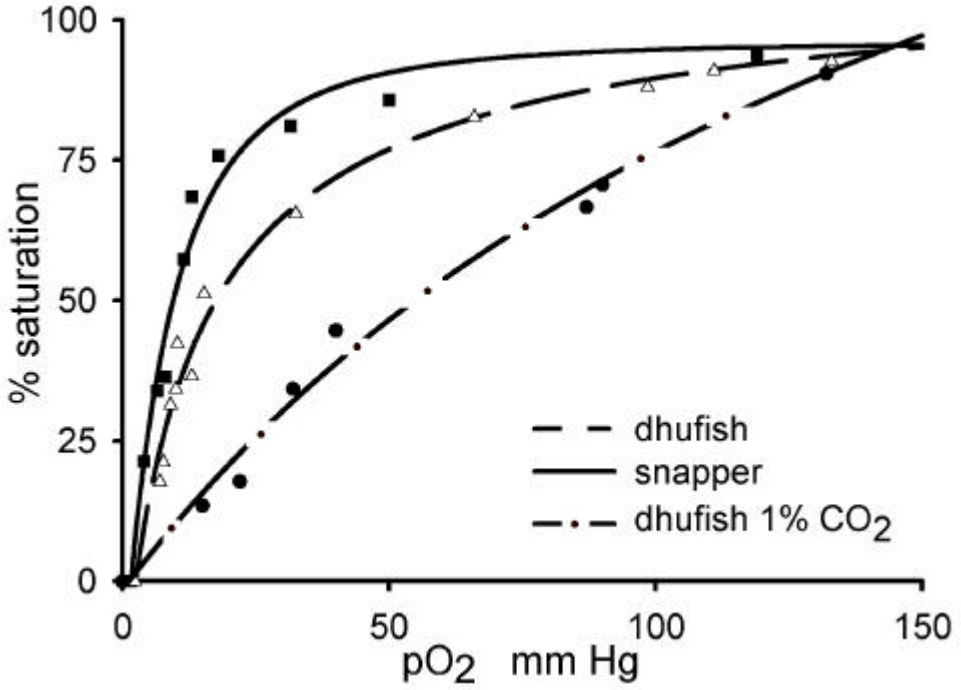

Figure 17. Oxygen dissociation curves of dhufish and snapper whole blood. Dhufish blood had a lower oxygen affinity (larger P_{50}) than snapper blood. Blood in contact with increased pCO_2 (7.6 mm Hg) significantly decreased oxygen affinity of dhufish blood, demonstrated by a right shift of the curve.

A comparison of three methods used to examine the Bohr effect of whole blood is demonstrated (Fig. 18). The results using the ABL 5 blood gas machine and calculation of P_{50} produced the expected linear regression and a Bohr factor of –1.43. The same process using snapper blood, but with an initial lower pH indicating a degree of lactic acidosis resulted in a Bohr factor of –1.10. A sigmoidal curve was obtained for blood equilibrated in 0.03% CO_2 in air and alteration of pH by the addition of acid and base, however, the same blood equilibrated in 1% CO_2 in air produced a linear plot and Bohr factor of – 0.66. The Hill coefficient, η, for dhufish whole blood calculated from the oxygen dissociation curve at 0.015% CO_2 was 1.25. The linear regression used in its calculation is displayed in Figure 19.

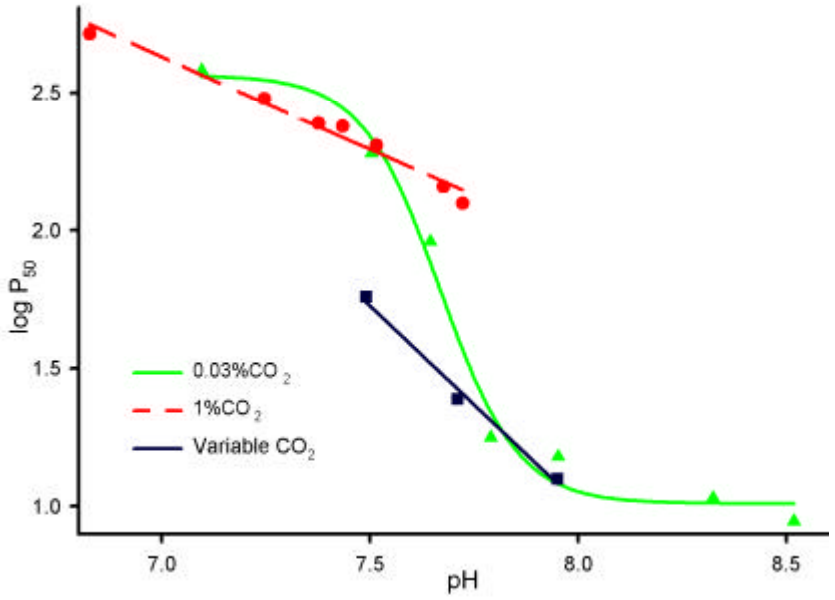

Figure 18. Bohr factor for dhufish whole blood derived using three methods. The sigmoidal curve was obtained from whole blood following pH modification by the addition of acid (lactic acid) and base (Tris) and analysis on the ABL 520. The linear regression is from the same blood equilibrated in 1% CO_2 following the addition of acid and base and analysis on the ABL 520. The lower linear regression was obtained from a different sample of blood following equilibration in .0015%, 0.2% and 1% CO_2, the use of the mixing technique and analysis on an ABL 5 blood gas machine.

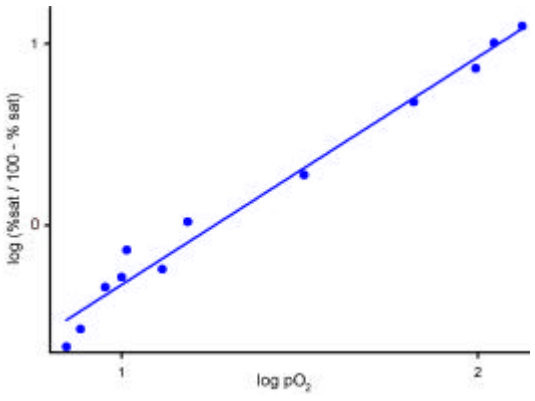

Figure 19. Hill coefficient for dhufish whole blood. The Hill coefficient, η, was calculated from the slope of the linear regression (R^2 = 0.98) of log (% saturation/ 100-% saturation) versus log pO_2 from data obtained by the mixing technique for calculating pO_2 at known % haemoglobin saturation.

Comparisons between blood obtained from dhufish in winter when water temperature was 16-17°C in preceding weeks, and in summer when minimum daily water temperature ranged from 22 to 24°C in the preceding weeks are displayed in Table 9. Oxygen dissociation curves constructed for whole blood from wild caught dhufish held in seawater during summer P_{50} was 60.4 ± 7.0, n=3 and in winter was 71.7 ± 9.3, n=2 in the presence of 1% CO_2. No seasonal trend was apparent in the haematology and haemoglobin oxygenation properties of dhufish blood in summer and winter as there was only one statistically significant result, blood ATP (student t-test, $p < 0.05$).

Table 9. Haematological parameters of dhufish in summer and winter. Whole blood was examined in late winter and summer when mean water temperatures were 16.5°C and 23°C, respectively.

	Winter, n=8	Summer, n=19
Hb (g L^{-1} ± S.D)	60.1 ± 3.3	57.1 ± 6.9
Hct (% ± S.D.)	21.1 ± 1.5	20.0 ± 2.8
RBC (10^6 μL^{-1} ± S.D.)	272.1 ± 27.6	264.4 ± 44.2
MCV (μm^3 ± S.D.)	77.4 ± 7.4	76.8 ± 13.6
MCHC (g L^{-1} ± S.D.)	286.0 ± 12.1	287.6 ± 25.1
MCH (pg ± S.D.)	22.1 ± 2.2	21.9 ± 3.7
ATP (mmol mL^{-1} ± S.D.)	1.3 ± 0.04	1.2 ± 0.1 *
ATP (μmol g^1 Hb ± S.D.)	21.4 ± 1.3	21.2 ± 1.7

* statistically significant difference between ATP in blood in summer and winter (student t-test, p= 0.036).

n=10 for summer ATP and ATP:Hb.

Water in tanks containing seawater and bore water had consistently different pH resulting from a difference in pCO_2. A linear association was found between pH and pCO_2 of bore water, seawater and seawater perfused with CO_2 (Fig. 20). A comparison of chemical properties of bore water and seawater is displayed in Table 10.

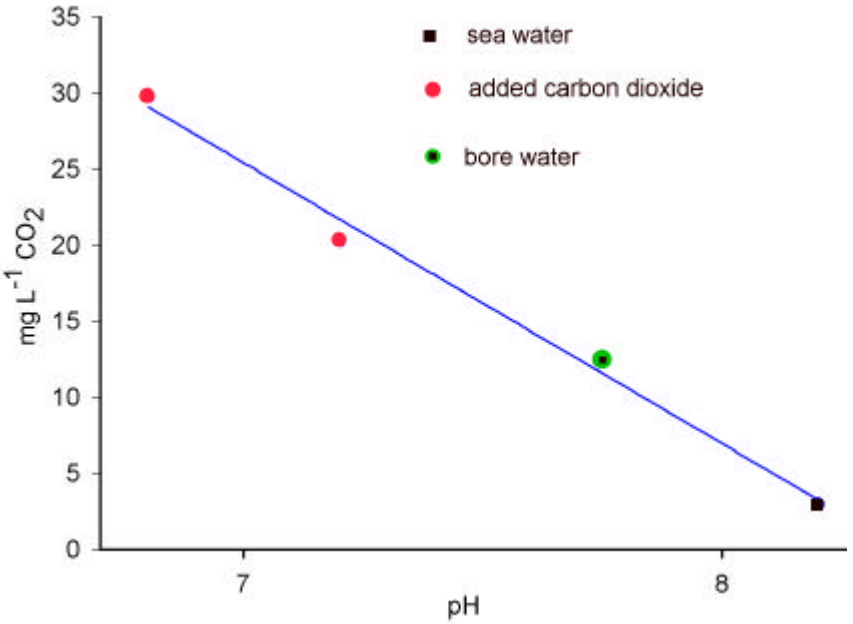

Figure 20. CO_2 content and pH of bore water, seawater and seawater perfused with CO_2.

Dhufish held in bore water had some differences in acid-base and haematological parameters (Table 10). pCO_2, HCO_3^-, haemoglobin, haematocrit and erythrocyte counts were significantly higher in bore water-held fish (Table 11).

Table 10. Water analysis results comparing properties of bore water and seawater. Results are expressed as means ± S.D. n=3.

water source 22°C	pH	alkalinity (mg $CaCO_3^-L^{-1}$)	free CO_2 (mg L^{-1})	pCO_2 (mm Hg)	Ca (mg L^{-1})
bore water	7.73±0.03	123 ± 1.3	11.7 ± 0.4	6.4 ± 0.3	388 ± 2
seawater	8.15±0.03	112 ± 1.4	2.7 ± 0.3	1.5 ± 0.2	404 ± 8

Table 11. Dhufish growth and blood respiratory and acid-base parameters following long term culture in seawater and high CO_2 bore water.

	Bore water, (n=18)	Seawater,(n=19)
fish weight (kg $\pm$ S.D.)	0.96 ± 0.21	1.04 ± 0.23
fish length (cm $\pm$ S.D.)	34.8 ± 2.8	35.8 ± 2.7
pH $\pm$ S.D.	7.62 ± 0.07	7.57 ± 0.10
pCO_2 (mm Hg $\pm$ S.D.)	9.8 ± 1.5 *	8.7 ± 1.3
HCO_3^- (mmol $L^{-1} \pm$ S.D.)	12.0 ± 0.8 **	9.6 ± 1.5
Cl^- (mmol $L^{-1} \pm$ S.D.)	149.7 ± 8.2	148.4 ± 5.6
Hb (g $L^{-1} \pm$ S.D.)	75.3 ± 11.5 **	61.9 ± 8.3
Hct (% $\pm$ S.D.)	27.0 ± 4.1 **	22.7 ± 3.0
RBC ($10^6 \mu L^{-1} \pm$ S.D.)	3.27 ± 0.61 **	2.85 ± 0.42
MCV ($\mu m^3 \pm$ S.D.)	84.4 ± 15.1	80.5 ± 7.8
MCHC (g $L^{-1} \pm$ S.D.)	280.2 ± 28.0	274.8 ± 42.5
MCH (pg $\pm$ S.D.)	23.5 ± 4.5	22.1 ± 3.7

** significantly different at the 0.01 level, * significantly different at the 0.05 level

Discussion

The thick retina and large choroid body supplied with oxygenated blood from the gills via the pseudobranch is a feature common to dhufish and many other marine teleosts. Although the afferent vasculature of the pseudobranch of dhufish was similar to that of rainbow trout and some other teleosts (Laurent and Dunel-Erb 1984), its ventral origin in the first branchial arch rather than the dorsal route from cephalic circle was dissimilar to the pattern identified in some species (Goodrich 1930). The significance of the ventral route of the artery in dhufish is unclear but is consistent with the choroid body being a vestige of a premandibular gill, and the pseudobranch a vestige of the mandibular gill arch (Goodrich 1930). The position of the pseudobranch, in series between the gill and choroid body, in dhufish suggests a function in oxygenation of the retina as in other species (Wittenberg and Wittenberg 1974; Hoffert and Ubels 1979; Copeland 1980).

The ventral route of the afferent pseudobranchial artery from the first gill arch and the presence of neurones within its wall (Fig. 13 B), suggests that the organ may also have a sensory function similar to that identified in rainbow trout (Perry and Heming 1981) where the pseudobranch acted as a pressure receptor although it did not regulate

cardiovascular or respiratory functions. In another study, McKenzie *et al.* (1991) demonstrated cardiovascular responses to hypoxia in an air breathing fish following pseudobranch ablation, however, the relative effects of pseudobranch ablation and branchial denervation could not be differentiated.

The anatomy of the dhufish eye was similar, but not identical to that of snapper and other teleosts (Wittenberg and Wittenberg 1974; Copeland 1980). The thickness of the dhufish retina was similar to that of the twenty six species of predominantly marine teleosts studied by Wittenberg and Wittenberg (1974), where mean retinal thickness was 360 μm (range 240-500 μm) and the distance between the choriocapillaris and the mitochondria-rich receptor cell layer of the retina was 106 μm (78-170 μm). As there are few or no capillaries within, or on the surface of the dhufish retina, oxygen diffuses into the retina from the choroid. The high pO_2 in the normal dhufish eye correlates with a similarly high pO_2 in other species with a large choroid body and thick retina (Wittenberg and Wittenberg 1962, 1974; Fairbanks *et al.* 1969). In rainbow trout with a mean retinal thickness of 324 μm (Desrochers *et al.* 1985), pO_2 decreased from 381 mm Hg at the choriocapillaris to 124 mm Hg at the junction of the vitreous humour and retina. Hypoxia from reduced ventilatory flow caused a rapid and severe decrease in pO_2 in the choroid (Hoffert and Ubels 1979), similar to the reduction in pO_2 in dhufish eyes when the oxygen sensor was pushed too vigorously against the retina thus interrupting blood flow in the choroid.

The dependence of oxygenation on carbonic anhydrase activity was not fully investigated in dhufish, however the results of its inhibition with acetazolamide were unequivocal as there was uncertainty about the dose rate and expected timing and duration of effect following treatment. However, it did not alter the course of the condition in the exophthalmic dhufish that were treated, unlike the finding for exophthalmic Atlantic cod (Dehadrai 1966).

The pronounced Root effect of dhufish haemoglobin suggests that it may have a function in deoxygenation of haemoglobin in the choroid rete, similar to that demonstrated in rete of the gas glands in the swim bladder of rainbow trout and eel, *Anguilla anguilla*, (Bridges *et al.* 1998; Pelster 1998) where a decrease in erythrocyte pH reduced the ability of haemoglobin to carry oxygen (Root 1931; Gilmour 1998). The pH within dhufish erythrocytes (Table 8) was below that of plasma, as in other fish (Brauner and Randall 1996), and any further fall in pH may increase oxygen unloading in the choriocapillaris.

The single dhufish haemoglobin appears to be an anodic haemoglobin as it had large Root and Bohr effects and sensitivity to nucleoside triphosphates (Table 7 and Fig 18). Anodic haemoglobins are also characterised by large Haldane effects (an increased capacity of deoxygenated haemoglobin to carry more CO_2 and protons in comparison with oxygenated haemoglobin) (Jensen 1989), sensitivity to temperature (Weber and de Wilde 1976) and low haemoglobin-oxygen affinity (Weber and Jensen 1988). Dhufish haemoglobin was not investigated to determine these properties.

The significant right shift of the dhufish oxygen dissociation curve in the presence of 1% CO_2 (Fig. 17), indicates a substantial decrease in haemoglobin-oxygen affinity, and correlates with the finding of a large Bohr factor (Fig. 18). A limitation of the use of the mixing technique to construct oxygen dissociation curves in fish is that the magnitude of the Root effect cannot be demonstrated unless blood is equilibrated in very high pO_2. The low pH of dhufish blood equilibrated at pO_2 160 mm Hg and 7.6 mm Hg CO_2 indicated that haemoglobin is unlikely to be fully saturated in such an environment. The dark purple colour of blood following equilibration in this gas mix also suggests that haemoglobin is not fully oxygenated. Saturation was over-estimated using the mixing technique in this high pCO_2 environment and the oxygen dissociation curve did not display a downwards shift as it should when the Root effect was active (Gilmour 1998). Had CO_2 in oxygen (pO_2 760 mm Hg) rather than air (pO_2 160 mm Hg) been used for equilibration, the magnitude of Root effect may have been visualised in this study of whole dhufish blood.

Snapper and dhufish whole blood in the present study consistently produced hyperbolic oxygen dissociation curves similar to those of Australian blackfish *Gadopsis marmoratus*, barramundi *Lates calcarifer*, tarpon *Megalops cyrinoides* and saratoga *scleropages jardinii* (Dobson and Baldwin 1982a; Wells *et al.* 1997). Hyperbolic oxygen dissociation curves indicate low cooperativity of binding between oxygen binding sites of haemoglobin (Riggs 1970) and a Hill coefficient near unity as in dhufish (Fig. 19). The oxygen dissociation curve of dhufish whole blood may, in fact, be slightly sigmoidal but may appear hyperbolic unless many recordings are obtained at the points of flexure. Similarly, some studies of human blood and haemoglobin have produced hyperbolic or almost hyperbolic oxygen dissociation curves (Riggs 1970) and a slightly sigmoidal curve was demonstrated for tench blood by Weber and Jensen (1988), whereas Eddy (1973) produced a hyperbolic oxygen dissociation curve in the same species. The relatively low Hill coefficient obtained for dhufish whole blood equilibrated in 0.015% CO_2 suggests the oxygen dissociation curve may be slightly sigmoidal rather than hyperbolic. A flexure at very low pO_2 may be difficult to demonstrate using the mixing technique because small quantities of blood were used, and dead space of the syringe and needle may affect pO_2.

The Root effect of dhufish haemoglobin in Tris buffers of known pH (Table 7) was much larger than that of Australian tropical reef fishes such as spangled emperor *Lethrinus nebulosus* or wire-netting cod *Epinephelus quoyanus* (Wells and Baldwin 1990), although a slightly different pH was used in their study. Two buffers were used to quantify the Root effect in dhufish. The buffers produced different results, although the relative magnitude of the Root effect between species was consistent, irrespective of the type of buffer used. Weber (1992) recommended the use of HEPES buffer as Cl^- in Tris buffering systems is an allosteric effector of haemoglobin oxygenation. Tris buffers were extensively used for investigations of the Root effect prior to Weber's investigation (1992). In the present study, the HEPES buffer adversely affected the stability of haemoglobin, with methaemoglobin levels being consistently higher than in Tris buffer.

The relevance of *in vitro* results using haemoglobin to haemoglobin oxygenation in erythrocytes *in vivo* is uncertain due to the complexity of processes regulating haemoglobin oxygenation within erythrocytes. Nucleoside triphosphates are one of the major allosteric effectors of haemoglobin oxygenation (Powers 1980; Weber and Jensen 1988), and although their concentration in the haemoglobin dilutions used in these experiments was negligible due to dilution, they are expected to have a large impact on haemoglobin oxygenation *in vivo*.

Without isoelectric focusing (Weber and de Wilde 1976; Weber *et al.* 1976; Dobson and Baldwin 1982a; Wells *et al.* 1997) it was not possible to functionally differentiate the haemoglobins in black bream, snapper and King George whiting haemolysate. The less pronounced Root effect in snapper, black bream and King George whiting haemolysates suggests these species may have both anodic and cathodic haemoglobins, or haemoglobins that are structurally different from dhufish haemoglobin (Brittain 1987; Weber and Jensen 1988; Pelster and Weber 1990).

The wavelength scans obtained for oxygenated and partially deoxygenated dhufish haemoglobin were similar in shape to those obtained for bullfrog haemoglobin (Riggs 1951). Examination of such wavelength scans may be an alternative method of assessing the magnitude of the Root effect in fish haemolysates, although absorbance at wavelengths 540, 560 and 576 nm remains the key feature in determining relative oxygenation of haemoglobin in any species.

The single haemoglobin of dhufish with its pronounced Root effect may have profound effects on the ability of the fish to adapt to changing environmental and physiological conditions as single haemoglobins have been linked to environmental stability (Riggs 1970) and sluggish bottom dwelling habits (Riccio *et al.* 2000). It was thought that anodic and cathodic haemoglobins in the same fish conferred the ability to adapt to a variety of internal metabolic conditions and external environmental conditions (Riggs 1970; Powers 1980), although this generalisation did not always apply (Weber and de Wilde 1976; Fyhn *et al.* 1979).

Assessment of whole blood or haemoglobin oxygen affinity, indicated by P_{50}, is highly dependent on temperature, pH and methods used in each study. Nevertheless, dhufish whole blood oxygen affinity was low compared with that of many fish species (Root 1931; Eddy 1973; Weber and de Wilde 1975; Cech *et al.* 1994), but higher than that of Australian blackfish (Dobson and Baldwin 1982b) and similar to rainbow trout (Cameron 1971a). The relatively low whole blood oxygen affinity of dhufish may indicate adaptation to a sedentary lifestyle and consistently high availability of oxygen in the environment (Powers *et al.* 1979; Powers 1980). However, allosteric effectors and ionic composition within erythrocytes have a large impact on the adaptability of blood oxygen transport mechanisms and it is an over-simplification to assert that a single property reflects adaptation to a particular habitat (Wells 1990).

ATP, GTP and CO_2 are allosteric effectors of haemoglobin-oxygen binding in dhufish as the blood had a large Bohr factor (Fig 18), the oxygen dissociation curve shifted to the right in the presence of CO_2 (Fig 17) and the Root effect was more pronounced in the presence of ATP and GTP (Table 7). In practice it is difficult to differentiate the effect of the CO_2 Bohr factor on haemoglobin-oxygen affinity from the Root effect, which decreases haemoglobin-oxygen carrying capacity (Bridges *et al.* 1983; Brittain 1987). Both effects were demonstrated in oxygen dissociation curves of rainbow trout blood by Cameron (1971a) who used the mixing technique and pCO_2 of 0 to 7-8 mm Hg. The Root effect in rainbow trout appeared less pronounced than that of dhufish, as indicated by the smaller Bohr factor of –0.49 (Tetens and Lykkeboe 1981) and the relatively small decrease in blood oxygen carrying capacity (less than 5%) at pCO_2 3 mm Hg (Cameron 1971a).

The Root effect of dhufish haemoglobin may be the reason for the sigmoidal curve obtained for the Bohr factor plot of blood in the presence of 0.03%CO_2 that was obtained on the ABL 520 blood gas analyser (Fig 18). Plots used to calculate Bohr factors are expected to be linear, as was dhufish blood in 1%CO_2 and blood from snapper and dhufish analysed on the ABL 5, however, sigmoidal curves are sometimes obtained in studies of Root haemoglobins (Brittain 1987) and fish blood (Noble *et al.* 1986). Brittain (1987) attributed the significant reduction in haemoglobin-oxygen affinity with decreasing pH to the Root effect and stated that cooperativity of Root haemoglobins decreased with decreasing pH. Other investigators, however, found variable dependence of cooperativity on pH (Weber *et al.* 1976; Tetens and Lykkeboe 1981; Noble *et al.* 1986; Cech *et al.* 1994; Wells *et al.* 1997) with no predictable pattern emerging. The reasons for the two different plots obtained for dhufish blood is uncertain but may relate to the method of calculation of P_{50}. The ABL 520 automatically calculated the value from a single blood sample using an algorithm and may be the more accurate reflection of the effect of CO_2 on dhufish blood. Analysis of all samples on the ABL 520 blood gas analyser, negating the requirement to use the mixing technique (Fig. 18), may have demonstrated a similar and larger Bohr factor in the region of physiological pH (the linear part of the green plot in Fig. 18) as the ABL 520 is more likely to be able to accurately assess blood-oxygen saturation in the presence of the Root effect.

There are several potential sources of error when using uncannulated fish and analysers developed for gas and acid base determination in human blood. They include: heating of blood by blood gas analysers and the use of algorithms derived from humans to calculate values for fish at their environmental temperature; delays between bbod collection and analysis necessitated by fish holding facilities being 10 km from Murdoch University Veterinary Hospital; capture and anaesthesia resulting in changes to erythrocyte volume, blood pH and pCO_2 in uncannulated fish.

Radiometer blood gas machines are commonly used to monitor anaesthesia in mammalian species (Day *et al.* 1995) but heating may have a deleterious effect on the fish blood. As the dhufish is a temperate/subtropical species this was not considered to be of major significance, and equilibration of replicates of dhufish blood in known gas mixtures validated the use of this the Radiometer ABL 5 blood gas analyser as pCO_2 and

pO_2 produced the expected results, reflecting that of the gas mixture used to equilibrate the samples. Another potential problem with the use of the analyser was the specification for pCO_2, which was 5 mm Hg for the ABL 5, whereas expected pCO_2 of venous fish blood was 2-4 mm Hg (Wood *et al.* 1979). However, following equilibration in air with pCO_2 of 2 mm Hg the results obtained for dhufish blood were the same as those of the equilibration gas and were highly accurate and repeatable.

The ABL 5 calculates HCO_3^- concentrations using the Henderson-Hasselbach equation and constants for human blood. They are not directly applicable to dhufish blood, however, the constant for dhufish is not expected to differ greatly (Boutilier *et al.* 1984). Differences between groups of dhufish, however, they should be interpreted as qualitative data rather than quantitative data.

The effect of delay in assessment of blood samples was a potential source of error, however, blood gases and pH of dhufish blood stored in plastic syringes in ice slurry did not alter significantly over a ninety minute period and were not considered to be of clinical significance in experiments where all samples received similar treatment. pCO_2 and pO_2 increased and pH decreased during storage and were similar to investigations of human blood where pH varied little (<0.01 unit decrease) over a two-hour period (Biswas *et al.* 1982; Mahoney *et al.* 1991; Liss and Payne 1993; Beaulieu *et al.* 1999). pO_2 was the most variable parameter in dhufish samples, increasing by approximately 3 mm Hg over a two-hour period, probably due to greater solubility at reduced temperature (Mahoney *et al.* 1991; Liss and Payne 1993; Beaulieu *et al.* 1999), brief exposure to air during serial analysis of the same sample and diffusion through the plastic wall of the syringe (Madiedo *et al.* 1980; Biswas *et al.* 1982). pCO_2 increased during storage of dhufish blood, presumably from metabolic activity within the blood (Biswas *et al.* 1982; Beaulieu *et al.* 1999).

The deep anaesthesia required for ease of handling and bleeding large dhufish resulted in reduced ventilatory volume and rate and was the most likely explanation for higher than expected pCO_2 in dhufish and snapper blood. Likewise, decreased ventilation during anaesthesia increased arterial pCO_2 to approximately 6 mm Hg in coho salmon and rainbow trout (Graham and Iwama 1990).

Large differences in blood pH in both dhufish and snapper were obtained from samples taken on different days and were likely to result from stress (Cameron 1984; Ling and Wells 1985). Handling and anaesthetic agents stress fish and increase blood cortisol, haemoglobin and lactate levels (Strange and Schreck 1978; Pickering *et al.* 1982; Graham and Iwama 1990; Molinero and Gonzalez 1995; Gerwick *et al.* 1999). Increased blood lactate of several hours duration was attributed to struggling or increased muscular activity during capture and handling (Pickering *et al.* 1982; Molinero and Gonzalez 1995), or struggling during induction of anaesthesia (Graham and Iwama 1990). The capture order of fish also has a large effect on the amount of lactate produced, with the first fish captured having significantly lower levels (Pickering *et al.* 1982; Molinero and Gonzalez 1995). As expected, increased blood lactate was associated with lower blood pH in anaesthetised fish (Soivio *et al.* 1977; Smit *et al.* 1979). As struggling sometimes

occurred during anaesthesia of dhufish, it is not surprising that similar changes in blood gases, acid-base balance and haematology were noted.

The consistent increase in venous pCO_2, the variable reduction in blood pH and occurrence of erythrocyte swelling in dhufish, can be attributed to anaesthesia-related activities similar to those described in rainbow trout during anaesthesia with AQUI-S (Davidson *et al.* 2000) and snapper following the stresses of handling and anaesthesia in 2-phenoxyethanol (Bollard *et al.* 1993).

These findings highlight the importance of using cannulated fish held in black boxes (Soivio and Nyholm 1975) to obtain blood samples free from the effects of handling and anaesthetic stresses. Cannulated fish were not used in this study because of the high value of large fish and the necessity to use the same fish in several, long-term investigations. Nevertheless, comparisons between treatments are considered valid as several replicates were used, and the technique of sampling and fish handling and the time between collection of blood and analysis were similar in each instance.

Erythrocyte swelling, evidenced by increased haematocrit and reduced MCHC, together with decreased plasma pH in snapper and dhufish blood, was particularly evident after prolonged handling or slow tank draining, similar to that demonstrated in rainbow trout and gilthead sea bream, *Sparus aurata,* following anaesthesia with MS 222 (Soivio *et al.* 1977; Molinero and Gonzalez 1995) and AQUI-S (Davidson *et al.* 2000).

Swelling of teleost erythrocytes is a response to stress or plasma acidosis (Houston and Mearow 1979; Tetens and Lykkeboe 1981; Ling and Wells 1985; Weber and Jensen 1988) and also occurs following delays between sampling and sample analysis (McLeay and Gordon 1977; Railo *et al.* 1985). For these reasons leucocrit has been suggested as a more useful indicator of chronic stress and health problems in fish than haematocrit (McLeay and Gordon 1977) as it is less affected by sampling and storage conditions.

Erythrocyte swelling in dhufish is expected to decrease the concentration of ATP or GTP in the erythrocyte, thereby reducing the magnitude of the Root effect (Pelster and Weber 1990) and assisting haemoglobin oxygenation at the gills during lactic acidosis and stress. This response is especially evident in species with an active Na^+/ proton pump that maintains pH_i at close to resting levels. As it is probable that dhufish erythrocytes do not rely on a Na^+/ proton pump, erythrocyte swelling may be accompanied by a fall in pH_i in response to decreased plasma pH, counteracting any reduction in ATP/GTP concentration (Railo *et al.* 1985). Therefore, any circumstances resulting in decreased plasma pH may also reduce pH_i in dhufish and result in an increase in magnitude of the Root effect.

Haematological parameters, oxygen dissociation curves and blood ATP levels (Table 9) in dhufish suggest that seasonal water temperature has little effect on dhufish blood oxygen transport properties. Although ATP content per mL of blood was statistically different between summer and winter, the results are unlikely to be biologically significant as this difference was not reflected in other haematological parameters such as the ATP:Hb ratio, suggesting unchanged haemoglobin-oxygen affinity. This conclusion is

supported by the oxygen dissociation curves of blood in winter and summer where little change in blood-oxygen affinity was evident. The statistically significant result of ATP content of blood (Table 9) may result from a Type I error caused by small sample size and low power of the statistical tests (Tacha *et al.* 1982).

It can be assumed that seasonal temperatures ranging from 17 to 24°C are within the optimal range for dhufish, however, the daily rate of change in water temperature may adversely affect dhufish by increasing the prevalence of exophthalmos or affecting reproductive development or performance.

The pH of bore water (Table 10) is unlikely to adversely affect to dhufish as a pH range of 7.5 to 8.5 is considered acceptable for marine fish (Boyd and Tucker 1998). The lower pH of bore water was associated with high pCO_2, a common feature of limestone aquifers (Boyd and Tucker 1998). The high pCO_2 is of potential significance to the dhufish as haemoglobin oxygenation may be compromised under hypercapnic conditions in species with large Root and Bohr effects (Randall 1970; Wedemeyer 1997). The increased haematocrit, haemoglobin content and erythrocyte count of dhufish held in bore water (Stephens *et al.* in press) supports this hypothesis.

In other marine fish, reduced plasma pH was compensated within 10 hours as HCO_3^- is taken from the aqueous environment and Cl^- is released into the water (Toews *et al.* 1983). The rapid compensation in marine fish is attributed to their ability to homoregulate in highly saline water because their plasma osmolality is less than the surrounding water and they are highly adapted to ion exchange rather than water retention as in freshwater fish (Ferguson 1989).

Plasma pH, MCV and MCH were not significantly different between dhufish held in seawater and bore water (Table 11), indicating that changes in haemoglobin-oxygen affinity did not result from swelling of erythrocytes. The oxygen carrying capacity of the blood of dhufish held in bore water was higher as a result of polycythaemia. Similar changes occurred during long-term tissue hypoxia in trout held in freshwater of low pO_2 and high pCO_2 (Phillips 1947). However, no long-term studies have demonstrated changes in plasma pH during environmental hypercapnia or acidity. In fact in most long-term studies (Soldatov 1996; Fivelstad *et al.* 1998), no increases in erythrocyte numbers were found following environmentally induced hypoxia, although Soldatov (1996) found that more rapid changeover of erythrocytes occurred under these conditions. It is, therefore, difficult to link changes in erythrocyte numbers in dhufish in bore water to changes in haemoglobin oxygenation resulting from the Root and Bohr effects. Nevertheless dhufish appear to be extremely susceptible to environmental hypercapnia as indicated by polycythaemia in the presence of relatively small changes in Cl^-, HCO_3^- and pCO_2 compared with salmon and sea bass in hypercapnic environments (Grøttum and Sigholt 1996; Fivelstad *et al.* 1998). This may have caused the anorexia noted in dhufish held in bore water.

The physical chemistry of gases may be significant to dhufish in aquaculture situations where low hydrostatic pressure may affect haemoglobin oxygenation. Each gas contributes a partial pressure to total gas pressure, and the partial pressures of the biologically important gases O_2 and CO_2 are expected to be much less in tanks than in the fishes natural environment where hydrostatic pressure results in significantly greater total and partial gas pressure (Vick 1984). This may have serious consequences for dhufish as it may be highly adapted to high pO_2 environments, and is an inactive species not likely to be regularly exposed to prolonged swimming activity and lactic acidosis.

Analysis of seawater pCO_2 using standard water laboratory techniques (Table 10) appeared to over-estimate values based on computations for saturated seawater at 20°C (Colt 1984; Fivelstad *et al.* 1998). However, for the purposes of this study, a comparison of the magnitude of the difference between the two water sources was considered to be acceptable.

Prior to commencement of this project, the cause of spontaneous exophthalmos in dhufish was thought to be mechanical trauma to the protruding eye followed fish handling or fright induction in brightly illuminated tanks. Partially covering tanks with black plastic and modifying handling and tank management practices to reduce sudden fright reactions and the use of foam to support fish during handling reduced the prevalence of the condition. However, trauma did not explain the presence of gas in exophthalmic eyes. In addition, fish accidentally dropped during handling or fish that 'jumping' from tanks up to 2 m in height were sometimes bruised but did not develop exophthalmos following the incident.

Gas bubble disease caused by supersaturation of fish tissues with nitrogen and oxygen was a possible explanation for the gas in exophthalmic eyes. The hypothesis that exophthalmos in dhufish was the result of gas bubble disease was rejected as gas bubbles were seen only in and around the eye and in no other tissues; water depth in tanks and fish behaviour indicated that high levels of supersaturation would be required to cause gas bubble disease; gas bubble disease was not seen in other species held in equivalent conditions at the same premises and supersaturation of water (indicated by dissolved oxygen of greater than saturation at given temperature and pressure) was not recorded in tanks during the study period.

Water supersaturated with both nitrogen and oxygen and total gas pressure in excess of 110% saturation is usually required for clinical disease to occur in salmonids (Weitkamp and Katz 1980; Colt *et al.* 1991). At the ADU, the most likely cause of supersaturation would be cavitation within pumps and incoming water lines, and would be indicated by high dissolved oxygen recordings. No such findings were made. Additionally, no grossly visible gas bubbles were seen in other dhufish tissues such as primary gill lamellae (Machado *et al.* 1987; Smith 1988; Speare 1998), although Elston *et al.* (1997) reported that gas bubbles dissipate following excision of tissues, and warned that false negative findings may result if fish tissues are not examined immediately during necropsy.

Fish compensate for supersaturation by moving to deeper water (Chamberlain *et al.* 1980; Knittel *et al.* 1980; Stevens *et al.* 1980) and every one metre increase in water depth reduces the probability of gas bubble disease (Weitkamp and Katz 1980; Elston *et al.* 1997). Thus supersaturation of approximately 125% would be required to cause gas bubble disease in a 1.5 m deep dhufish tank, assuming that dhufish susceptibility to supersaturation is similar to that of salmonids (Colt and Bouck 1984).

Following analysis of epidemiological information and examination of affected eyes it was hypothesised that exophthalmos resulted from localised supersaturation of the choroid with oxygen and the formation of gas bubbles during certain physiological and physical events (Stephens *et al.* 2001). In the only other published report of high pO_2 in bubbles in fish eyes prior to the present study, the condition was experimentally induced following supersaturation of the water with oxygen (Engelhorn 1943).

In dhufish the condition was frequently associated with increases in water temperature during heat waves when water temperatures rose by up to 2°C in the afternoon and evening (Fig. 4). This increase in temperature reduces gas solubility (le Chatelier's principle) and possibly blood pH (Albers 1970; Cech *et al.* 1976; Hoffert and Ubels 1979; Cameron 1984). The development of exophthalmos in three dhufish when risk factors were experimentally reproduced supports the hypothesis that water temperature and/or blood pH may pre-dispose dhufish to exophthalmos.

Biochemical reaction rates increase in ectothermic animals when water temperatures increase (Cech *et al.* 1976; Wood 1980). The increased production of metabolites such as lactic acid and CO_2 in the retina and choroid from such increased metabolic activity (Cech *et al.* 1976, 1994; Dobson and Baldwin 1982a) may have profound effects on plasma pH and haemoglobin oxygenation of dhufish as a result of the Root and Bohr factors (Stephens *et al., in press*). Increased muscular tissue during increased swimming activity may have similar effect (Black 1958; Milligan and Wood 1986).

This may increase the amount of oxygen released from oxyhaemoglobin in the choroid when blood pH in choroid rete is reduced, as in rete in the gas glands of the swim bladder of the eel (Steen 1963). The efficiency of pH compensation in the pseudobranch under conditions of reduced plasma pH has not been thoroughly investigated, although Bridges *et al.* (1998) found that the pH of blood leaving the pseudobranch was relative to the pH of blood entering the pseudobranch, suggesting that complete pH compensation may not occur in this organ. Oxygen released from haemoglobin will result in increased pO_2 in the choroid, increasing supersaturation further and increasing the risk of gas bubble formation should gas micronuclei be present.

Epidemiological data (Fig. 2 and Table 5) suggests that both the physical chemistry of gases in water and the physiological status of the dhufish may contribute to the development of exophthalmos. Decreased gas solubility occurs at elevated temperature (Colt 1984) and/or from 'salting out' when lactate concentration increases during increased glycolysis (Pelster 1998). In supersaturated tissues that contain bubble micronuclei (Hills 1977), this results in expansion of bubbles when dissolved gases return

to the gaseous phase. As bubbles grow their surface tension is decreased, which further increases diffusion of gases into the bubble (Foster *et al.* 1998). Gas bubbles may coalesce, resulting in local oedema, haemorrhage, endothelial damage and thrombus formation as reported in decompression sickness and gas bubble disease (Speare 1991; James 1993; Kitano 1995). Figure 21 illustrates the events within the choroid that may contribute to the pathogenesis of exophthalmos.

The high prevalence of spontaneous exophthalmos in dhufish compared with snapper under similar culture conditions may be the result of lower supersaturation of the choroid and attributes such as the magnitude of the Root effect. In dhufish, pre-disposition of oxyhaemoglobin to become deoxygenated following minor pH changes may be a precursor to increased oxygen saturation in the choroid and subsequent exophthalmos.

That the condition is usually unilateral suggests that a localised predisposition such as the presence of bubble seeds or micronuclei may be necessary for initiation of bubble formation, however once gas bubbles form they rapidly increase in size.

There has been little investigation into the effect of biochemical, physical or physiological factors on oxygen secretion to the fish retina, although the effects of carbonic anhydrase inhibition were investigated by Fairbanks *et al.* (Fairbanks *et al.* 1974), and Hoffert and Ubels (1979) investigated the effect of temperature on arterial pH and pO_2 and choroidal pO_2 in trout. Increasing water temperature increased choroidal pO_2 until 20°C, where it fell below initial levels, as did arterial pO_2. This was attributed to increased metabolic and oxygen demand without a compensatory increase in ventilatory rate. The results cannot be directly applied to dhufish as the trout were anaesthetised and 20°C is extreme for trout not adapted warm regions, whereas temperatures experienced by dhufish in tanks were not as extreme. Nevertheless, the findings suggest that temperature, ventilatory rate and flow can significantly and rapidly affect arterial and choroidal pO_2.

The events precipitating exophthalmos in dhufish may only be of several hours duration. Williams *et al.* (1998) suggested that exophthalmos in Atlantic halibut often occurred following handling and may have been trauma or temperature-related. Atlantic cod also appeared to develop spontaneous exophthalmos following capture and handling (Dehadrai 1966). Atlantic cod have large choroid bodies and high retinal-vitreal pO_2 (Wittenberg and Wittenberg 1962; 1974) and may be predisposed to exophthalmos formation in decreased hydrostatic pressure during capture in a similar mechanism to that hypothesised for dhufish.

Gaseous exophthalmos accompanying abdominal distension in red grouper, *Epinephelus morio*, (Brulé *et al.* 1996) was attributed to swim bladder stress syndrome (SBSS). This condition has been reported in other species following stressful culture conditions such as shallow culture tanks, increased water temperature, low pO_2, or less than ideal salinity and light intensity (Johnson and Katavic 1984; Kolbeinshavn and Wallace 1985). The pathogenesis of SBSS and exophthalmos in species such as dhufish may have similarities,

as they both may be associated with increased pO_2 secretion in the rete of the gas glands and choroid respectively.

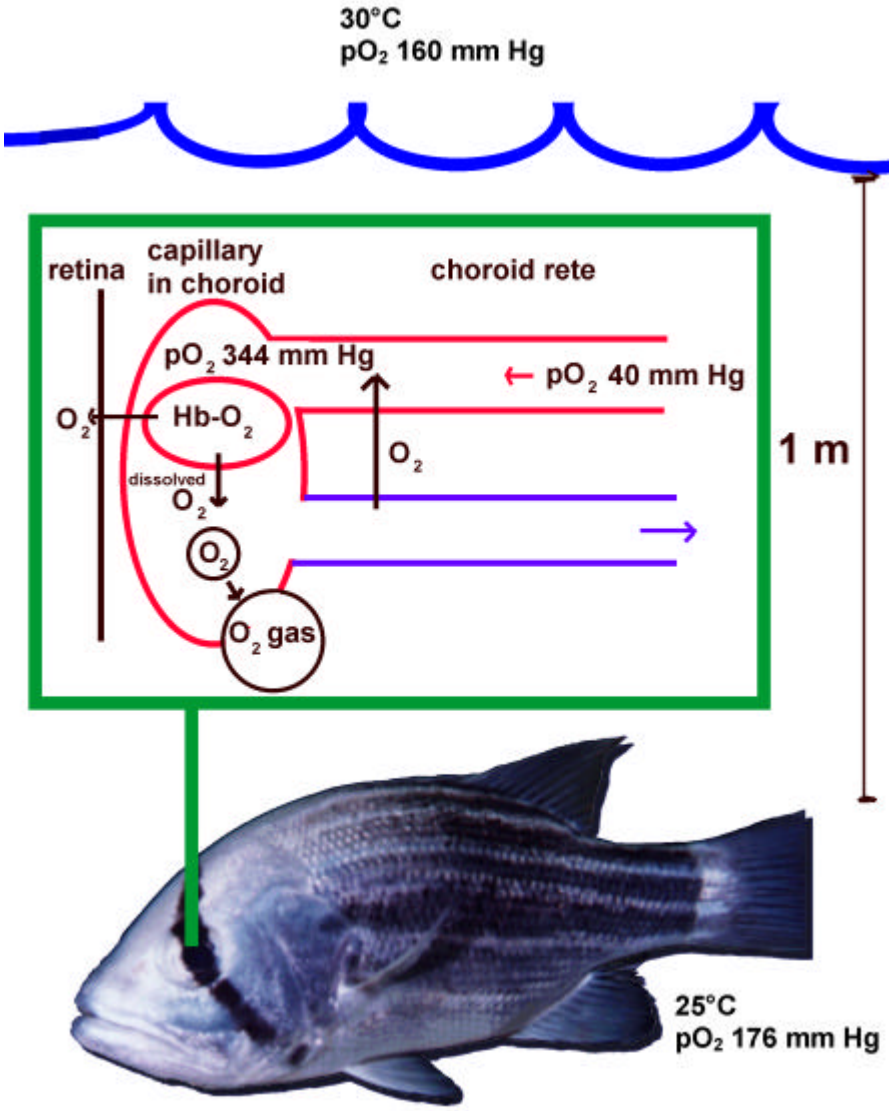

Figure 21. Schematic diagram of the physiological events that may occur in the choroid of dhufish held in aquaculture tanks when water temperatures increase. Supersaturation of the choroid (pO_2 344 mm Hg) from deoxygenation of haemoglobin, countercurrent exchange between adjacent arterial and venous capillaries in the choroid rete and decreased gas solubility may cause pre-existing minute gas bubbles to increase in size. Eventually capillary walls rupture, causing distension of the eyeball and/or retrobulbar tissue with gas and haemorrhage.

The buoyancy problems reported in exophthalmic Atlantic cod may have been caused by greater than usual haemoglobin deoxygenation occurring concurrently in both the eye and swim bladder (Dehadrai 1966). Although buoyancy problems occasionally accompanied exophthalmos in dhufish, the large size of the swim bladder and its extremely thick fibrous wall may partially protect the fish from severe distension of the swim bladder. Alternatively, decreased gas solubility and the presence of gas micronuclei in the supersaturated eye may be a more frequent underlying cause of exophthalmos in dhufish, unrelated to disturbances of physiological or biochemical processes.

Monogenea

The gill monogenean, described as *Haliotrema abaddon* during this project (Kritsky and Stephens 2001), was a recurring problem. The parasite (Fig. 22) was common on both wild-caught and hatchery-reared dhufish, and high rates of infestation were associated with mortality on several occasions. Fifty three of one hundred and fifty three fish (35%) of fish in group 3 (Table 5) died between June and August 2000 (Fig. 1 B) with heavy *H. abaddon* burdens. No other primary disease agent was identified although some fungal hyphae and *Flavobacterium*-like bacteria were seen on gill smears. Diffuse, severe hyperplasia of epithelial cells of secondary lamellae was a consistent feature of heavy infestation with *H. abaddon* in this and other disease outbreaks.

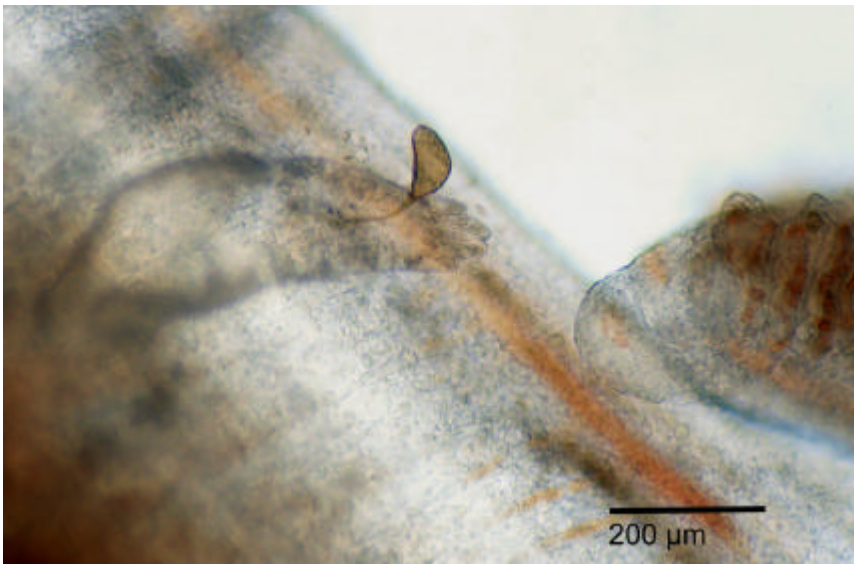

Figure 22. A mature *Haliotrema abaddon* with attached egg in an unstained gill biopsy.

The parasite was described (Kritsky and Stephens 2001) and named *Haliotrema abaddon*. The stages of the life-cycle are illustrated in Fig. 23. Eggs hatched after 7 to 10 days at ambient temperature of approximately 25°C. The oncomiracidia were motile, moving forward in a direct line with the rear of the oncomiracidia moving from side to side at an angle of approximately 45º. Oncomiracidia attached to the base of the petri dish and cilia ceased moving within hours of hatching.

Heavily parasitised fish developed severe, diffuse hyperplasia of gill lamellae (Fig. 24). Strands containing multiple eggs visible to the naked eye (Fig. 25) were entwined in gill lamellae, at time appearing to strangulate the lamellar blood supply, as evidenced by white necrotic areas within the egg strand.

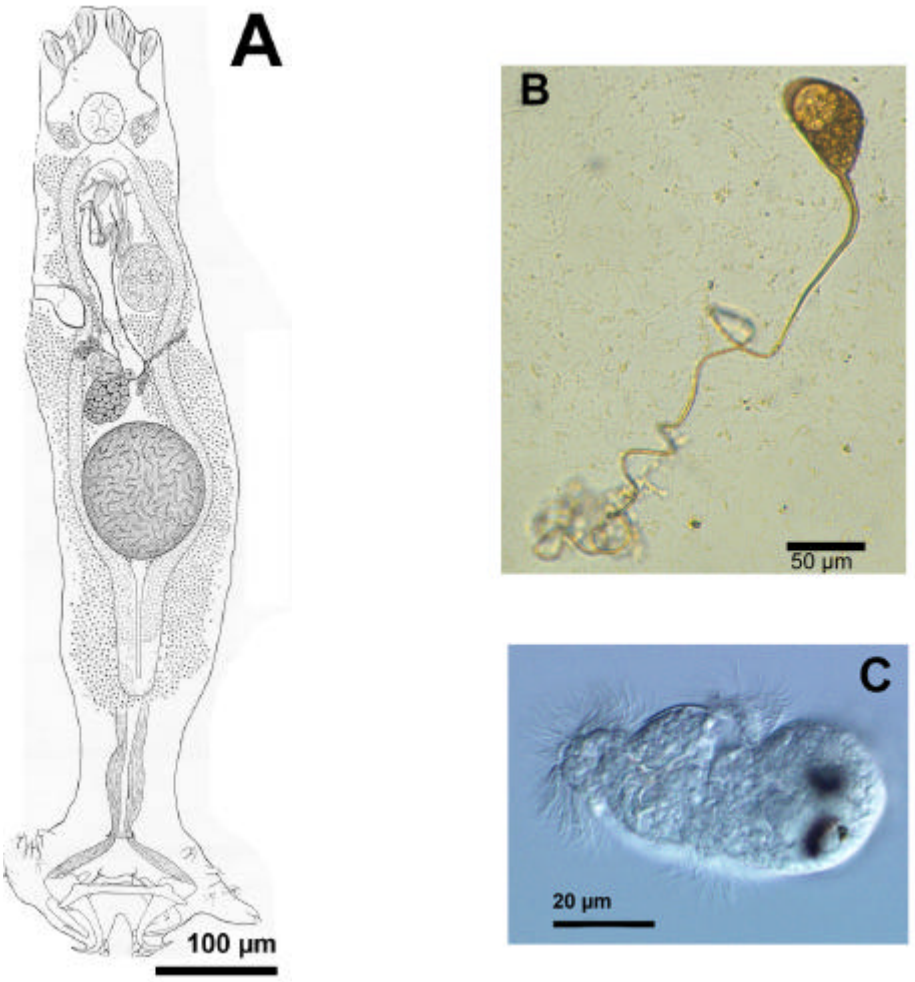

Figure 23. Stages of the *H. abaddon* life-cycle. A. Adult from (Kritsky and Stephens 2001). B. Egg with developing oncomiracidium. C. Oncomiracidium.

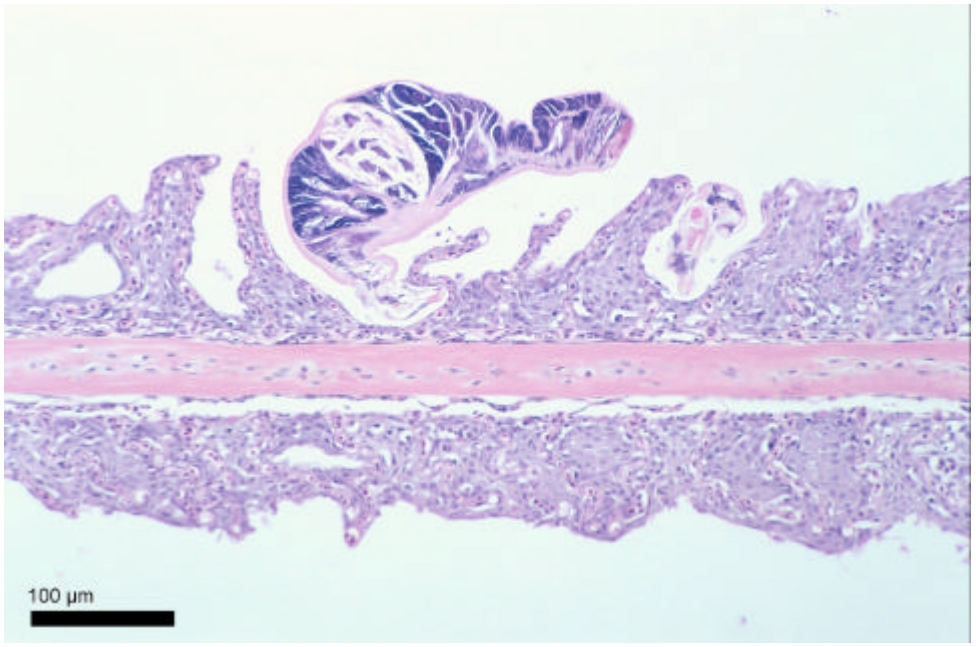

Figure 24. Histological section of a primary gill lamella infested with *H. abaddon*. Severe, diffuse hyperplasia and metaplasia of epithelial cells is evident with scattered necrotic epithelial cells and infiltration of lymphocytes, macrophages and granulocytes.

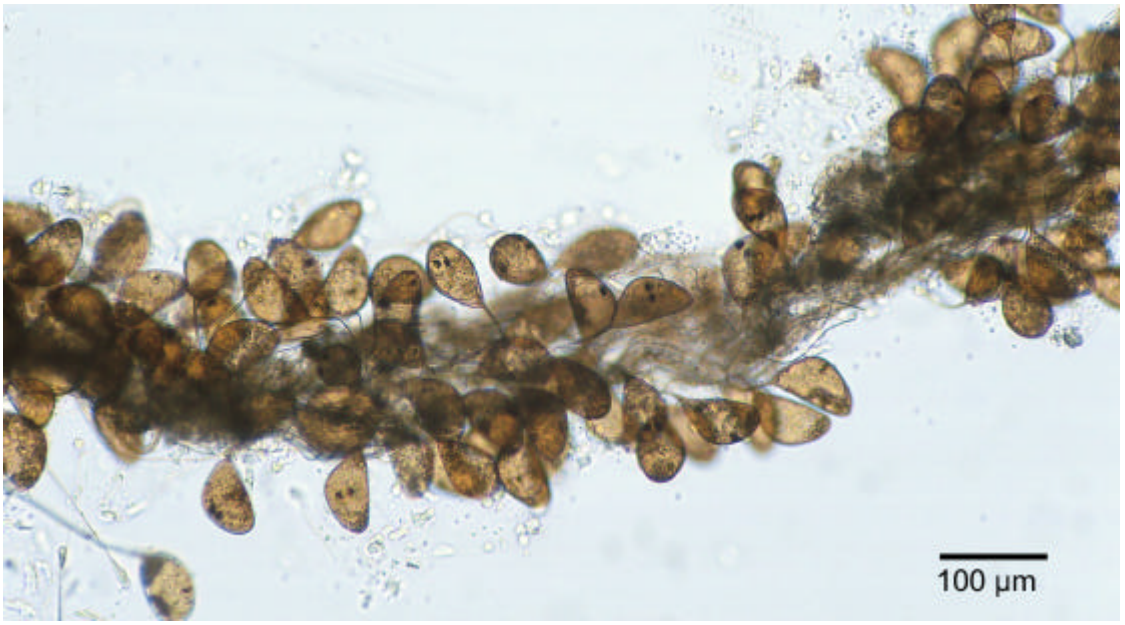

Figure 25. A cluster of *H. abaddon* eggs from the gills of a heavily parasitised dhufish. Eyespots of developing oncomiracidia can be seen in many of the eggs.

Prevalence of *H. abaddon* on wild fish (n=32) was 100% but the intensity of infestation was low (Table 12). Prevalence, based on biopsy was 100% in wild-caught, captive fish parasite intensity varying from low to high in dhufish within the same tank. Healthy F1 fish with no apparent health problems had low parasite intensity and high prevalence.

Table 12. Prevalence and abundance of *H. abaddon* on various groups of fish. Prevalence taken from means of over 50 primary gill lamellae (ocean-caught fish) or more than 5 lamellae (gill biopsy on tank held fish).

Dhufish history	Haliotrema prevalence				Water temperature ^{o}C
	0	+	++	+++	
Ocean dwelling (>40cm), n= 27	0	26	1	0	21-22
Tank held, ocean reared, n= 13	3	5	4	1	22-25
F1 3 year old, n=5 (held in seawater)	0	5	0	0	22-25
F1 3 year old, n=5 (held in bore water)	2	3	0	0	19.5-20

0= nil + = mean of <2 per lamella

++ = mean of 2-10 per lamella +++ = mean of > 10 per lamella

Trials over several hours using gills infested with *H. abaddon* to visualise the effects of various potential chemotherapeutic agents on the parasite produced variable results. The results are summarised and categorised in Table 13.

Table 13. Results of *in vitro* chemotherapeutic trials.

A. Treatments with some evidence of activity against *H. abaddon.*

Treatment	Dose rate mgL^{-1}	Trial No.	Comments
Praziquantel	50	1, 2	Parasites were slow moving or twitching, short and many became detached from gill lamellae
	25-30	1, 2, 3	Parasites were slow moving or twitching, short and many became detached from gill lamellae
Toltrazuril	50-70	1, 2	Parasites were normal size but much more slowly moving than normal
	25-30	1, 3	Parasites were normal size but much more slowly moving than normal
Low salinity	4 ppt	2	Parasites were translucent, of normal size and most appeared to be dead.
garlic	25-50	3	Parasites appeared to be reduced in length and dead
closantel	37.5-75	2, 3	Parasites appeared to be dead
AQUI-S	5-10	2	Parasites were translucent, of normal size and with markedly reduced activity levels.

B. Treatments with indeterminate activity against *H. abaddon.*

Treatment	Dose rate	Trial	Comments
low salinity	8 ppt	2	Parasite motility appeared reduced and parasites were translucent.
moxidection	1-2 mg L^{-1}	1, 3	Parasite motility appeared to be slightly reduced and in one trial were more elongated than normal

C. Treatments showing no activity against *H. abaddon.*

Treatment	Dose rate	Trial
netobimin	70-140 mg L^{-1}	2
ivermectin	1-2 mg L^{-1}	2
isopropanolol	3 mL L^{-1}	2
triclabendazole	120 mg L^{-1}	1
flubendazole	5-10 mg L^{-1}	3

The results of the first preliminary trial to assess the safety of potential treatments are presented in Table 14. Closantel at the dose used was extremely toxic, resulting in mortality within minutes, but other treatments were well tolerated by fish.

Table 14. Small scale trial to test the safety of potential treatments for *H. abaddon.*

Treatment	Mortality
closantel 18.8 mg L^{-1}	Yes
praziquantel 5 mg L^{-1}	No
toltrazuril 12.5 mg L^{-1}	No
trichlorphon 30 mg L^{-1}	No

The results of the second preliminary trial to assess the safety and efficacy of some potential treatments are summarised in Table 15. Formalin at 250 ppm for one hour was toxic and resulted in the death of two thirds of treated fish within twelve hours of treatment. There were many remaining parasites on formalin treated fish despite evidence that many had been removed. Praziquantel and fenbendazole were well tolerated by the fish, but only praziquantel appeared to have any activity against *H. abaddon*.

Table 15. Small scale trials of infested dhufish to trial the efficacy and safety of potentially useful treatments for *H. abaddon.*

Treatment	Mortalities	Parasites per lamella mean ± S.D. (n=3)	Parasites in 1 mL sediment mean ± S.D. (n=3)
control	0	1.7 ± 1.4	0.7 ± 0.6
formalin 250 ppm	8	1.4 ± 1.2	537 ± 114
fenbendazole 25 mg L^{-1}	0	0.5 ± 0.3	0.3 ± 0.6
praziquantel 5 mg L^{-1}	0	1.1 ± 0.5	24 ± 10

Sediment results of treatments using infested fish indicated a statistical difference ($P<0.05$) between control and treatment groups for 0.5 mg L^{-1} trichlorphon treatments of 24 and 44 hour duration and indicated that significant numbers of parasites were removed from gills during these treatments (Fig. 26). Biopsy results for both 24 and 44 hour praziquantel 2 mg L^{-1} treatments and freshwater bath of less than 1.5 ‰ were statistically significant (Fig. 27). Results from both sediment and biopsy indicated that a 24 hour, 2 mg L^{-1} praziquantel treatment was most effective in reducing the number of *H. abaddon* present on gills (Stephens *et al.* in press).

Results of sediment analysis were not normally distributed and were analysed and displayed as box plots. The results from replicates of control fish and freshwater bath treatments with salinities greater than and less than 1.5‰ salinity were grouped to permit comparisons between similar treatments.

Fish mortalities occurred in several treatments and are summarised in Table 16. Many fish that died had histological evidence of severe pre-existing conditions suggesting that the stress of handling, holding in small tanks and treatment may have significantly contributed to mortality. Two of the five fish that died after the completion of the 15 mg L^{-1} trichlorphon treatment had severe, chronic, diffuse myocardial necrosis and mineralisation. Control fish that died had heavy *H. abaddon* infestations and multifocal moderate to severe hyperplasia of lamellar epithelial cells, suggesting stress and pre-existing pathological changes contributed to mortality.

Table 16. Dhufish mortalities during and after treatment. Treatments not represented in the table had no fish mortalities. Each replicate contained three fish. The fish that died in the freshwater treatment in trial one was held in 0.6 ‰ salinity. Fourteen of two hundred and seventy fish used in the trials died during the trials.

Treatment	Replicate						Total
	1	2	3	4	5	6	
control, trial 1	0	0	0	0	0	1	1
trichlorphon, 15 mg L^{-1}, 2 hour	1	1	0	0	1	1	4
freshwater, 1.5 hour	1	0	0	0	0	0	1
praziquantel, 5 mg L^{-1}, 5 hour	0	0	0	0	1	0	1
control, trial 2	0	1	0	0	0	0	1
control, trial 3	0	0	0	1	0	0	1
praziquantel, 2 mg L^{-1}, 44 hour	1	1	0	1	0	1	4
trichlorphon, 0.5mg L^{-1}, 44 hour	0	1	0	0	0	0	1

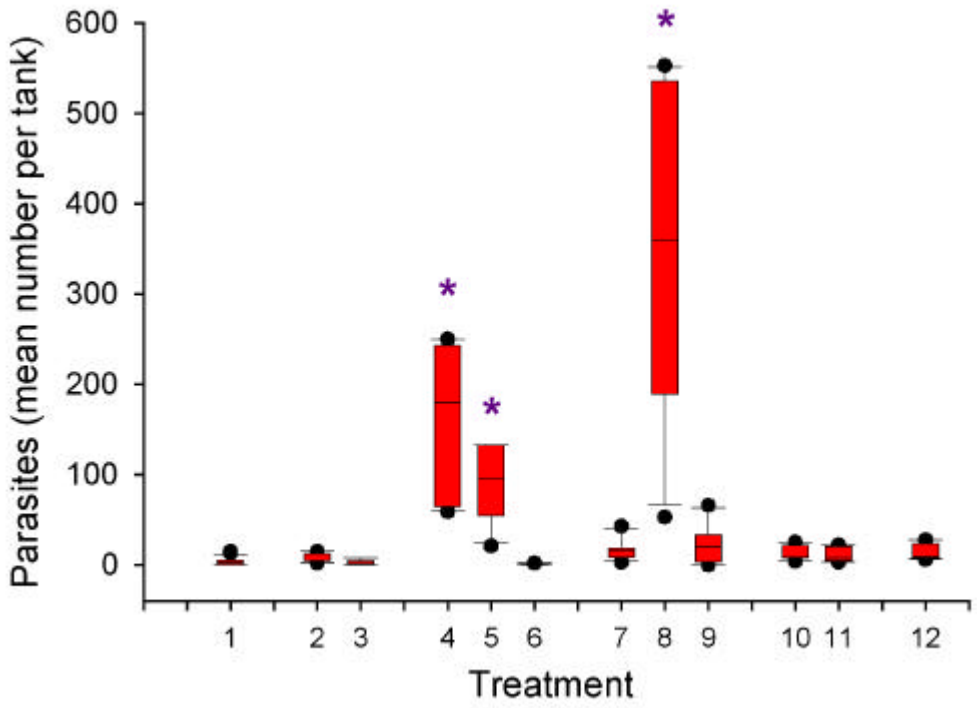

Figure 26. Sediment analysis results plotted as a box plot for each treatment. The number of *H. abaddon* found in 2 mL of sediment filtered from each tank is recorded for each treatment. Boxes represent values within the 25th and 75th quartile, error bars represent 10 and 90th percentiles and the dots indicate results that were outside the 10 and 90th percentiles.

Treatments.

1 control n=24 **2** freshwater <1.5‰ n=7 **3** freshwater 2-4‰ n=5

4 trichlorphon 0.5 mg L^{-1} for 24 hours n=6

5 trichlorphon 0.5 mg L^{-1} for 44 hours n=6

6 trichlorphon 15 mg L^{-1} for 2 hours n=6

7 praziquantel 5 mg L^{-1} for 5 hours n=6

8 praziquantel 2 mg L^{-1} for 24 hours,n=6

9 praziquantel 2 mg L^{-1} for 44 hours n=6

10 formalin 25ppm for 10 hours n=6

11 formalin 25 ppm for 24 hours n=6

12 toltrazuril 12.5 mg L^{-1} for 6 hours n=6

Statistically significant results ($p<0.05$) are marked *

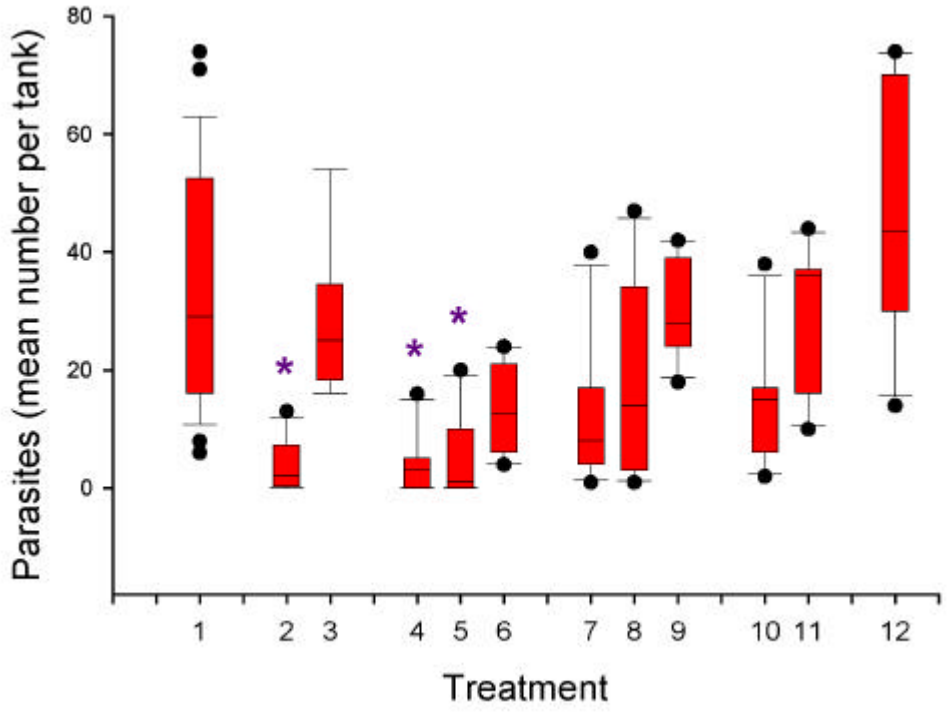

Figure 27. Biopsy results displayed as box plots. The number of *H. abaddon* per tank (mean ± S.D.) assessed by gill biopsy on the day following treatment is plotted for each treatment type.

Treatments.

1 control n=24

2 freshwater <1.5‰ n=7

3 freshwater 2-4 ‰ n=5

4 praziquantel 2 mg L^{-1} for 44 hours n=6

5 praziquantel 2 mg L^{-1} for 24 hours,n=6

6 praziquantel 5 mg L^{-1} for 5 hours n=6

7 trichlorphon 0.5 mg L^{-1} for 44 hours n=6

8 trichlorphon 0.5 mg L^{-1} for 24 hours n=6

9 trichlorphon 15 mg L^{-1} for 2 hours n=6

10 formalin 25 ppm for 24 hours n=6

11 formalin 25ppm for 10 hours n=6

12 toltrazuril 12.5 mg L^{-1} for 6 hours n=6.

Statistically significant results ($p<0.05$) are marked *

The freshwater bath caused anxiety in the fish, as evidenced by constant swimming activity following the commencement of freshwater flow until salinity was returned to initial levels. The fish became pale in colour and after approximately 1 hour some fish progressively lost buoyancy and were recumbent with intermittent darting swimming activity.

Analysis of tank sediment at the completion of freshwater treatment did not produce large numbers of parasites indicating that either the parasites moved to a more inaccessible site during treatment or did not fall from the gills until some time after treatment.

Histological examination of fish that died following the freshwater bath had sub acute to chronic multifocal gill lesions, with clubbed primary lamellae, hyperplasia of epithelial cells and necrosis and fibrosis of tissue surrounding the cartilagenous matrix. Hydropic degeneration of hepatocytes was also evident.

Biopsy results for 25 ppm formalin bath for 24 hours indicated that this treatment was partially effective in reducing parasite numbers, as did sediment results from both the 10.5 and 24 hour treatments. Ammonia could not be detected in tanks that had formalin added, suggesting an interaction between formalin, ammonia and/or the test reagents.

15 mg L^{1} trichlorphon was toxic to fish and had no significant effect on *H. abaddon* burdens. Fish mortalities started soon after completion of treatment. Rapid flushing of aquaria five hours after the completion of treatment resulted in recumbent fish regaining normal appearance and swimming activity within several hours. Trichlorphon at 0.5 mg L^{-1} was relatively safe for fish and sediment examination indicated the removal of significant numbers of parasites after more than 24 hours of exposure. Biopsy results were not significantly different from controls in any trichlorphon treatments, however, the lower number of parasites present in gill biopsies after 24 and 44 hour treatment support the results of sediment analysis where large numbers of parasites were found in the tank water following treatment.

Livers from fish that died following trichlorphon treatment were congested with distended sinusoids on histological examination. Coagulative necrosis of the cytoplasm and nuclear changes of hepatocytes were also evident.

Toltrazuril was safe for the host and removed some parasites as evidenced by the results of sediment examination at the completion of treatment. Biopsy results, however, indicated no reduction in *H. abaddon* following treatment.

Low dose, long-term praziquantel treatments were most effective in removing parasites from fish with a significant reduction in parasites on gill biopsies after 24 and 44-h treatments at 2 mg L^{-1}. This was supported by the significant increase in parasites found in tank sediment after 24 hours. A large amount of organic material was found in sediment following praziquantel treatments of 44 hour duration, and this was thought to contain *H. abaddon* that had decomposed beyond recognition during the course of the relatively long treatment.

After 36 hours some fish in 2 mg L^{-1} praziquantel died and water in the base of aquaria had a white cloudy appearance. Ammonia levels that had been increasing over the first thirty hours, in line with those of control tanks, could no longer be detected. Histology of mortalities revealed changes to the base of epithelial cells of renal tubules consistent with the presence of oedema.

Discussion

Approximately one third of the monogeneans currently described are within three genera: *Gyrodactylus* (approximately 900 species); *Dactylogyrus* (approximately 450 species); *Haliotrema* (approximately 156 described species) (Cribb *et al.* 2001). *Haliotrema* is a miscellaneous group that probably includes several, as yet uncharacterised genera, (Delane Kritsky, personal communication). The life-cycle and life stages of *H. abaddon* (Fig. 23) were similar to those of other dactylogyrids (Cone 1995) although the strands of eggs entwined on gill lamellae of heavily infested dhufish (Fig. 25) have not been reported in monogenean infestations of other cultured fish. The egg filaments may aid auto-infection of the host as do adaptations of other monogenean eggs such as the thorn-like filament of eggs of *Lamellodiscus acanthopagrus* (Roubal 1994), and the egg bundles of *Dionchus remorae* that have a loop of egg shell material for attachment to gill lamellae (Whittington 1990).

Adult *H. abaddon* appear to be fecund in water temperatures of over 20°C when large number of eggs and immature *H. abaddon* are often seen on dhufish gills. Similarly, other Monogenea such *Haliotrema spariensis* (Roubal 1994) and *Gyrodactylus salaris* (Jansen and Bakke 1991) reproduced more rapidly in warmer water.

The high prevalence and low intensity of *H. abaddon* on wild dhufish (Table 12) was a common finding in many studies of other populations (Symons 1989), suggesting the existence of a long-term host-parasite relationship with little affect on the host (Cone 1995). The absence of gill hyperplasia in wild dhufish supports this epidemiological argument although the hyperplasia and hyperplastic nodules of lamellar epithelial cells evident in gills of wild yellowfin bream, *Acanthopagrus australis*, infested with *Haliotrema spariensis* and *Lamellodiscus major* (Roubal 1986; 1989) did not support this theory. The more severe gill pathology in those fish suggests that other toxins or irritants may have been affecting the fish gills or the mode of attachment of the parasites to gill lamellae may cause severe damage.

The seasonal changes in mean intensity of *H. abaddon* on captive dhufish contrasts with that seen in monogenean infestations of pond-reared and caged yellowfin bream *Acanthopagrus australis* and snapper, *Pagrus auratus* (Roubal 1995; Roubal *et al.* 1996) where parasite intensity remained relatively low. A possible reason for the differences between species may be related to host factors such as stress and immuno-competence during culture.

The reasons for seasonal and water temperature effects on the population of *H. abaddon* remain unclear, with both host and parasite factors likely to contribute to changes in parasite intensity and abundance. The findings of studies of *Gyrodactylus salaris* (Jansen and Bakke 1991) where parasite numbers increased in summer months in the absence of heightened host immune response appears also to apply to dhufish.

The balance of host, parasite and environmental factors appears to differ in captive and wild dhufish. In addition, individual captive dhufish appear to have highly variable host responses, indicated by extreme variability of parasite intensity between fish in the same tank (Table 12). The reasons for the large range of parasite intensities are unclear, although a similar pattern of parasitism is common in helminth and ectoparasitic parasitism of farmed livestock (Symons 1989), with poor condition of the host evident prior to heavy parasitism rather than poor condition being the result of parasitism. Dhufish that are more stressed, for example those low in the social order of dominance, evident at feeding time when such fish are reluctant to aggressively compete for food, are more likely to be immuno-suppressed (Pickering 1998) and have high parasite burdens. Latent infections with other disease agents such as bacteria may also occur in such fish.

Stress and decreased immune response appeared to increase parasite intensity in other fish species (Jokinen *et al.* 1995; Bagge and Valtonen 1996), and although the immune response to monogenean infestations is poorly understood it appears to be largely a non specific immune response involving factors such as complement (Harris *et al.* 1998) and cytokines (Buchmann and Lindenstrøm 2001).

Genetic resistance of dhufish to *H. abaddon* is unlikely to vary greatly as the wild population is confined to a relatively small area and F1 dhufish were derived from a small number of spawnings. Genetic resistance to monogenean infestation appears to play an important role in other fish such as Atlantic salmon where Norwegian strains are highly susceptible to *Gyrodactylus salaris* whilst Finnish strains are minimally affected (Heggberget *et al.* 1993; Rintamaki-Kinnunen and Valtonen 1996). The prevalence and intensity of *H. abaddon* on wild dhufish reflects that of a long-term, balanced host-parasite relationship. As F1 dhufish from the same spawning exhibited the same marked differences in parasite intensity as did captive wild fish, stress-related host factors offer a more feasible explanation for individual differences in parasite intensity than genetic differences.

The effects of heavy burdens of *H. abaddon* on dhufish are unclear, although severely infested fish were often anorexic and sometimes anaemic. In some instances, however, suboptimal pH and pCO_2 of culture water may also have contributed to clinical signs. Some F1 fish had concurrent infection with epitheliocystis, *Caligus* sp. or pathogenic bacteria suggestive of immuno-suppression and the contribution of each disease agent to anorexia and anaemia was uncertain. Nevertheless, anorexia and anaemia are clinical signs of many parasitic infections and infestations of mammals (Symons 1989) and most pair-feeding trials demonstrate that clinical signs are more marked in parasitised animals. Although little work has been done on the effects of parasitism on fish, similar mechanisms appear to result in decreased production. A study of rainbow trout

Oncorhynchus mykiss infected with *Cryptobia salmositica*, demonstrated that uninfected fish ate more than infected fish (Thomas and Woo 1992). Antibody titres against sheep red blood cells of pair-fed uninfected fish following inoculation was intermediate between that of uninfected and infected fish fed to satiety and it was concluded that anorexia was a clinical sign of infection and contributed to immunosuppression.

Anorexia may result from increased cholecystokinin production acting on the hypothalamus and on other unidentified factors such as pain and irritation caused by the parasitic infection (Symons 1989). Depressed growth rate may result not only from anorexia, but also from impaired nutrient utilisation and diversion of protein synthesis from productive processes to protective processes in response to parasitism (Symons 1989).

Similarly anaemia is thought to be greater than that resulting from blood loss from parasitism. Anaemia may be a secondary effect of long-term anorexia and a declining nutritional status, the result of unidentified toxins produced during parasitism or may be a primary response to concurrent, unidentified subclinical infections (Symons 1989). Anaemia in dhufish with heavy *H. abaddon* infestations appears to be a secondary effect of parasitism as the parasite is not haemophagus.

It remains unclear whether anaemia and anorexia are clinical signs of *H. abaddon* infestation or result from other factors such as immunosuppression, concurrent subclinical infections or less than ideal environmental conditions such as low pH and high pCO_2 of culture water.

The large differences in predisposition of individual, captive dhufish to infestation with *H. abaddon* were similar to those found between roach *Rutilis rutilis* in lakes in Finland (Koskivaara and Valtonen 1992; Bagge and Valtonen 1996). Stress and decreased immune response when fish were transferred to a polluted lake caused a decrease of 64% in IgM levels in roach five weeks and increased mean abundance of several dactylogyrids (Jokinen *et al.* 1995). IgM levels were still 29% below those of fish in the unpolluted lake eight weeks after transfer.

Similarly, maintaining good health and the provision of a relatively stress-free environment with excellent water quality may help to prevent heavy *H. abaddon* infestation in dhufish. In practice, however, this may not be easy to achieve, as commercial considerations such as the need to increase stocking densities are important in aquaculture. Likewise, growth rate is increased in warmer water temperatures which at the same time may favour more rapid multiplication of pathogens such as *H. abaddon*.

An understanding of the epidemiology of *H. abaddon* infestations is important in deciding control strategies. Models used for controlling nematodes in sheep (Anderson *et al.* 1978) are worthy of consideration when formulating control strategies for *H. abaddon* in dhufish as both hosts have 100% parasite prevalence all of the time, the parasites are host specific with direct life-cycles, have relatively hardy eggs that are released into the environment, are relatively fecund under ideal conditions and resistance to anthelmintics

is a major potential problem in both the livestock and aquaculture industries. Strategic drenching when parasite numbers in the host are low, but prior to predicted periods of high egg output is one strategy used to control nematodes in sheep (Anderson *et al.* 1978; Dash 1988). Using correct dose rates, minimising the number of treatments, the use of new anthelmintics, mixing anthelmintics and using highly effective anthelmintics are strategies used to reduce the development of resistance to anthelmintics (Martin 1988). Another common strategy is to spatially separate the infective free-living stages of the parasite from the host. In sheep, this is achieved by grazing pastures, crops and stubbles that have not been recently grazed by infected sheep (Anderson *et al.* 1978).

Equivalent strategies may be applied to control of *H. abaddon* infestations of dhufish. Vacuuming the bottom of the tank daily to remove eggs that have detached from gills, and moving fish to newly cleaned tanks every five or six days in summer when water temperatures are high are two potentially useful strategies for preventing newly emerged oncomiracidia from finding a host. Similar strategies are recommended for control of protozoan parasites such as *Cryptocaryon* sp. and *Ichthyophthirius* sp. (Noga 1996). In Japan, a similar strategy is regular changes or cleaning of net on net pens to reduce egg numbers of *Benedenia seriolae* as fouling of nylon sea cages entraps eggs and significantly increases the intensity of infestation of *Seriola* spp. (Whittington *et al.* 2001).

Paperna (1987) suggested the spread of parasites on equipment is the most likely method of infestation of previously uninfested hosts in aquaculture situations. Juvenile dhufish may be infested by this method, rather than from eggs and oncomiracidia from wild fish entering the culture system in seawater as they were parasite-free for several months but eventually became infested. The tanning of monogenean eggs makes them highly resistant to chemical treatments (Thoney 1990; Yoshinaga *et al.* 2000), although Diggles *et al.* (1993) found that hyposalinity combined with 200 mg L^{-1} formalin reduced egg viability. It is imperative that separate equipment is reserved for use with each group of fish and that transfer of eggs by other means such as via personnel or water is prevented if juvenile fish are to remain parasite-free.

Strategic treatments for *H. abaddon* in winter before the annual rise in seawater temperatures may prevent heavy infestation in summer. No treatment removed all *H. abaddon* from dhufish, therefore, there is a real possibility that the parasite may rapidly develop resistance to treatment chemicals. The use of the most efficacious treatments, together with the possible use of a mixture of anthelmintics and rotation of treatments is important if the development of resistance is to be avoided (Martin 1988). As it was suggested that parasite resistance emerged following a single treatment of *Benedeniella posterocolpa* with trichlorphon (Thoney 1990) and following seven treatments of eels *Anguilla anguilla* with mebendazole at monthly intervals (Buchmann and Roepstoff 1992), it may be prudent to combine treatments (Goven and Amend 1982; Kim *et al.* 1998).

Single oral treatments (up to 600 mg kg^{-1}) of a small number of dhufish with praziquantel did not appear to be effective in removing *H. abaddon* although a single dose of up to

500 mg kg^{-1} was reported to be effective in other species (Székely and Molnár 1991; Stoskopf 1993; Kim *et al.* 1998). The poor efficacy of a single dose in dhufish may indicate that tissue levels in gill epithelium were insufficient to adversely affect the parasite, although oral praziquantel has been used successfully to treat other tissue grazing Monogenea (Al Dove, New York Aquarium, personal communication). Alternatively the fish may have re-gurgitated the praziquantel following recovery from anaesthesia, or repeated doses may be required to achieve clinical levels in the target tissue (Stoskopf 1993).

Long term, low–dose treatments in water were much more effective in removing *H. abaddon* from dhufish than higher dose treatments of only a few hours as reported for the benzimidazoles (Treves-Brown 2000). Treatment trials indicated that formalin (250 mg L^{-1} for one hour, Table 15) and trichlorphon (15 mg L^{-1} for two hours, Table 16) were toxic to dhufish. Freshwater baths below 1.5 ppt were effective but stressful and must be used with care. The reason for the low number of parasites present in sediment vacuumed from aquaria immediately following the cessation of treatment is uncertain but may indicate delayed detachment following treatment. It may also be useful to examine fish for remaining parasites forty eight or more hours after cessation of various treatments as continued mortality may follow treatment. Praziquantel appeared to kill the majority of *H. abaddon* within the first twenty four hours of treatment, as opposed to trichlorphon where many parasites had fallen from the host after twenty four hours but may have been paralysed rather than dead as they remained identifiable after forty four hours. In practical terms, paralysed parasites are unlikely to re-infest the host and the distinction between dead and paralysed detached parasites is of academic interest only. The cause of toxicity of 2 mg L^{-1} praziquantel after forty four hours is unclear as a forty hour treatment of 2 mg L^{-1} praziquantel is routinely used to treat fish at the New York Aquariums (Leslie Chisholm, personal communication).

The *in vitro* trials may have resulted in the rejection of some potentially useful treatments as viability of the parasites in control replicates was low after three hours, preventing long-term assessment of the effects of some chemicals. In particular, flubendazole (Untergasser 1989), mebendazole dissolved in formic acid (Buchmann and Roepstoff 1992) and fenbendazole (Munday 1996) are worthy of further investigation. The effect of fish anaesthetics such as AQUI-S and 2-phenoxyethanol on Monogenea requires further investigation as some parasites may detach from fish during anaesthesia. Other treatments showing no activity against *H. abaddon* during *in vitro* trials do not warrant further investigation on the basis of their lack of efficacy in trials of other species infested with Monogenea and their efficacy against platyhelminths in studies of mammalian parasites (Vanden Bossche 1985; Campbell 1986).

Liver pathology

Hepatocytes in the hepatopancreas of rapidly growing juvenile dhufish were often rounded with distinct, round vacuoles visible within basophilic cytoplasm (Fig. 28 C).

Frozen sections stained with oil red O or osmium tetroxide confirmed the vacuoles were filled with lipid.

Juveniles fed a pelleted diet designed for snapper had more severe hepatocellular fatty change. Hepatocytes were distorted and angular, cytoplasm was no longer basophilic, and irregular unstained areas with a feathery appearance predominated. Nuclei were distorted and compressed at the margins of the cytoplasm with indistinct nucleoli (Fig. 28 D). The fish had poor growth rates and arteritis, epicarditis and myopathy of muscle in the region of the pharynx and acicular clefts in hepatocytes were also noted in several fish at 145 days of age. The poor growth rate and persistent doubts about the ability of the fish to resist disease resulted in the diet being changed to freshly thawed seafood in January 2000. As demonstrated in Figure 1 A, growth rates rapidly increased, however, it is not evident whether the increase in growth rate was the result of warmer seasonal water temperatures, improved nutrition or both.

Juveniles from all groups of dhufish spawned in 1999 also had granulomas in the liver, first noted at 145 days of age. Macrophages and a fibrous wall enclosed areas of lipid-like material (Fig. 28 D) and acicular clefts were present in some lesions. The appearance was unlike granulomas of microbial origin and may have been within the walls of blood vessels (Assoc. Professor Jennet Harvey, Pathology Department, The University of Western Australia, personal communication). However, Factor 8 immunohistochemistry performed by the Pathcentre, Queen Elizabeth II Medical Centre, failed to confirm the presence of endothelial cells using rabbit-anti human Von Willebrand factor (Dako A/S, Denmark). Masson's trichrome stain highlighted the presence of collagen in the periphery of granulomas. Surrounding hepatocytes were often more fatty in appearance than those in other lobes of the same liver.

Small crystal-like structures, possibly disintegrating erythrocytes, were present in F1 fish at 10 months of age. They stained red with naphthol green B stain and may have been haemoglobin, but because, other chemicals such as zymogen in the pancreas were similarly stained the origin of the material remained uncertain. However, when fish from the same tank were sampled three months later there was no evidence of the material but there were many melanomacrophage centres that may have resulted from the earlier pathology. The nuclei of many hepatocytes had marginated chromatin, however, viral particles were not evident on transmission electron microscopy of affected livers that had been reprocessed from paraffin blocks (Dr Shane Raidal, personal communication).

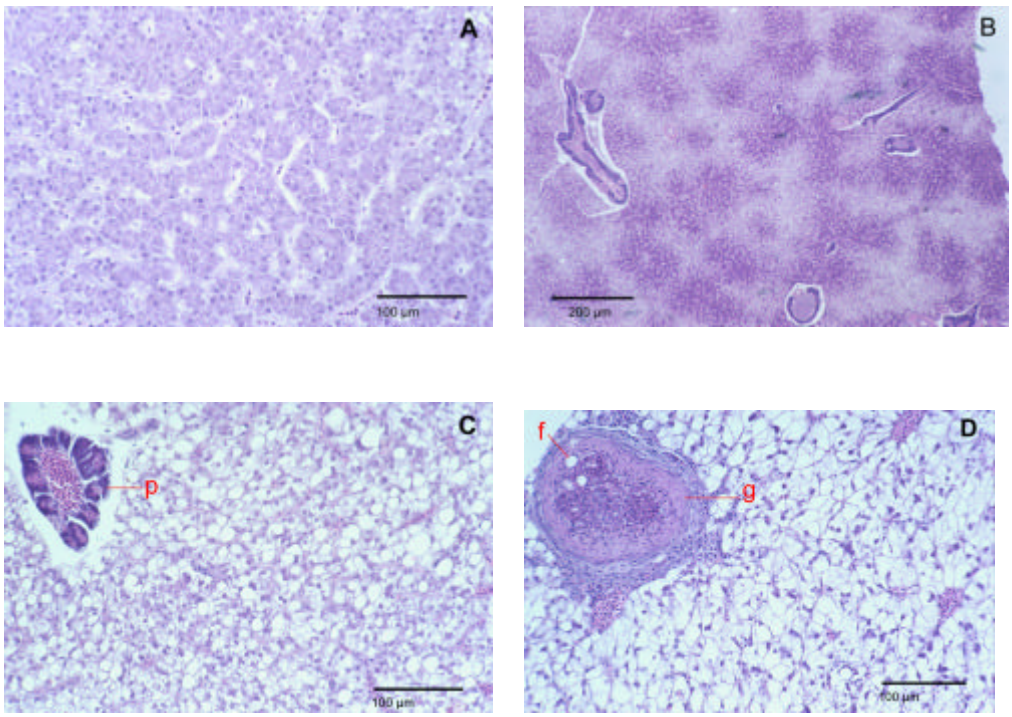

Figure 28. Histological sections of dhufish livers. A. Liver of wild-caught dhufish. Hepatocytes have large nuclei with prominent nucleoli and relatively basophilic cytoplasm. B. Liver from a wild-caught dhufish that died in captivity, possibly from bacterial septicaemia. Severe, diffuse multifocal, coalescing necrosis of hepatocytes is evident. C. Fatty liver from a healthy, rapidly growing, captive, juvenile dhufish. Nuclei of hepatocytes are rounded with prominent nucleoli. Fat appears as large rounded vacuoles within cytoplasm. Pancreatic tissue (p) is unaffected. D. Liver of a juvenile dhufish with poor growth rate. There is severe, diffuse fatty degeneration of irregularly-shaped hepatocytes. Cytoplasm is pale and nuclei are small and appear to be compressed at the periphery of hepatocytes. A granuloma (g) contains vacuoles with a lipid-like appearance (f).

Discussion

Fatty liver of juvenile dhufish was a perplexing problem. In particular, the cause of ill thrift of pellet-fed fish and cause of granulomas in the livers of juveniles from one spawning season (groups 1 to 4, Table 5) were unable to be unequivocally identified. The histological appearance of the livers of juvenile hatchery-reared dhufish invites speculation about the effect of nutrition on hepatocyte function and the overall health of the fish. Livers of wild dhufish are a dark red colour, similar to those of healthy mammalian liver, with no fat globules present in histological sections (Fig. 28 A). The livers of apparently healthy, rapidly growing juvenile dhufish fed a flesh diet, and healthy snapper fed a commercial pelleted diet designed for snapper and held in a coolroom were both a pale tan-orange colour with fat globules present in hepatocytes in histological sections (Fig. 28 C). In contrast, juvenile dhufish fed a pelleted diet failed to thrive and their hepatocytes were distorted and abnormal in appearance (Fig. 28 D).

Although the fatty livers of both healthy and ill thrifty juvenile dhufish resemble those of salmonids with lipoid liver disease or lipidosis from the feeding of rancid fats (Ferguson 1989), their physiological significance remains unclear. The high level of polyunsaturated fats in fish diets (De Silva and Anderson 1995) pre-disposes them to oxidative changes, especially following storage in moist, warm conditions, however, pellets fed to dhufish were stored in a coolroom and supplemented with antioxidants such as vitamin E. Anaemia caused by increased erythrocyte fragility may develop in fish once hepatocytes accumulate lipid products, including ceroid, and increased amounts of haemosiderin are deposited in organs such as the spleen. Lipid peroxidation within hepatocytes may also be exacerbated by the consumption of endogenous antioxidants such as glutathione and vitamin E/selenium in fish food (Ferguson 1989).

The presence of granulomas in fish from all four batches of juveniles from the 1999 spawning season suggests a nutritional problem common to all groups. The most likely source is peroxidation of polyunsaturated fatty acids in the enrichment media supplied to *Artemia* nauplii, possibly the result of storage in warm conditions (Sargent *et al.* 1997; Sargent *et al.* 1999).

The dietary requirements of dhufish are poorly understood. Dhufish flesh has relatively large amounts of docosahexanoic acid (DHA; 22:6n-3) and less eicosapentanoic acid (EPA; 20:5n-3) than most species of fish (CSIRO 1999). In addition it has a relatively large amount of arachidonic acid (ARA; 20:4n-6). As the validity of assessing the dietary requirements of dhufish from the fatty acid content of its flesh is uncertain, an alternative method of assessing dietary requirements was to examine the diet of wild dhufish. The stomach contents of wild dhufish included a variety of prey, with reef fish such as wrasse, algae and cephalopods commonly found (Marr 1980; Robinson 1987). The aetiology of liver pathology in hatchery-reared juvenile dhufish was difficult to identify, and imbalances of C, E or B vitamins or minerals were suspected contributory factors.

Granulomas in juvenile dhufish may have originated from occluded blood vessels or from within hepatocytes. There were some similarities between these lesions and atherosclerosis lesions in the intima of arteries in man where atherosclerotic plaques in

arteries follow peroxidation of low density lipoproteins and result in several changes within arterial intima, including proliferation of smooth muscle cells, conversion of blood monocytes to macrophages and foam cell formation and initiation of a humoral and cell-mediated immune response (Witztum 1994; Aviram 1995). Research into the protective effects of antioxidants such as vitamins E and C and β-carotene is ongoing in humans, with several studies suggesting that diets high in such antioxidants may reduce the rate of development of atherosclerosis (Halliwell 1994).

The relevance of hepatic lesions in dhufish to lipid peroxidation in arteries of man is unclear because lesions were found only in livers of dhufish whereas atherosclerosis occurs in arteries in multiple organs, however, the acicular clefts, foamy, lipid-like material and macrophages within lesions in dhufish were similar to those seen in atherosclerosis. The negative results of Factor 8 testing prevented confirmation that the lesions were within blood vessels, although the negative results were inconclusive as dhufish Von Willebrand factor may be antigenically distinct from the human factor. Lipid peroxidation in hepatocytes is, however, the most likely event initiating the pathogenesis of the granulomas in dhufish livers.

The marginated chromatin and large number of melanomacrophage centres found at various times in juvenile dhufish from the final spawning season during the tenure of the current project did not appear to result from viral infection. Granulomas were not seen in the livers of this group of fish, however, the fish failed to thrive and frequently succumbed to bacterial septicaemia or died following short periods of suboptimal water conditions. The reasons for the general ill thrift of this group are unclear.

Copepods

An undescribed parasitic copepod (Fig. 29) was identified as *Caligus* sp. by Dr Brian Jones, Senior Fish Pathologist, Fisheries WA. It caused significant health problems in all tanks of wild-caught fish in the second summer of the current project, and in one tank of wild-caught fish in the first summer.

Affected fish frequently had ovoid erosions on the midline of the dorsal cranium above the eyes (Fig. 30 A). Heavily parasitised fish (Fig. 30 B) became anorexic and were visibly distressed by skin irritation, evidenced by frequent 'flashing'. Hatchery reared dhufish held in the same water supply were unaffected with the possible exception of one outbreak in fish held in bore water in the second winter of the project when the parasite was not detected although lesions were similar to those caused by *Caligus* sp. Incomplete removal of the parasite from newly introduced wild-caught stock during quarantine was thought to be the source of parasites in confirmed outbreaks.

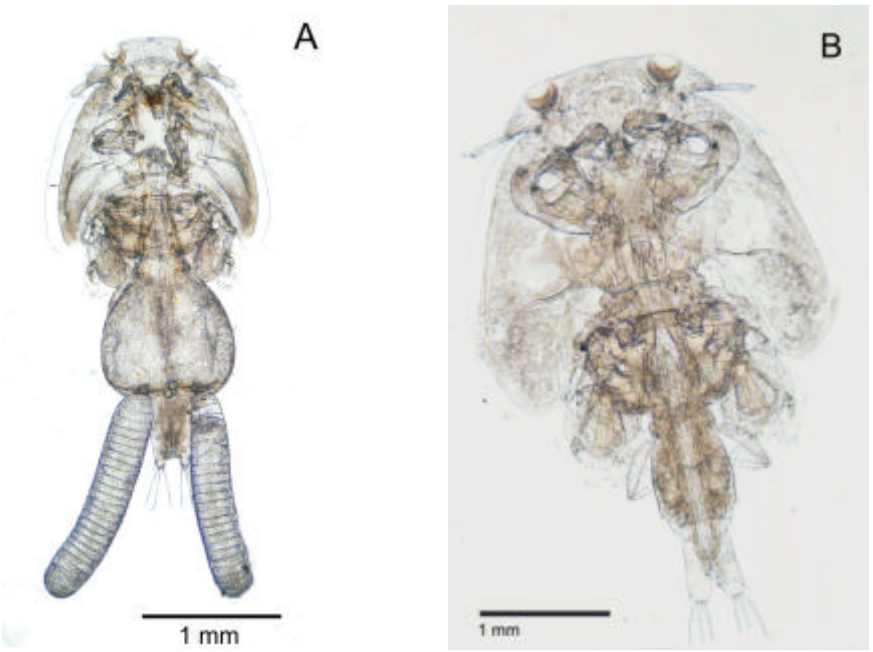

Figure 29. *Caligus* sp. removed from the skin of captive dhufish. A. Female. B. Male

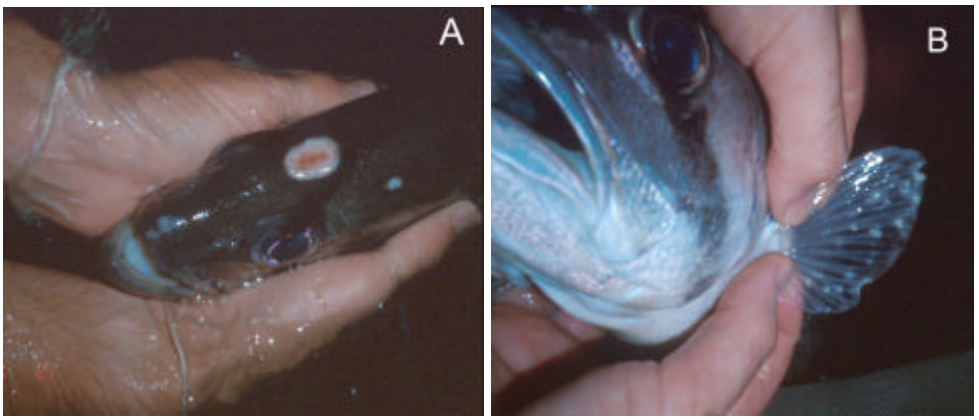

Figure 30. Dhufish with heavy infestation of *Caligus* sp. A. Erosion of skin on the midline of the dorsal surface of the head. B. Multifocal pale areas on pectoral fins.

Treatment with a 90-minute freshwater bath of approximately 3‰ salinity resulted in removal of large numbers of parasites and immediate resumption of normal feeding by affected fish. Skin lesions resolved within days.

Discussion

Infestation of cultured dhufish with the undescribed copepod, *Caligus* sp., occurred on several occasions and affected fish were successfully treated with a ninety minute low salinity bath. In contrast, the parasitic copepods of farmed salmon, *Lepeoptheirus salmonis* and *Caligus elongatus,* are significant problems in north America, Europe and

Chile (Roy *et al.* 2000; Treasurer and Pope 2000; Treves-Brown 2000). A range of control methods have evolved for treating sea-caged salmon, including the use of single year classes, fallowing, the use of cleaner fish and treatment with a range of chemicals (Treasurer and Pope 2000). Programs monitoring the number of parasites at each developmental stage on the host have been developed to predict optimal timing of treatments (Treasurer and Pope 2000). The following treatments have been used: organophosphorus compounds such as trichlorphon, dichlorvos and azamethiphos that are active against the post-chalimus stages of parasites (Treves-Brown 2000); hydrogen peroxide (Treves-Brown 2000); oral ivermectin (Johnson and Margolis 1993; Smith *et al.* 1993; Treves-Brown 2000); pyrethroids such as deltamethrin and cypermethrim (Treves-Brown 2000); oral teflubenzuron, a chemical that interferes with chitin synthesis (Treves-Brown 2000) and emamectin benzoate (Roy *et al.* 2000). Cleaner fish are sometimes used for more ecologically sustainable parasite control (Gorlick 1984).

Epitheliocystis

Heavy mortality occurred in juveniles in May/June 2000 (group 1, Table 5) held in bore water and many finely granular, basophilic intracytoplasmic inclusions were found in gill epithelial cells, together with large numbers of *H. abaddon*. Epitheliocystis was diagnosed (Fig. 31 A). The intracytoplasmic inclusions stained negative with Feulgens stain and positive with Giemsa. Lesions were not seen in dhufish at any other time or in fish in other tanks at the time of the outbreak.

Immunoperoxidase staining, using flourescein-labelled monoclonal antibody to *Chlamydia* lipopolysaccharide (Cellabs Pty Ltd, Brookvale, NSW, Australia) was performed by Mr David Lines at Murdoch University. Gills from four affected fish produced one positive result. Although positive and negative controls indicated successful staining, the single positive result neither proved nor disproved that the organism was a *Chlamydia* sp.

In a survey of thirty-two wild fish in January/February, 44% of fish had lesions resembling epitheliocystis on a histology section of 2 cm of the second gill arch. As lesion intensity was low (less than five lesions on each section) and there was no tissue or inflammatory response no clinical significance was attributed to the lesions.

Treatment for 10 consecutive days for 2 hours with 150 mg L^{-1} oxytetracycline resulted in a rapid reduction in mortalities. Surviving fish sampled six weeks after treatment had no evidence of epitheliocystis lesions but there was extensive disruption of normal gill morphology (Fig. 31 B). Fusion of the distal parts of secondary lamellae appeared to produce debris-containing spaces surrounded by hyperplastic epithelial cells resembled the swirling hyperplastic epithelial cells surrounding infected cells seen in gills prior to treatment (Fig. 31 A).

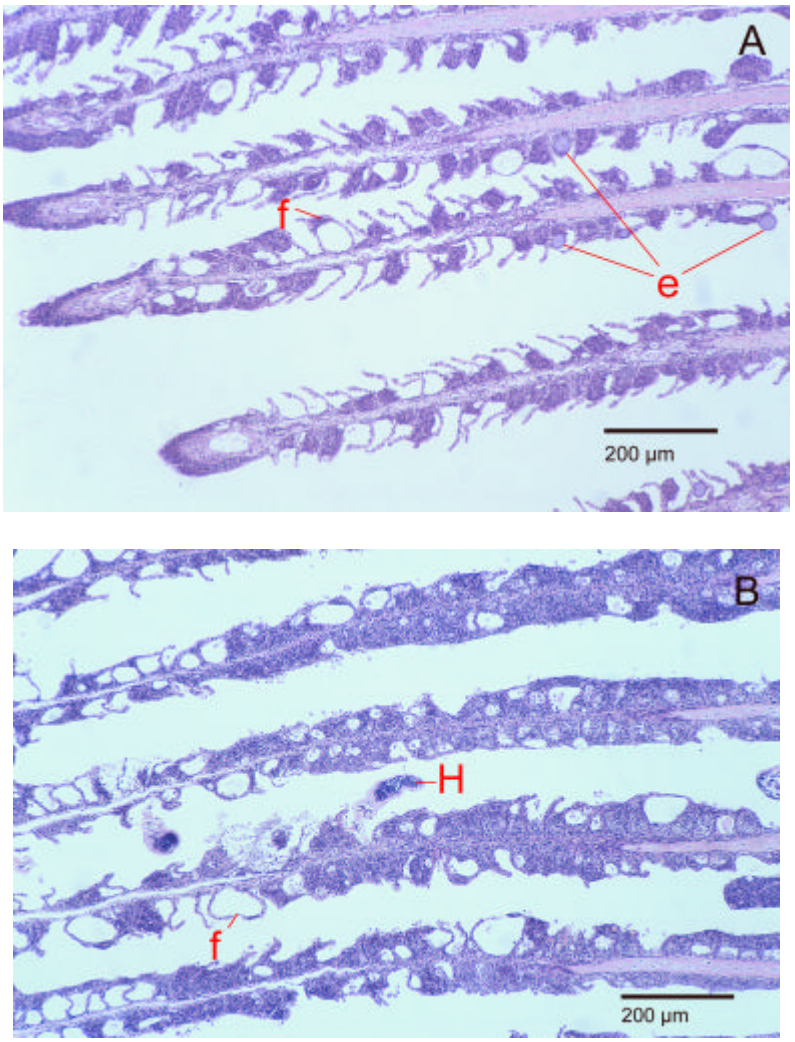

Figure 31. Histological sections of gills of dhufish with epitheliocystis. A. Moderate, multifocal hyperplasia of gill epithelium with lamellar fusion (f) with numerous basophilic areas within cytoplasm of epithelial cells infected with epitheliocystis (e). B. Gills of a dhufish six weeks later, following treatment with oxytetracycline. Severe, diffuse hyperplasia of epithelial cells is evident. Metazoan parasites, *H. abaddon* (H) are present and lamellar fusion (f) remains although no epitheliocystis is visible.

Discussion

Epitheliocystis is thought to be caused by a rickettsia- or chlamydia- like organism (Ferguson 1989), and often occurs in aquatic species in association with other diseases. *H. abaddon* may have facilitated spread of infection in dhufish and stress and immuno-suppression may pre-dispose fish to severe infection (Dr Brian Jones, Department of Fisheries, Western Australia, personal communication). Although epitheliocystis is generally thought to be a benign infection with no evidence of inflammatory or hyperplastic response in surrounding tissues and no clinical signs (Ferguson 1989), Noga (1996) reported heavy mortality associated with high numbers of lesions.

In Australia, lesions occurred in up to 12.5% of Atlantic salmon from several farms sampled in different seasons in Tasmania, but it was concluded that the disease was of little clinical significance as intensity of lesions in affected fish was low (Nowak and Clark 1999). Likewise, Langdon *et al.* (1991) described epitheliocystis in a leafy sea dragon in a Western Australian public aquarium but also suggested that the infection was of little clinical significance because of the lack of necrosis, hyperplasia or inflammation of branchial tissue and respiratory distress of the animal. In contrast, the large number of cysts on gill lamellae of affected dhufish, associated with a severe, generalised hyperplastic and inflammatory response and high mortality suggest at least some role in ill thrift and mortality resulting from epitheliocystis despite the presence of relatively large numbers of *H. abaddon*. The histological appearance of gills in fish several weeks after treatment suggests that disruption of normal gill morphology (Fig. 31 B) was not rapidly repaired following epitheliocystis infection or that other toxic agents were present, although similar lesions were not seen in fish in other tanks with the same water supply and management.

Infected fish tissue tested by Nowak and Clark (1999) and Langdon *et al.* (1991) produced negative results to monoclonal antibody immuno-fluorescence testing for chlamydial antigen as did three of the four dhufish samples. The negative Feulgens stain in this study suggests that the organism may not contain DNA. These findings suggest that either *Chlamydia* spp. did not cause the lesions or were antigenically distinct from the antigen used in the production of the commercially available antibody.

Fungal disease

Significant mortality occurred in juvenile dhufish at nine months of age (group 4, Table 5). *Vibrio fluvialis* was initially isolated from skin ulcers and fish were treated daily for ten days with 'in water' oxytetracycline (150 mg L^{-1}) for 2 hours. There was a significant reduction in mortality, however 2 weeks after completion of treatment, pale, necrotic ulcers with a haemorrhagic border occurred on the operculum, caudo-lateral aspect of the head, and around the eyes of many fish (Fig. 32). Mortalities began and lesions progressed to brown-black fistulae several millimetres in diameter. The dematiaceous yeast, *Exophiala* sp. (McGinnis and Ajello 1974) was isolated from these lesions and identified by the Mycology section, Pathcentre, Queen Elizabeth II Medical Centre, Perth. Retrospective sampling of formalin-fixed fish that died prior to isolation of the yeast found one fish with typical hyphae in gill and liver lesions that had died during the

oxytetracycline treatment, indicating that *Exophiala* sp. was present and clinically affecting some fish at least two months before it was isolated.

Fish that died with no external signs of disease often had large granulomas containing hyphae typical of the organism in liver and kidney (Fig. 33). Fungal hyphae were stained with PAS and Grocott methenamine silver stain. The organs were grossly enlarged and in some cases little normal tissue remained.

Following diagnosis, fish were treated with a series of two hour 75 ppm malachite green/formalin baths. Lesions resolved and the last lesions were reported three months after the initiation of treatment. Mortality peaked in the month following diagnosis. Following the outbreak, fish that were not affected by exophthalmos achieved high growth rates.

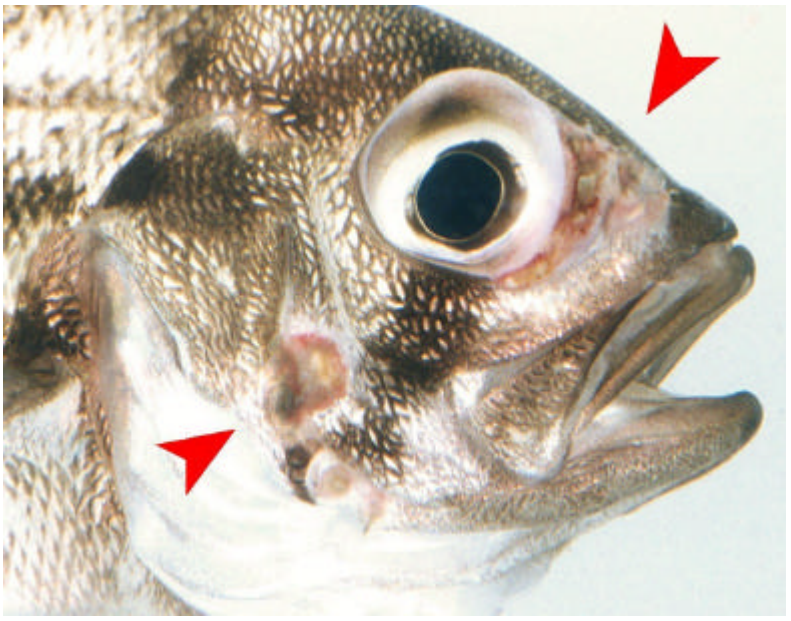

Figure 32. A juvenile dhufish infected with *Exophiala* sp. Multifocal yellow/brown areas of necrosis surrounded by hyperaemia are present on the operculum and in the periorbital area. Fungal hyphae were visible in scrapings from these lesions. Lesions progressed to discrete, brown/black, slightly raised nodules.

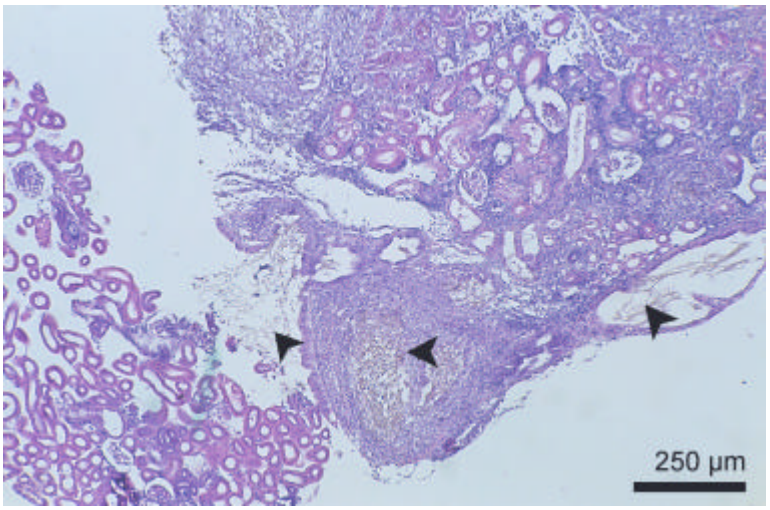

Figure 33. A histological section of a dhufish kidney infected with *Exophiala* sp. A granuloma can be seen (lower centre) infiltrated with hyphae (arrowheads).

Discussion

Lesions resulting in significant mortalities of juvenile dhufish from infection with the dematiaceous yeast, *Exophiala* sp. were similar to those attributed to *Exophiala pisciphila* in Atlantic salmon parr in Australia (Langdon and McDonald 1987). Lesions in dhufish may also be situated in canals of the lateral line system as suggested by Langdon and McDonald (1987). *Exophiala* sp. has also been isolated from cultured King George whiting in South Australia (Dr Ruth Reuter, South Australia, personal communication) and similar lesions were observed in snapper and another fish from aquaria in the Perth metropolitan area (Dr Brian Jones, Department of Fisheries, personal communication), suggesting the infection may be more common than published reports suggest.

Dematiaceous yeasts have been recorded in many species of fish including Atlantic salmon: *Exophiala salmonis* (Richards *et al.* 1978); *Exophiala psychrophila* (Pedersen and Langvad 1989); *Phialophora* sp. (Ellis *et al.* 1983) and *Exophiala*-like fungi were isolated from five species of captive marine fish (Blazer and Wolke 1979) with granulomas in several internal organs.

Identification of dematiaceous yeasts in the above reports and in the present study was made on the basis of morphology. Molecular biology may significantly alter the taxonomic classification of these organisms. An unsuccessful attempt was made to describe the yeast isolated from dhufish using molecular biology techniques at Westmead Hospital in Sydney.

Dematiaceous yeasts are ubiquitous in the terrestrial and marine environment and may form biofilms on surfaces in the aquaculture system as they have been found colonizing

marine bryozoans (Schotz 1998) and the marine sponge, *Mycale adhaerens* (Doshida *et al.* 1996). They are also found on wallpaper and paint in damp buildings and in rock statues and monuments in central Europe and Mediterranean basin (Urzi *et al.* 1995).

Dematiaceous yeasts cause skin infections in humans and other mammals. They usually occur in immunologically compromised hosts or following long-term steroid or immunosuppressive therapy (Yager *et al.* 1993; Norton Rossman *et al.* 1996), although they also occur in the vicinity of puncture wounds that were contaminated with soil (Norton Rossman *et al.* 1996; Mounzer *et al.* 1999). A similar epidemiological pattern may occur in fish, with clinical infection following stress and immunsuppression (Pickering 1998).

Bacterial disease

Dhufish often died with no external lesions and no disease signs other than anorexia of variable duration. When gill and skin lesions were present, bacteria were sometimes cultured (Fig. 34). The cause of death was attributed to bacterial septicaemia based on isolation of pathogens from tissue and/or histological appearance of lesions.

Lesions associated with septicaemia included severe multifocal necrosis of hepatocytes, fibrinous epicarditis and necrosis of renal tubular epithelium, a lesion thought to relate to hypoxia (Maxie and Prescott 1993). The majority of fish also had heavy burdens of *H. abaddon*.

Bacteria isolated by Agriculture WA included several *Vibrio* species, including *V. harveyi* and *V. fluvialis*, *Photobacterium damselae* and *Flexibacter*-like bacteria. Vibrios were the most commonly isolated bacterial pathogens. As the bacteria are present in seawater, they may form biofilms on tank surfaces and in water-supply equipment. Each *Vibrio* species has an optimal temperature for growth and those from the temperate region inhabited by dhufish are likely to be more prevalent in the warmer summer months. Snieszko (1974) correlated the occurrence of overt bacterial infections with stressed fish and certain water temperatures. This explanation of pre-disposition to bacterial septicaemia appears to be equally applicable to dhufish.

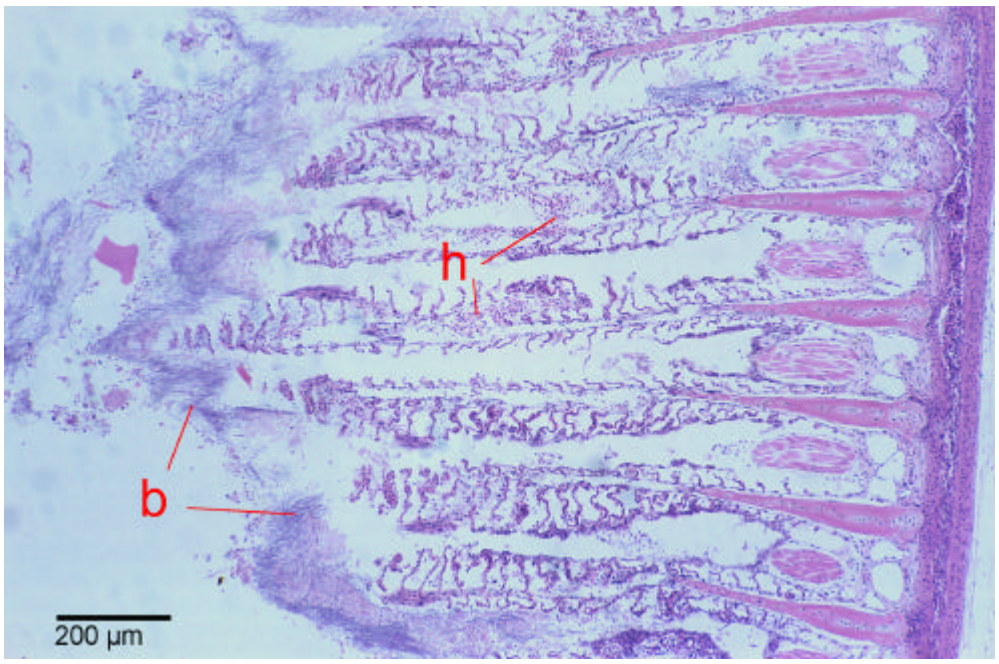

Figure 34. Branchitis caused by *Flexibacter*-like bacteria. Severe, diffuse necrosis and haemorrhage (h) of gill lamellae can be seen. Parallel strands of bacteria (b) cover the distal surfaces of affected primary lamellae.

Miscellaneous parasites

The following parasites were seen during the project, however, they were not associated with pathology and are unlikely to occur when dhufish are spawned and raised in land-based holding tanks as, with the exception of *Argulus* sp., they probably have a multi-host life cycle.

A single specimen of *Argulus* sp. (Fig. 35), similar to an earlier one identified by Dr Brian Jones, was recovered from the flank of a wild-caught male dhufish that had been held in captivity for several months.

Sanguinicola sp. were found in the heart of one sexually mature dhufish that had been recently captured. Fish caught in the wild and necropsied within months of capture had large numbers of unidentified larval nematodes in the stomach wall and in fat and mesentery of the gastro-intestinal tract. These parasites were not seen in fish that had been spawned and raised in captivity.

Figure 35. *Argulus* sp. removed from the skin of a wild-caught dhufish several months after capture.

Live specimens of *Philometra lateolabracis* were seen in the gonads of a female 2.9 kg fish during January. The fish had been captured in July of the previous year. In other large, wild-caught fish there was often evidence of dead parasites in gonads. The parasite was not seen in fish that had been spawned and raised in captivity but is frequently found in the gonads of sexually mature wild dhufish (Hesp and Potter 2000).

Overall discussion

Exophthalmos in dhufish may have a multifactorial pathogenesis. Based on the scientific evidence produced during this project, the most logical explanation for the majority of lesions is that bubbles form from bubble micro-nuclei in the supersaturated choroid of fish in relatively shallow culture tanks, especially during periods of decreasing gas solubility coinciding with increases in water temperature. Increased pO_2 from increased deoxygenation of haemoglobin may also occur following decreasing pH in erythrocytes or plasma in the choroid body or increased carbonic anhydrase activity.

As the dhufish is a deep water species that lives in a well-aerated habitat and has a life-style with low physical activity, its ability to adapt to environmental and/or internal chemical and biochemical changes may be marginal. Some properties of the physiology of blood oxygen transport of dhufish may decrease its ability to adjust to certain aquaculture conditions. in particular its single haemoglobin with pronounced Root and Bohr effects may result in poor ability to maintain haemoglobin oxygenation and acid-base homeostasis in the choroid during periods of lactic acidosis following intense exercise, rapid changes in water temperature or environmental hypercapnia. The low hydrostatic pressure in tanks at sea level compared with the fishes natural habitat may pre-dispose dhufish to gas bubble formation in the supersaturated choroid.

Heavy infestation with *H. abaddon* was probably the result of stress and immuno-suppression of the host. Praziquantel was identified as the most effective treatment, however, rotating the three most effective treatments praziquantel, trichlorphon and freshwater baths, and treating for *H. abaddon* in spring appears to offer the most effective method of preventing heavy parasitism in summer months. It is equally important to optimise the overall health of the fish by providing a well balanced diet, relatively stress-free surroundings and water with physico-chemical characteristics within the ideal range for the host.

Benefits

This study highlights the difficulties of rearing dhufish in captivity because of the number and severity of health problems to which the species succumb. The health problems of dhufish that occurred during the tenure of the study were more numerous and severe than those experienced by other species held under equivalent conditions at the same facility during the same time period. The most probable reason for the increased susceptibility of dhufish to health problems in captivity are poor physiological adaptation to an environment in which water quality, diet and social conditions vary from those of their natural habitat. This appears to cause immunosuppression as a result of stress with resultant increased susceptibility to a range of infectious diseases. The non-infectious health problem, exophthalmos, appears to be largely the result of poor physiological adaptation to low hydrostatic pressure in tanks, altered activity patterns and fluctuations in water temperature that occur in captivity. The study indicates that careful attention to detail in the design and management of dhufish holding facilities is required if commercial aquaculture of the species is to be viable.

Despite the health problems occurring in captive dhufish, the study also indicated that certain diseases prevalent in wild dhufish are unlikely to be a problem in captive fish. The most notable example is the parasite, *Philometra* sp. which was not seen in hatchery-spawned dhufish or in captive dhufish caught in the wild before reaching sexual maturity. The parasite appears to require an intermediate host for completion of its life cycle, and this and other parasites with indirect life cycles are unlikely to be present in captive fish which are not exposed to infected intermediate hosts.

Further development

Improved understanding of the physiology of the dhufish would assist the development of optimal culture conditions for the species. Investigations of erythrocyte intracellular pH, and factors that affect it, may improve understanding of the effects of various water quality parameters and exercise on dhufish. The use of cannulated fish (Soivio and Nyholm 1975) to minimise the effects of stress from capture and anaesthesia, and measurement of intracellular pH using fluorescent probes and ratiometric imaging or flow cytometry (Rothe and Valet 2000) to assess the effect of allosteric effectors of

haemoglobin oxygenation on erythrocyte pH may clarify some of the physiological and biochemical responses demonstrated in Chapter 4. *In vitro* and *in vivo* measurement of plasma pH, pO_2, and pCO_2 using equipment appropriate to determination of these parameters in ectotherms (Tucker 1967; Cameron 1971b) would further elucidate blood transport physiology and the potential effect of environmental conditions on dhufish. An investigation of respiratory and acid base characteristics of dhufish held at high hydrostatic or atmospheric pressures simulating those in their natural habitat may improve understanding of the effects of low hydrostatic pressure on the species and its susceptibility to exophthalmos in captivity.

A scientifically designed long term study to determine the effects of stabilising water temperature and decreasing light intensity to reduce fish activity on the prevalence of exophthalmos, and nutritional studies appear warranted if commercial aquaculture of the species is to be successful.

Strategic management practices for controlling *Haliotrema abaddon* are dependent on understanding the epidemiology of the parasite population. A technique similar to that used by Chisholm and Whittington (2001) would provide further information about lifecycle parameters. Naïve fish, or fish from which all parasites have been removed following two praziquantel baths (2 to 5 mg L^{-1} for 40 hours), were obtained and freshly produced eggs or oncomiracidia added to the holding tank. Progression of infestation at different temperatures by recording the appearance of immature and mature egg laying adults on the host (Ernst *et al.* 2001) would provide important epidemiological information.

In vitro studies using adult parasites or eggs in 12 or 48 well disposable microtitre, cell culture plates (Carter *et al.* 2001) or perspex dishes (Kearn 1973) to monitor parasite activity or fecundity, or egg hatching time at different temperatures or in different treatments may provide valuable information on parasite control measures. Eggs or parasites are placed in wells and incubated with daily water changes or the use of antimicrobial agents (Roubal 1994) and natural photoperiod as oncomiracidia of most species investigated have hatched early in the morning.

Further study of treatments, including benzimidazoles such as mebendazole dissolved in formic acid and albendazole (Buchmann and Bjerregaard 1990), 'in feed' praziquantel, niclosamide or bithionol (Vanden Bossche 1985) at the dose rates suggested by Stoskopf (1993) may increase the range of useful treatments.

Investigations of the pharmacology of treatment chemicals, including residues in meat, their effect on biological filters and run-down times in continuously flowing water is important if chemicals are to be used in commercial dhufish aquaculture. Experimental design and methods used should be similar to that described by Diggles *et al.* (1993) with individual fish killed at specified time intervals following treatment and total number of parasites counted both on the fish and in sediment.

Planned outcomes

The research undertaken during this project has resulted in improved understanding of the pathogenesis, control and treatment of health problems of captive dhufish. The research confirmed that the species is relatively difficult to maintain in captivity. Successful commercial aquaculture of dhufish in the future will be dependent on the provision of exacting environmental and management conditions that minimise infectious and non infectious health problems and maximise growth.

Conclusion

Rearing and breeding the West Australian dhufish in captivity presented several challenges, including the health problems reported and investigated during the present study.

The development of exophthalmos in a significant number of otherwise healthy dhufish remains a serious threat to their successful aquaculture. Other problems, included several outbreaks of disease caused by a range of agents, ill thrift and poor growth rates of juveniles that were spawned and reared at the ADU during the tenure of the current project were also a cause for concern. The poor performance of many juveniles indicates that dietary and water quality requirements are not yet completely understood. Although significant advances have been made in understanding the requirements of larval dhufish, problems such as the fatty liver and granulomas emerged in juvenile dhufish following experimentation with diets and water temperature. Earlier batches of juveniles that had negligible disease problems and better growth rates were fed freshly thawed, uncooked seafood supplemented with minerals and vitamins and were held in seawater heated to a constant temperature of 23°C by electric immersion heaters. However, it would be uneconomical to raise dhufish on seafood diet in a commercial aquaculture enterprise.

Ill thrift and fish mortality associated with high mean intensity of infestation with the monogenean *Haliotrema abaddon* was a frequent occurrence and was difficult to treat in both wild-caught and hatchery-reared dhufish throughout the tenure of the present project. Such health problems were more common during periods of warmer water temperature and when fish were anorexic due to other poorly understood factors that may include the stress of capture and rearing in tanks in the case of recently caught wild fish. Holding dhufish in water outside the optimal range of physico-chemical requirements for the species, including incompletely aerated bore water may also have contributed to the development of health problems. Granuloma formation associated with severe fatty change, observed in many histology sections of liver from one batch of juvenile dhufish with poor growth rates suggested a nutritional imbalance.

The development of spontaneous exophthalmos resulting from gas and haemorrhage in the choroid, retrobulbar and periorbital tissues unassociated with gas bubble formation or haemorrhage in other tissues indicated that neither trauma nor supersaturation of water was the cause of exophthalmos. Investigations of the haemoglobin and haemoglobin-

oxygen transport properties of dhufish and the role of oxyhaemoglobin in oxygenation of the retina were supported by the finding of high concentrations of oxygen in the choroid and retina of normal dhufish. The high oxygen content of gas bubbles in dhufish eyes with recent exophthalmos suggested that the oxygen may initially have been derived from oxyhaemoglobin. Comparison of dhufish with other species that were less susceptible to exophthalmos in the same aquaculture facility, found a particularly marked Root effect in the single dhufish haemoglobin suggesting potential for poor physiological compensation to water quality parameters and activity patterns that differ from those experienced by wild fish in their natural habitat. The species appears to be highly adapted to its demersal habitat and relative inactivity. Conditions to which it is exposed during aquaculture, including more variable water quality parameters, the necessity for a different social structure and altered patterns of swimming activity may act as stressors.

The reasons for gas bubble formation, particularly the high prevalence of unilateral lesions in affected dhufish remain poorly understood. Nevertheless, the epidemiological association of exophthalmos with periods of rapidly rising water temperatures in active, healthy fish suggests that falling intracellular pH within erythrocytes, decreasing gas solubility and the formation of gas bubble nuclei may initiate formation of large gas bubbles in the choroid. Seasonal changes in seawater temperature appeared to have little effect on wild dhufish held in tanks, however, changes in dissolved gas resulting from low hydrostatic pressure in tanks at sea level, rapidly rising water temperature and altered gas ratios in bore water may adversely affect wild-caught dhufish. The ability of some hatchery spawned and reared dhufish to thrive in similar conditions may reflect selection pressure early in the larval rearing process.

Haliotrema abaddon was described and stages of its life-cycle identified. The effects of factors such as water temperature on parasite fecundity and generation time remain poorly understood, although increased parasite intensity often occurred in warmer water. Stress and immunosuppression of fish may also increase the susceptibility of individual fish to severe infestation. Praziquantel was identified as an effective ‘in water’ treatment.

Anorexia caused by clinical or subclinical disease or exophthalmos results in ill thrift or fish mortality and is a major source of high production costs in dhufish aquaculture where expenditure on equipment, services, labour and feed is high. The use of expensive feeds based on uncooked, whole, frozen seafood; relatively low stocking densities; the provision of seawater with stable temperature and physico-chemical parameters and darkened, relatively deep holding tanks have produced the best growth rates in cultured juvenile dhufish to date. It is perhaps not surprising that these conditions are similar to those in the natural habitat of dhufish.

References

Albers C. (1970). Acid-base balance. In *Fish Physiology*, Vol. 4 Ed. W. S. Hoar and D. J. Randall, pp. 173-208. Academic Press, New York.

Anderson N., Dash K. M., Donald A. D., Southcott W. H. & Waller P. J. (1978). Epidemiology and control of nematode infections. In *The epidemiology and control of gastrointestinal parasites of sheep in Australia.* Ed. A. D. Donald, W. H. Southcott and J. K. Dineen. Division of Animal Health, CSIRO, Melbourne.

APHA. (1995) Standard methods for the examination of water and wastewater. American Public Health Association, American Water Works Association, Water Environment Federation: Washington.

Aviram M. (1995). Macrophage-mediated oxidation of low density lipoprotein and atherosclerosis. In *Free radicals, liporpotein oxidation and atherosclerosis.* Ed. G. Bellomo, G. Finardi, E. Maggi and C. Rice-Evans, pp. 101-137. Richelieu Press, London.

Bagge A. M. & Valtonen E. T. (1996). Experimental study on the influence of paper and pulp mill effluent on the gill parasite communities of roach (*Rutilus rutilus*). *Parasitology* **112**, 499-508.

Beaulieu M., Lapointe Y. & Vinet B. (1999). Stability of pO2, pCO2 and pH in fresh blood samples stored in plastic syringe with low heparin in relation to various blood-gas and haematological parameters. *Clinical Biochemistry* **32**, 101-107.

Benesch R. E., Benesch R. & Yung S. (1973). Equations for the spectrophotometric analysis of hemoglobin mixtures. *Analytical Biochemistry* **55**, 245-248.

Biswas C. K., Ramos J. M., Agroyannis B. & Kerr D. N. S. (1982). Blood gas analysis: effect of air bubbles in syringe and delay in estimation. *British Medical Journal* **284**, 923-927.

Black E. C. (1958). Hyperactivity as a lethal factor in fish. *Journal of the Fisheries Research Board of Canada* **15**, 573-586.

Blazer V. S. & Wolke R. S. (1979). An *Exophiala*-like fungus as the cause of a systemic mycosis of marine fish. *Journal of Fish Diseases* **2**, 145-152.

Bollard B. A., Pankhurst N. W. & Wells R. M. G. (1993). Effects of artificially elevated plasma cortisol levels on blood parameters in the teleost fish *Pagrus auratus* (Sparidae). *Comparative Biochemistry and Physiology* **106A**, 157-162.

Boutilier R. G., Heming T. A. & Iwami G. K. (1984). Physicochemical parameters for use in fish respiratory physiology. In *Fish Physiology*, Vol. X, Part A Ed. W. S. Hoar and D. J. Randall, pp. 403-426. Academic Press, Orlando.

Boyd C. E. & Tucker C. S. (1998) Pond aquaculture water quality management. Kluwer Academic Publishers: Boston.

Brauner C. J. & Randall D. J. (1996). The interaction between oxygen and carbon dioxide movements in fishes. *Comparative Biochemistry and Physiology* **113A**, 83-90.

Bridges C. R., Berenbrink M., Müller R. & Waser W. (1998). Physiology and biochemistry of the pseudobranch - an unanswered question. *Comparative Biochemistry & Physiology* **119A**, 67-77.

Bridges C. R., Hlastala M. P., Riepl G. & Scheid P. (1983). Root effect induced by carbon dioxide and by fixed acid in the blood of the eel, *Anguilla anguilla. Respiration Physiology* **51**, 275-286.

Bridges C. R. & Morris S. (1989). Respiratory pigments: interactions between oxygen and carbon dioxide transport. *Canadian Journal of Zoology* **67**, 2971-2985.

Brittain T. (1987). The Root effect. *Comparative Biochemistry and Physiology* **86B**, 473-481.

Brulé T., Aldana Aranda D., Sánchez Crespo M. & Colas Marrufo T. C. (1996). A preliminary study on the growth performance of juvenile red grouper reared in a recirculating-water system. *The Progressive Fish Culturist* **58**, 192-202.

Buchmann K. & Bjerregaard J. (1990). Comparative efficacies of commercially available benzimidazoles against *Pseudodactylogyrus* infestations in eels. *Diseases of Aquatic Organisms* **9**, 117-120.

Buchmann K. & Lindenstrøm T. (2001) Interactions between monogenean parasites and their fish host. Paper presented at the *4th International Symposium on Monogenea*: Brisbane.

Buchmann K. & Roepstoff A. (1992). Experimental selection of mebendazole-resistant gill monogeneans from the European eel, *Anguilla anguilla* L. *Journal of Fish Diseases* **15**, 393-400.

Cameron J. N. (1971a). Oxygen dissociation characteristics of the blood of the rainbow trout, *Salmo gairdneri. Comparative Biochemistry and Physiology* **38A**, 699-704.

Cameron J. N. (1971b). Rapid method for determination of total carbon dioxide in small blood samples. *Journal of Applied Physiology* **31**, 632-634.

Cameron J. N. (1984). Acid-base status of fish at different temperatures. *American Journal of Physiology* **246**, R452-R459.

Campbell W. C. (1986). The chemotherapy of parasitic infections. *Journal of Parasitology* **72**, 45-61.

Carter P., Sommerville C., Gibson D. & Shinn A. (2001) The impact of cadmium on *Dactylogyrus extensus.* Paper presented at the *4th International Symposium on Monogenea*: Brisbane.

Cech J. J., Bridges D. W., Rowell D. M. & Balzer P. J. (1976). Cardiovascular responses of the winter flounder, *Pseudopleuronectes americanus* (Walbaum), to acute temperature increase. *Canadian Journal of Zoology* **54**, 1383-1388.

Cech J. J., Castleberry D. T. & Hopkins T. E. (1994). Temperature and CO_2 effects on blood O_2 equilibria in northern squawfish, *Ptychocheilus oregonensis. Canadian Journal of Fisheries and Aquatic Science* **51**, 13-19.

Chamberlain G. W., Neill W. H., Romanowsky P. A. & Strawn K. (1980). Vertical responses of Atlantic croaker to gas supersaturation and temperature change. *Transactions of the American Fisheries Society* **109**, 737-750.

Chisholm L. & Whittington I. (2001) Host invasion and development of monocotylid monogeneans from the shovelnose ray. Paper presented at the *4th International Symposium on Monogenea*: Brisbane.

Cleary J. J. & Jenkins G. (2000) Development of aquaculture techniques for the production of the West Australian dhufish. Aquaculture Development Unit, Challenger TAFE: Fremantle.

Colt J., (1984). Computation of dissolved gas concentrations in water as functions of temperature, salinity, and pressure. *American Fisheries Society Special Publication 14* , 1-65.

Colt J. (1986). Gas supersaturation- impact on the design and operation of aquatic systems. *Aquacultural Engineering* **5**, 49-85.

Colt J. & Bouck G. (1984). Design of packed column for degassing. *Aquacultural Engineering* **3**, 251-273.

Colt J. E., Orwicz K. & Brooks D. (1991). Gas supersaturation in the American river. *California Fish and Game* **77**, 41-50.

Cone D. K. (1995). Monogenea (Phylum Platyhelminthes). In *Fish Diseases and Disorders, Volume 1: Protozoan and metazoan infections* Ed. P. T. K. Woo, pp. 289-327. CAB International, Wallingford.

Copeland D. E. (1980). Functional vascularisation of the teleost eye. *Current Topics in Eye Research* **3**, 219-280.

Cribb T., Chisholm L. & Bray R. (2001) External and direct (Monogenea) *versus* internal and indirect (Digenea): contrasting patterns of success or do the monogenea just suck? Paper presented at the *4th International Symposium on Monogenea*: Brisbane.

CSIRO. (1999) Australian seafood handbook (Ed. G. K. Yearsley, P. R. Last and R. D. Ward), pp. 461. CSIRO Marine Research: Hobart.

Dacie J. V. & Lewis S. M. (1975) Practical Haematology. Churchill Livingstone: Edinburgh.

Dafré A. L. & Wilhelm F° D. (1989). Root effect hemoglobins in marine fish. *Comparative Biochemistry and Physiology* **92A**, 467-471.

Dash K. M. (1988). Helminth control strategies. In *Sheep health and production. The T G Hungerford refresher course for veterinarians. Proceedings 110*, pp. 295-305. Post Graduate Committee in Veterinary Science, Sydney.

Davidson G. W., Davie P. S., Young G. & Fowler R. T. (2000). Physiological responses of rainbow trout *Oncorhynchus mykiss* to crowding and anesthesia with AQUI-S. *Journal of the World Aquaculture Society* **31**, 105-114.

Day T. K., Gaynor J. S., Muir W. W., Bednarski R. M. & Mason D. E. (1995). Blood gas values during intermittent positive pressure ventilation and spontaneous ventilation in 160 anesthetized horses positioned in lateral or dorsal recumbency. *Veterinary Anesthesia* **24**, 266-276.

De Silva S. & Anderson T. A. (1995) Fish nutrition in aquaculture. In *Aquaculture series 1*, pp. 319. Chapman Hall: London.

De Young A., Kwiatkowski L. D. & Noble R. W. (1994). Fish hemoglobins. *Methods in Enzymology* **231**, 124-150.

Dehadrai P. V. (1966). Mechanism of gaseous exophthalmia in the Atlantic cod, *Gadus morhua* L. *Journal of th e Fisheries Research Board of Canada* **23**, 909-914.

Desrochers P. E., Pratt K. A., Fromm P. O. & Hoffert J. R. (1985). Oxygen diffusion in the trout retina. *Experimental Eye Research* **41**, 607-618.

Dhindsa D. S., Hoversland A. S., Neill W. A. & Metcalfe J. (1971). Changes in blood oxygen affinity and hemodynamics in anemic dogs. *Respiration Physiology* **11**, 346-353.

Diggles B. K., Roubal F. R. & Lester R. J. G. (1993). The influence of formalin, benzocaine and hyposalinity on the fecundity and viability of *Polylabroides multispinosus* (Monogenea: Microcotylidae) parasitc on the gills of *Acanthopagrus australis* (Pisces: Sparidiae). *International Journal of Parasitology* **23**, 877-884.

Dobson G. P. & Baldwin J. (1982a). Regulation of blood oxygen affinity in the Australian blackfish *Gadopsis marmoratus*. *Journal of Experimental Biology* **99**, 245-254.

Dobson G. P. & Baldwin J. (1982b). Regulation of blood oxygen affinity in the Australian blackfish *Gadopsis marmoratus*. *Journal of Experimental Biology* **99**, 223-243.

Doshida J., Hasegawa H., Onuki H. & Shimidzu N. (1996). Exophilin A, a new antibiotic froma marine microorganism *Exophiala pisciphila*. *The Journal of Antibiotics* **49**, 1105-1109.

Eddy F. B. (1973). Oxygen dissociation curves of the blood of the tench, *Tinca tinca*. *Journal of Experimental Biology* **58**, 281-293.

Edwards M. J. & Martin R. J. (1966). Mixing technique for the oxygen-haemoglobin equilibrium and Bohr effect. *Journal of Applied Physiology* **21**, 1898-1902.

Ellis A. E., Waddell I. F. & Minter D. W. (1983). A systemic fungal disease in Atlantic salmon parr, *Salmo salar* L., caused by a species of *Phialophora*. *Journal of Fish Diseases* **6**, 511-23.

Elston R., Colt J., Abernethy S. & Maslen W. (1997). Gas bubble reabsorption in chinook salmon: pressurisation effects. *Journa l of Aquatic Animal Health* **9**, 317-321.

Engelhorn O. R. (1943). Die gasblasenkrankheit bei Fischen. *Zeitschrift fur Fischeri* **41**, 297-317.

Ernst I., Whittington I., Corneillie S. & Talbot C. (2001) A simple deterministic model for understanding the epidemiology of *Benedenia seriolae* on sea-caged *Seriola* spp. in Japan. Paper presented at the *4th International Symposium on Monogenea*: Brisbane.

Fairbanks M. B., Hoffert J. R. & Fromm P. O. (1969). The dependence of the oxygen-concentrating mechanism of the teleost eye (*Salmo gairdneri*) on the enzyme carbonic anhydrase. *The Journal of General Physiology* **54**, 203-211.

Fairbanks M. B., Hoffert J. R. & Fromm P. O. (1974). Short circuiting of the ocular oxygen concentrating mechanism in the teleost *Salmo gairdneri* using carbonic anhydrase inhibitors. *The Journal of General Physiology* **64**, 263-273.

Farmer M., Fyhn H. J., Fyhn U. E. H. & Noble R. W. (1979). Occurrence of Root effect haemoglobins in Amazonian fishes. *Comparative Biochemistry and Physiology* **62A**, 115-124.

Ferguson H. W. (1989) Systemic pathology of fish. Iowa State University Press: Ames.

Fisheries WA. (2000) State of the fisheries report 1998/2000. pp. 138. Fisheries Research Division: Fisheries Western Australia: Perth.

Fivelstad S., Haavik H., Løvik G. & Olsen A. B. (1998). Sublethal effects and safe levels of carbon dioxide in seawater for Atlantic salmon postsmolts (*Salmo salar* L.): ion regulation and growth. *Aquaculture* **160**, 305-316.

Foster P. P., Conkin J., Powell M. R., Waligora J. M. & Chikara R. S. (1998). Role of metabolic gases in bubble formation during hypobaric exposures. *Journal of Applied Physiology* **84**, 1088-1095.

Fyhn U. E. H., Fyhn H. J., Davis B. J., Powers D. A., Fink W. L. & Garlick R. L. (1979). Hemoglobin heterogeneity in Amazonian fishes. *Comparative Biochemistry and Physiology* **62A**, 39-66.

Gerwick L., Demers N. E. & Bayne C. J. (1999). Modulation of stress hormones in rainbow trout by means of anesthesia, sensory deprivation and receptor blockade. *Comparative Biochemistry and Physiology* **124A**, 329-334.

Gilmour K. M. (1998). Gas exchange. In *The Physiology of Fishes.* Ed. D. H. Evans, pp. 101-127. CRC Press, New York.

Goodrich E. S. (1930) Studies on the structure and development of vertebrates. Macmillan and Co., Ltd.: London.

Gorlick D. L. (1984). Preference for ecto-parasite-infected host fishes by the Hawaiian cleaning wrasse, *Labroides phthirophagus* (Labridae). *Copeia* **1984**, 758-762.

Goven B. & Amend D. F. (1982). Mebendazole/trichlorfon combination a new anthelminthic for removing monogenetic trematodes from fish. *Journal of Fish Biology* **20**, 373-378.

Graham M. S. & Iwama G. K. (1990). The physiologic effects of the anesthetic ketamine hydrochloride on two salmonid species. *Aquaculture* **90**, 323-331.

Grøttum J. A. & Sigholt T. (1996). Acute toxicity of carbon dioxide on European seabass (*Dicentrarchus labrax*): mortality and effects on plasma ions. *Comparative Biochemistry and Physiology* **115A**, 323-327.

Halliwell B. (1994). Free radicals, antioxidants, and human disease; curiosity, cause, or consequence? *The Lancet* **344**, 721-724.

Hamilton R. D., Crockett A. J. & Alpers J. H. (1978). Arterial blood gas analysis: potential errors due to the addition of heparin. *Anaesthesia and Intensive Care* **6**, 251-255.

Harris P. D., Soleng A. & Bakke T.A. (1998). Killing of *Gyrodactylus salaris* (Platyhelminthes, Monogenea) mediated by host complement. *Parasitology 117* **117**, 137-143.

Heggberget T. G., Johnsen B. O., Hindar K., Johnson B., Hansen L. P., Hvidsten N. A. & Jensen A. J. (1993). Interactions between wild and cultured Atlantic salmon: A review of the Norwegian experience. *Fisheries Research (Amsterdam)* **18**, 123-146.

Hesp S. A. & Potter I. C. (2000) Determination of the biological parameters required for managing the fishery of West Australian dhufish. Fisheries Research and Development Corporation Report FRDC Project No.96/103.

Hills B. A. (1977) Decompression sickness: the biophysical basis of prevention and treatment, Vol. 1, pp. 289. John Wiley & Sons: Chichester.

Hoffert J. R. & Ubels J. L. (1979). The intraocular pO_2 and electroretinogram of the trout as affected by temperature and ventilatory flow. *Comparative Biochemistry and Physiology* **62A**, 563-568.

Houston A. H. & Mearow K. M. (1979). Temperature-related changes in the erythrocytic carbonic anhydrase (acetazolamide-sensitive esterase) activity of goldfish, *Carassus auratus*. *Journal of Experimental Biology* **78**, 255-264.

Hussain N. A. & Higuchi M. (1980). Larval rearing and development of the brown spotted grouper, *Epinephelus tauvina* (Forskal). *Aquaculture* **19**, 339-350.

Ingermann R. L. & Terwilliger R. C. (1982). Presence and possible function of Root effect hemoglobins in fishes lacking functional swim bladders. *The Journal of Experimental Zoology* **220**, 171-177.

James P. B. (1993). Dysbarism: the medical problems from high and low atmospheric pressure. *Journal of the Royal College of Physicians of London* **27**, 367-374.

Jansen P. A. & Bakke T. A. (1991). Temperature-dependent reproduction and survival of *Gyrodactylus salaris* Malmberg, 1957 (Platyhelminthes: Monogenea) on Atlantic salmon (*Salmo salar* L.). *Parasitology* **102**, 105-112.

Jensen F. B. (1989). Hydrogen ion equilibria in fish haemoglobins. *Journal of Experimental Biology* **143**, 225-234.

Johnson D. W. & Katavic I. (1984). Mortality, growth and swim bladder stress syndrome of sea bass (*Dicentrarchus labrax*) larvae under varied environmental conditions. *Aquaculture* **38**, 67-78.

Johnson S. C. & Margolis L. (1993). Efficacy of ivermectin for control of the salmon louse *Lepeophtheirus salmonis* on Atlantic salmon. *Diseases of Aquatic Organisms* **17**, 101-105.

Jokinen E. I., Aaltonen T. M. & Valtonen E. T. (1995). Subchronic effects of pulp and paper mill effluents on the immunoglobulins synthesis of roach, *Rutilus rutilus*. *Ecotoxicology and Environmental Safety* **32**, 219-225.

Kailola P. J., Williams M. J., Stewart P. C., Reichelt R. E., McNee A. & Grieve C. (1993) Australian Fisheries Resources. Commonwealth of Australia, Bureau of Resource Sciences and the Fisheries Research and Development Corporation: Canberra.

Kearn G. C. (1973). An endogenous circadian hatching rhythm in the monogenean skin parasite *Entobdella solae*, and its relationship to the activity rhythm of the host (*Solea solea*). *Parasitology* **66**, 101-122.

Kim K. H., Park S.-I. & Jee B.-Y. (1998). Efficacy of oral administration of praziquantel and mebendazole against *Microcotyle sebastis* (Monogenea) infestation of cultured rockfish (*Sebastes schlegeli*). *Fish Pathology* **33**, 467-471.

Kitano M. (1995). Pathological aspects of decompression sickness. In *Decompression sickness in divers Occasional papers No.25* Ed. M. Kitano, pp. 47-59. Kagoshima University Research Center for the South Pacific.

Knittel M. D., Chapman G. A. & Garton R. R. (1980). Effects of hydrostatic pressure on steelhead survival in air-saturated water. *Transactions of the American Fisheries Society* **109**, 755-759.

Kolbeinshavn A. & Wallace J. C. (1985). Observations on swim bladder stress syndrome in arctic charr (*Salvellinus alpinus*), induced by inadequate water depth. *Aquaculture* **46**, 259-261.

Koskivaara M. & Valtonen E. T. (1992). *Dactylogyrus* (Monogenean) communities on the gills of roach in three lakes in Central Finland. *Parasitology* **104**, 263-272.

Kritsky D. C. & Stephens F. J. (2001). *Haliotrema abaddon* n. sp. (Monogenoidea:Dactylogyridae) from the gills of wild and maricultured West Australian dhufish *Glaucosoma hebraicum* (Teleostei:Glaucosomatidae), in Australia. *Journal of Parasitology* **87**, 749-754.

Langdon J. S., Elliott K. & Mackay B. (1991). Epitheliocystis in the leafy sea-dragon. *Australian Veterinary Journal* **68**, 244.

Langdon J. S. & McDonald W. L. (1987). Cranial *Exophiala pisciphila* infection in *Salmo salar* in Australia. *Bulletin of the European Association of Fish Pathologists* **7**, 35-37.

Laurent P. & Dunel-Erb S. (1984). The pseudobranch: morphology and function. In *Fish Physiology*, Vol. X, Part B, Part B: Ion and water transfer Ed. W. S. Hoar and D. J. Randall, pp. 285-323. Academic Press, Orlando.

Lied E., Gjerde J. & Braekkan O. R. (1975). Simple and rapid technique for repeated blood sampling in rainbow trout (*Salmo gairdneri*). *Journal of the Fisheries Research Board of Canada* **32**, 699-701.

Ling N. & Wells R. M. G. (1985). Plasma carecholamines and erythrocyte swelling following capture stress in a marine teleost fish. *Comparative Biochemistry and Physiology* **82C**, 231-234.

Liss H. P. & Payne C. P. (1993). Stability of blood gases in ice and at room temperature. *Chest* **103**, 1120-1122.

Machado J. P., Garling D. L., Kevern N. R., Trapp A. L. & Bell T. G. (1987). Histopathology and the pathogenesis of embolism (gas bubble disease) in rainbow trout (*Salmo gairdneri*). *Canadian Journal of Fisheries and Aquatic Science* **44**, 1985-1994.

Madiedo G., Sciacca R. & Hause L. (1980). Air bubbles and temperature effect on blood gas analysis. *Journal of Clinical Pathology* **33**, 864-867.

Mahoney J. J., Harvey J. A., Wong R. J. & van Kessel A. L. (1991). Changes in oxygen measurements when whole blood is stored in iced plastic or glass syringes. *Clinical Chemistry* **37**, 1244-1248.

Marr F. (1980) Growth, reproduction and dietary preference of the jewfish, *Glaucosoma hebraicum*. B.Sc. project report, *Biology Department*. Curtin University: Perth.

Martin P. J. (1988). Anthelmintic resistance. In *Sheep health and production. The T G Hungerford refresher course for veterinarians. Proceedings 110*, pp. 347-367. Post Graduate Committee in Veterinary Science, Sydney.

Maxie M. G. & Prescott J. F. (1993). The urinary system. In *Pathology of domestic animals*. Vol. 2. Ed. K. V. F. Jubb, P. C. Kennedy and N. Palmer, pp. 447-538. Academic Press Inc., San Diego.

McGinnis M. R. & Ajello L. (1974). A new species of *Exophiala* isolated from channel catfish. *Mycologia* **66**, 518-520.

McKay R. J. (1997) Pearl perches of the world (Family Glaucosomatidae). In *FAO Species Catalogue*, Vol. 17. Food and Agriculture Organisation of the United Nations: Rome.

McKenzie D. J., Burleson M. L. & Randall D. J. (1991). The effects of branchial denervation and pseudobranch ablation on cardioventilatory control in an air-breathing fish. *Journal of Experimental Biology* **161**, 347-365.

McLeay D. J. & Gordon M. R. (1977). Leucocrit: a simple hematological technique for measuring acute stress in salmonid fish, including stressful concentrations of pulpmill effluent. *Journal of the Fisheries Research Board of Canada* **34**, 2164-2175.

Milligan C. L. & Wood C. M. (1986). Tissue intracellular acid-base status and the fate of lactate after exhaustive exercise in the rainbow trout. *Journal of Experimental Biology* **123**, 123-144.

Molinero A. & Gonzalez J. (1995). Comparative effects of MS 222 and 2-phenoxyethanol on gilthead sea bream (*Sparus aurata* L.) during confinement. *Comparative Biochemistry and Physiology* **111A**, 405-414.

Mounzer K. C., Rhoads S. & Blank J. E. (1999). Infection due to *Exophiala jeanselmei*. *Clinical Infectious Diseases* **29**, 1380, 1559-1560.

Munday B. (1996). Treatment of finfish disease. In *Fish health workshop*, pp. 179-198. Post Graduate Foundation in Veterinary Science, The University of Sydney, Sydney.

Noble R. W., Kwiatkowski L. D., De Young A., Davis B. J., Haedrich R. L., Tam L. & Riggs A. F. (1986). Functional properties of haemoglobins from deep-sea fish: correlations with depth distribution and presence of a swimbladder. *Biochimica et Biophysica Acta* **870**, 552-563.

Noga E. J. (1996) Fish Disease: diagnosis and treatment. Mosby: St Louis.

Norton Rossman S., Cernoch P. L. & Davis J. R. (1996). Dematiaceous fungi are an increasing cause of human disease. *Clinical Infectious Diseases* **22**, 73-80.

Nowak B. F. & Clark A. (1999). Prevalence of epitheliocystis in Atlantic salmon, *Salmo salar* L., farmed in Tasmania, Australia. *Journal of Fish Diseases* **22**, 73-78.

Nowak B. F. & Lucas C. T. (1997). Diagnosis of structural changes in fish gills-can biopsy replace necropsy? *Aquaculture* **159**, 1-10.

Palmer P. J., Burke J. B., Willett D. J. & Simpson R. R. (1992) Development of a low-maintenance technique for rearing barramundi (Bloch) larvae. In *Information Series Q192036*. Department of Primary Industries, Queensland.

Paperna I. (1987). Solving parasite-related problems in cultured marine fish. *International Journal of Parasitology* **17**, 327-336.

Payne M. F., Rippingale R. J. & Cleary J. J. (2001). Cultured copepods as food for West Australian dhufish (*Glaucosoma hebraicum*) and pink snapper (*Pagrus auratus*) larvae. *Aquaculture* **194**, 137-150.

Pearce A. (1986). Sea temperatures off Western Australia. *Fins* **19**, 6-9.

Pearce A. F. (1991). Eastern boundary currents of the southern hemisphere. *Journal of the Royal Society of Western Australia* **74**, 35-45.

Pedersen O. A. & Langvad F. (1989). *Exophia la psychrophila* sp. nov., a pathogenic species of the black yeasts isolated from farmed Atlantic salmon. *Mycological Research* **92**, 153-156.

Pelster B. (1998). Buoyancy. In *The Physiology of Fishes* Ed. D. H. Evans, pp. 25-43. CRC Press, New York.

Pelster B. & Weber R. E. (1990). Influence of organic phosphates on the Root effect of multiple fish haemoglobins. *Journal of Experimental Biology* **149**, 425-437.

Perry S. F. & Heming T. A. (1981). Blood ionic and acid-base status in rainbow trout (*Salmo gairdneri*) following rapid transfer from freshwater to seawater: effect of pseudobranch denervation. *Canadian Journal of Zoology* **59**, 1126-1132.

Phillips A. M. (1947). The effect of asphyxia upon the red cell content of trout blood. *Copeia* **3**, 183-186.

Pickering A. D. (1998). Stress responses of farmed fish. In *Biology of farmed fish* Ed. K. D. Black and A. D. Pickering, pp. 222-255. Sheffield Academic Press, Sheffield.

Pickering A. D., Pottinger T. G. & Christie P. (1982). Recovery of brown trout, *Salmo trutta* L., from acute handling stress; a time-course study. *Journal of Fish Biology* **20**, 229-244.

Pironet F. N. & Jones J. B. (2000). Treatments for ectoparasites and diseases in captive Western Australian dhufish. *Aquaculture International* **8**, 349-361.

Pironet F. N. & Neira F. J. (1998). Hormone-induced spawning and development of artificially reared larvae of the West Australian dhufish, *Glaucosoma hebraicum* (Glaucosomatidae). *Marine and Freshwater Research* **49**, 133-142.

Powers D. A. (1980). Molecular ecology of teleost fish hemoglobins: strategies for adapting to changing environments. *American Zoologist* **20**, 139-162.

Powers D. A., Fyhn H. J., Fyhn U. E. H., Martin J. P., Garlick R. L. & Wood S. C. (1979). A comparative study of the oxygen equilibria of blood from 40 genera of Amazonian fishes. *Comparative Biochemistry and Physiology* **62A**, 67-85.

Railo E., Nikinmaa M. & Soivio A. (1985). Effects of sampling on blood parameters in the rainbow trout, *Salmo gairdneri* Richardson. *Journal of Fish Biology* **26**, 725-732.

Randall D. J. (1970). Gas exchange in fish. In *Fish Physiology*, Vol. 4 Ed. W. S. Hoar and D. J. Randall, pp. 253-292.

Renaud S. M. & Parry D. L. (1994). Microalgae for use in tropical aquaculture II: Effect of salinity on growth, gross chemical composition and fatty acid composition of three species of marine microalgae. *Journal of Applied Phycology* **6**, 347-356.

Riccio A., Tamburrini M., Carratore V. & Di Prisco G. (2000). Functionally distinct haemoglobins of the cryopelagic Antarctic teleost *Pagothenia borchgrevinki*. *Journal of Fish Biology* **57**, Supplement A, 20-32.

Richards R. H., Holliman A. & Helgason S. (1978). *Exophiala salmonis* infection in Atlantic salmon *Salmo salar* L. *Journal of Fish Diseases* **1**, 357-368.

Riggs A. (1951). The metamorphosis of haemoglobin in the bullfrog. *Journal of General Physiology* **35**, 23-33.

Riggs A. (1970). Properties of fish haemoglobins. In *Fish Physiology*, Vol. 4 Ed. W. S. Hoar and D. J. Randall, pp. 209-246. Academic Press, New York.

Riggs A. (1981). Preparation of blood hemoglobins of vertebrates. *Methods in Enzymology* **76**, 5-29.

Rintamaki-Kinnunen P. & Valtonen E. T. (1996). Finnish salmon resistant to *Gyrogactylus salaris*: a long-term study at fish farms. *International Journal for Parasitology* **26**, 723-732.

Robinson P. (1987). The morphology and histology of the alimentary tract; the dietary preference of the Western Australian Jewfish: *Glaucosoma hebraicum*. B.Sc. project report, *Biology Department*, pp. 42. Curtin University: Perth.

Root, R.W. (1931). The respiratory function of the blood of marine fishes. *Biological Bulletin of the Marine Biological Laboratory (Woods Hole)* **61**, 427-456.

Rothe G. & Valet G. (2000). Biochemical parameters of cell function. In *Flow cytometry and cell sorting* Ed. A. Radbruch. Springer, Berlin.

Roubal F. R. (1986). Studies of monogeneans and copepods parasitizing the gills of the sparid, *Acanthopagrus australis* (Gunther) in northern New South Wales. *Canadian Journal of Zoology* **64**, 841-849.

Roubal F. R. (1989). Comparative pathology of some monogenean and copepod ectoparasites on the gills of *Acanthopagrus australis* (Family Sparidie). *Journal of Fish Biology* **34**, 503-514.

Roubal F. R. (1994). Observations on the eggs and fecundity of dactylogyrid and diplectanid monogeneans from the Australian marine sparid fish, *Acanthopagrus australis*. *Folia Parasitologica* **41**, 220-222.

Roubal F. R. (1995). Changes in monogenean and copepod infestation on captive *Acanthopagrus australis* (Sparidae). *Journal of Fish Biology* **46**, 423-431.

Roubal F. R., Quartararo N. & West A. (1996). Spatial and temporal variation in populations and community of ectoparasties on young snapper, *Pagrus auratus* (Bloch & Schneider) (Sparidae), from the wild and captivity at Port Hacking, Sydney, Australia. *Marine and Freshwater Research* **47**, 585-593.

Roy W. J., Sutherland I. H., Rodger H. D. M. & Varma K. J. (2000). Tolerance of Atlantic salmon *Salmo salar* L., and rainbow trout, *Oncorhynchus mykiss* (Walbaum), to emamectin benzoate, a new orally administered treatment for sea lice. *Aquaculture* **184**, 19-29.

Sargent J., McEvoy L., Estevez A., Bell G., Bell M., Henderson J. & Tocher D. (1999). Lipid nutrition of marine fish during early development: current status and future directions. *Aquaculture* **179**, 217-229.

Sargent J. R., McEvoy L. A. & Bell J. G. (1997). Requirements, presentation and sources of polyunsaturated fatty acids in marine fish larval feeds. *Aquaculture* **155**, 117-127.

Scheid P. & Meyer M. (1978). Mixing technique for study of oxygen-hemoglobin equilibrium: a critical evaluation. *Journal of Applied Physiology* **45**, 818-822.

Schotz J. (1998) Fungal infection and bryozoan morphology. Abstract from the *11th Conference of the International Bryozoology Association*: Brisbane.

Smit G. L., Hattingh J. & Burger A. P. (1979). Haematological assessment of the effects of the anaesthetic MS 222 in natural and neutralized form in three freshwater fish species; interspecies differences. *Journal of Fish Biology* **15**, 633-643.

Smith C. E. (1988). Histopathology of gas bubble dieases in juvenile rainbow trout. *The Progressive Fish Culturist* **50**, 98-103.

Smith P. R., Moloney M., McElligott A., Clarke S., Palmer R., O'Kelly J. & O'Brien F. (1993). The efficiency of oral ivermectin in the control of sea lice infestations of farmed Atlantic salmon. In *Pathogens of wild and farmed fish* Ed. G. A. Boxshall and D. Defaye, pp. 296-307. Ellis Horwood, Chichester.

Snieszko S. F. (1974). The effects of environmental stress on outbreaks of infectious diseases of fish. *Journal of Fish Biology* **6**, 197-208.

Soivio A. & Nyholm K. (1975). A technique for repeated sampling of the blood of individual resting fish. *Journal of Experimental Biology* **62**, 207-217.

Soivio A., Nyholm K. & Huhti M. (1977). Effects of anaesthesia with MS 222, neutralized MS 222 and benzocaine on the blood constituents of rainbow trout *Salmo gairdneri*. *Journal of Fish Biology* **10**, 91-101.

Soldatov A. A. (1996). The effect of hypoxia on red blood cells of flounder: a morphologic and autoradiographic study. *Journal of Fish Biology* **48**, 321-328.

Speare D. J. (1991). Endothelial lesions associated with gas bubble disease in fish. *Journal of Comparative Pathology* **104**, 327-335.

Speare D. J. (1998). Disorders associated with exposure to excess dissolved gases. In *Fish diseases and disorders.* Vol. 2: Non-infectious disorders Ed. J. F. Leatherhead and P. T. K. Woo, pp. 207-224. CAB International Publishing, Wallingford, Oxon.

Spotte S. (1992) Captive Seawater Fishes; Science and Technology, pp. 942. John Wiley and Sons, Inc.: New York.

Steen J. B. (1963). The physiology of the swimbladder in the eel *Anguilla vulgaris. Acta Physiologica Scandinavica.* **59**, 221-241.

Stephens F. J., Cleary J. J., Jenkins G., Jones B., Raidal S. R. & Thomas J. B., The effect of CO_2 rich ground water on the West Australian dhufish (*Glaucosoma hebraicum*). *Aquaculture (in press)* .

Stephens F. J., Cleary J. J., Jenkins G., Jones B., Raidal S. R. & Thomas J. B., Treatments to control *Haliotrema abaddon* in the West Australian dhufish, *Glaucosoma hebraicum. Aquaculture (in press)* .

Stephens F. J., Cleary J. J., Jenkins G., Jones B., Raidal S. R. & Thomas J. B., Haemoglobin and oxygen transport: apossible role in gas bubble formation in the eye of the West Australian dhufish, *Glaucosoma hebraicum* and other species. *Journal of Fish Diseases* (*in press*).

Stephens F. J., Cleary J. J., Jenkins G., Jones B., Raidal S. R. & Thomas J. B., (2001). Pathogenesis and epidemiology of spontaneous exophthalmos in the West Australian dhufish, *Glaucosoma hebraicum* Richardson,1845. *Journal of Fish Diseases* **24**, 515-522.

Stevens D. G., Nebeker A. V. & Baker R. J. (1980). Avoidance responses of salmon and trout to air-supersaturated water. *Transactions of the American Fisheries Society* **109**, 751-754.

Stoskopf M. K. (1993) Fish Medicine. W.B.Saunders Company: Philadelphia.

Stottrup J. G. (1999) The elusive copepods. Their production and suitability in marine aquaculture. Paper presented at *World Aquaculture '99*, pp. 727. World Aquaculture Society: Sydney, Australia.

Strange R. J. & Schreck C. B. (1978). Anesthetic and handling stress on survival and cortisol concentration in yearling chinook salmon (*Oncorhynchus tshawytscha*). *Journal of the Fisheries Research Board of Canada* **35**, 345-349.

Symons L. E. A. (1989) Pathophysiology of endoparasitic infection compared with with ectoparasitic infestation and microbial infection, pp. 331. Academic Press: Sydney.

Székely C. & Molnár K. (1991). Praziquantel (Droncit) is effective against diplostomosis of grasscarp *Ctenopharyngodon idella* and silver carp *Hypophthalmichthys molitrix*. *Diseases of Aquatic Organisms* **11**, 147-150.

Tacha T. C., Warde W. D. & Burnham K. P. (1982). Use and interpretation of statistics in wildlife journals. *Wildlife Society Bulletin* **10**, 355-362.

Tetens V. & Lykkeboe G. (1981). Blood respiratory properties of rainbow trout, *Salmo gairdneri*: responses to hypoxia acclimation and anoxic incubation of blood in vitro. *Journal of Comparative Physiology B* **145**, 117-125.

Thomas P. T. & Woo P. T. K. (1992). Anorexia in rainbow trout, *Oncorhynchus mykiss* (Walbaum), infected with *Cryptobia salmositica* (Sarcomastigophora: Kinetoplastida): its onset and contribution to the immunodepression. *Journal of Fish Diseases* **15**, 443-447.

Thoney D. A. (1990). The effects of trichlorphon, praziquantel and copper sulphate on various stages of the monogenean *Benedeniella posterocolpa*, a skin parasite of the cownose ray, *Rhinoptera bonasus* (Mitchill). *Journal of Fish Diseases* **13**, 385-389.

Toews D. P., Holeton G. F. & Heisler N. (1983). Regulation of the acid -base status during environmental hypercapnia in the marine teleost fish *Conger conger*. *Journal of Experimental Biology* **107**, 9-20.

Treasurer J. W. & Pope J. A. (2000). Selection of host sample number and design of a monitoring programme for ectoparasitic sea lice (Copepoda: Caligidae) on farmed Atlantic salmon, *Salmo salar*. *Aquaculture* **187**, 247-260.

Treece G. D. (1995). The production of live-food organisms for fishes. In *Production of aquatic animals: fishes.* World Animal Science, C8 Ed. C. E. Nash and A. J. Novotny. Elsevier, Amsterdam.

Treves-Brown K. M. (2000) Applied Fish Pharmacology. In *Aquaculture Series*, Vol. 3, pp. 309. Kluwer Academic Publishers: Dordrecht.

Tucker V. A. (1967). Method for oxygen content and dissociation curves on microliter blood samples. *Journal of Applied Physiology* **23**, 410-414.

Turton M. (1983). Heparin solution as a source of error in blood gas determination. *Clinical Chemistry* **29**, 1562-1563.

Untergasser D. (1989) Handbook of Fish Diseases, pp. 160. T.F.H.Publications, Inc.: Neptune City.

Urzi C., Wollenzien U., Criseo G. & Krumbein W. E. (1995). Biodiversity of the rock inhabiting microbiota with special reference to black fungi and black yeasts. In *Microbial diversity and ecosystem function.* Ed. D. Allsop, R. R. Colwell and D. L. Hawksworth, pp. 289-302. CAB International: Oxford.

Vanden Bossche H. (1985). Pharmacology of anthelmintics. In *Handbook of experimental pharmacology*, Vol. 77 Ed. G. Born, A. Farah, H. Herken and A. Welch. Springer-Verlag, Berlin.

Vick R. L. (1984) Contemporary Medical Physiology, pp. 1003. Addison Wesley Publishing Co. Ltd, Medical Division: Menlo Park.

Weber R. E. (1992). Use of ionic and zwitterionic (Tris/BisTris and HEPES) buffers in studies on hemoglobin function. *Journal of Applied Physiology* **72**, 1611-1615.

Weber R. E. & de Wilde J. A. M. (1975). Oxygenation properties of haemoglobins from the flatfish plaice (*Pleuronectes platessa*) and flounder (*Platichthys flesus*). *Journal of Comparative Physiology* **101**, 99-110.

Weber R. E. & de Wilde J. A. M. (1976). Multiple haemoglobins in plaice and flounder and their functional properties. *Comparative Biochemistry and Physiology* **54B**, 433-437.

Weber R. E. & Jensen F. B. (1988). Functional adaptations in hemoglobins from ectothermic vertebrates. *Annual Review of Physiology* **50**, 161-179.

Weber R. E., Wood S. C. & Lomholt J. P. (1976). Temperature acclimation and oxygen-binding properties of blood and multiple haemoglobins of rainbow trout. *Journal of Experimental Biology* **65**, 333-345.

Wedemeyer G. A. (1997). Effects of rearing conditions on the health and physiological quality of fish in intensive culture. In *Fish stress and health in aquaculture.* Ed. G. K. Iwama, A. D. Pickering, J. P. Sumpter and C. B. Schreck, pp. 35-71. Cambridge University Press, Cambridge.

Weitkamp D. E. & Katz M. (1980). A review of dissolved gas supersaturation literature. *Transactions of the American Fisheries Society* **109**, 659-702.

Wells R. M. G. (1990). Hemoglobin physiology in vertebrate animals: a precautionary approach to adaptationist thinking. In *Advances in comparative and environmental physiology: vertebrate gas exchange from environment to cell.* Vol. 6 Ed. R. G. Boutilier. Springer-Verlag, Berlin.

Wells R. M. G. & Baldwin J. (1990). Oxygen transport potential in tropical reef fish with special reference to blood viscosity and haematocrit. *Journal of Experimental Marine Biology and Ecology* **141**, 131-143.

Wells R. M. G., Baldwin J., Seymour R. S. & Weber R. E. (1997). Blood oxygen transport and hemoglobin function in three tropical fish species from northern Australian freshwater billabongs. *Fish Physiology and Biochemistry* **16**, 247-258.

Whittington I., Ernst I., Corneillie S. & Talbot C. (2001) An overview of *Benedenia seriolae* infections of *Seriola* spp. (Carangidae) in Japanese aquaculture. Paper presented at the *4th International Symposium on Monogenea*: Brisbane.

Whittington I. D. (1990). The egg bundles of the monogenean *Dionchus remorae* and their attachment to the gills of the remora *Echeneis naucrates. International Journal of Parasitology* **20**, 45-49.

Wilhelm D. F. & Reischl E. (1981). Heterogeneity and functional properies of hemoglobins from south Brazilian freshwater fish. *Comparative Biochemistry and Physiology* **69B**, 463-470.

Williams D. L., Hopcroft T., Pantel U. & Brancker W. M. (1998). Levels of choroidal body carbonic anhydrase activity and glycogen in farmed halibut. *The Veterinary Journal* **156**, 223-229.

Wittenberg J. B. & Wittenberg B. A. (1962). Active secretion of oxygen into the eye of fish. *Nature* **194**, 106-107.

Wittenberg J. B. & Wittenberg B. A. (1974). The choroid rete mirabile of the fish eye. I. Oxygen secretion and structure: comparison with the swimbladder rete mirabile. *Biological Bulletin of the Marine Research Laboratory (Woods Hole)* **146**, 116-136.

Wittenberg J. B. & Wittenberg B. A. (1975). A hemeprotein implicated in oxygen transport into the eye of fish. *Comparative Biochemistry and Physiology* **51A**, 425-429.

Witztum J. L. (1994). The oxidation hypothesis of atherosclerosis. *The Lancet* **344**, 793-795.

Wood C. M., McMahon B. R. & McDonald D. G. (1979). Respiratory gas exchange in the resting starry flounder, *Platichthys stellatus*: a comparison with other teleosts. *Journal of Experimental Biology* **78**, 167-179.

Wood S. C. (1980). Adaptation of red blood cell function to hypoxia and temperature in ectothermic vertebrates. *American Zoologist* **20**, 163-172.

Yager J. A., Scott D. W. & Wilcock B. P. (1993). The skin and appendages. In *Pathology of domestic animals.* Vol. 1. Ed. K. V. F. Jubb, P. C. Kennedy and N. Palmer, pp. 668. Academic Press Inc., San Diego.

Yoshinaga T., Segawa I., Kamaishi T. & Sorimachi M. (2000). Effects of temperature, salinity and chlorine treatment on egg hatching of the monogenean *Neoheterobothrium hirame* infecting Japanese flounder. *Fish Pathology* **35**, 85-88.

Appendix 1

The results of the study on praziquantel may constitute intellectual property that may be used to support a future application for the registration of praziquantel for use as an 'in water' treatment for fish with the National Registration Authority

Appendix 2

Analysis of blood samples was performed with the aid of equipment in the clinical pathology section of Murdoch University Veterinary Hospital. Staff of the Zoology Department of the University of Western Australia, especially Dr Jamie O'Shea gave invaluable advice in the use of techniques to demonstrate the vasculature of the dhufish head. Associate Professor Jennet Harvey viewed histology slides and gave her opinion of the liver lesions in juvenile dhufish. Mr David Lines and Mr Michael Platten at the Pathcentre performed immunohistochemical analysis of samples. Ms Nicky Buller, Animal Health Laboratory, Agriculture WA provided assistance with microbiological work-ups and pathogen identification. Mr Ian Arthur at the Mycology section of the Pathcentre at Queen Elizabeth II Medical Centre identified the black yeast infesting juvenile dhufish and Dr Delane Kritsky described the most significant monogenean parasite of dhufish. Mr Russ Hobbs, Mr Peter Fallon and Mr Ken Chong provided assistance in experimental techniques throughout the project.

Successful completion of the project would not have been possible without the interest and support of staff at the Aquaculture Development Unit of Challenger TAFE, in particular Mr Greg Jenkins, Mr Ken Frankish, Dr Jennifer Cleary, Mr Anthony Aris and Ms Tahryn Mackrill.