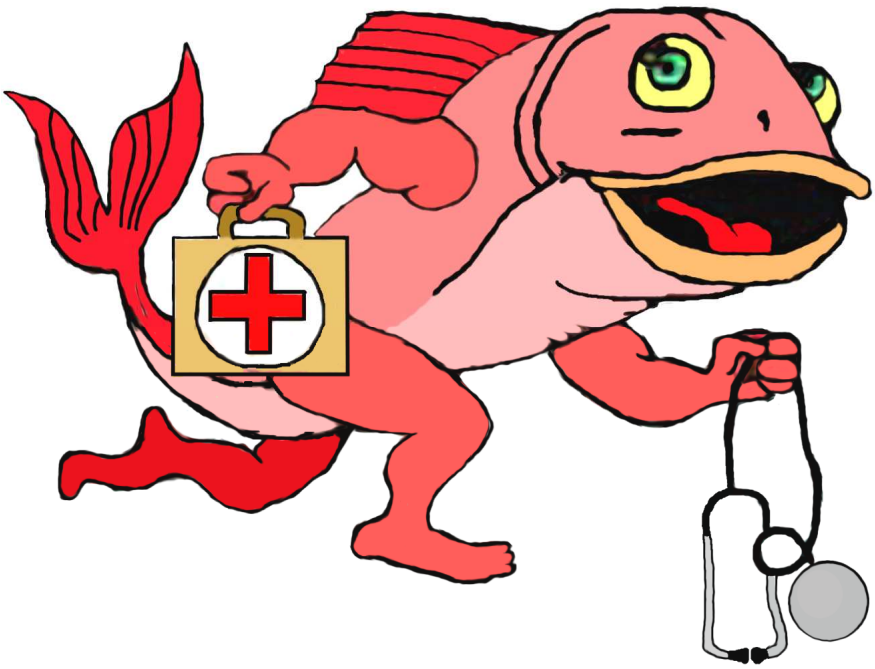
FISH VETTING ESSENTIALS



Dr Richmond Loh
&
Dr Matt Landos

ISBN

Published by:
Richmond Loh Publishing
Perth, Western Australia, Australia.

For orders
Phone: +61 (0)421 822 383
Email: thefishvet@gmail.com
Web: http://www.thefishvet.com.au

Preferred way to cite this publication:
Loh, R. and Landos M. (2011) ***Fish Vetting Essentials.*** Richmond Loh Publishing, Perth.

Publication designed and typeset by Richmond Loh.
Proofread by John Loh and Agatha Foo.

Front cover: The Fish Vet's logo. Part fish, part vet.

FOREWORD

This is a revised version of the self-published "Australian Fish Vetting Essentials" (2007) by Drs Richmond Loh & Matt Landos. The purpose of this manual is to collate the knowledge that aquarists, aquaculturalists, public aquaria, local fish shops and veterinarians already have, and to filter out misinformation and then provide this information in a readily digestible form. The information contained in this publication has been in the process of compilation since 2001. This manual is not prescriptive, but rather, it is a collection from our combined knowledge to promote to the industry that veterinarians are best equipped to deal with aquatic animal health.

Worthy of note is that many diseases found in aquatics can be classified as emerging diseases since an "emerging disease" is one that has appeared in a population for the first time, or that may have existed previously but is rapidly increasing in incidence of geographic range.

The Authors

The Fish Vet
Dr Richmond Loh
BSc BVMS MPhil MACVSc DipPM CMAVA
+61 (0)421 822 383
thefishvet@gmail.com
http://www.thefishvet.com.au

Future Fisheries Veterinary Consulting
Dr Matt Landos
BVSc HonsI, MACVSc
+61 (0)437 492 863
matty.landos@gmail.com

Contributors

Dr Michael Chia

Dr Stephen Pyecroft

Dr Judith Handlinger

Dr Shane Raidal

Dr Fran Stephens

ABOUT THE AUTHORS

Dr Richmond Loh

Dr Loh has always been interested in animals, nature and medicine, so naturally, he studied to become a veterinarian at Murdoch University. However, his passion for all things fish was strong and so his first job was as a veterinary fish pathologist for the Tasmanian state laboratory, providing diagnostic services for the large aquaculture farms including species such as salmon, trout, ornamental fishes, abalones and oysters. At the same time, he was offering veterinary services to owners of ornamental fishes.

In 2006, he passed the examinations for Aquatic Animal Health for the Australian College of Veterinary Scientists (at this stage the highest level of specialised examinations for fish anywhere in the world). In the same year, he was awarded a Master of Philosophy degree for cancer research in Tasmanian devils. To increase his depth of knowledge in the area of diseases, he studied for and passed the examinations for Pathobiology for the Australian College of Veterinary Scientists in 2009.

So far, he has given numerous talks at seven National Veterinary Conferences and also to the Pet Industry Australia Association delegates and at the New Zealand Companion Animal Conference. He regularly writes for aquarium and pet publications. These are an initiative to generate interest within the respective professions and industry to apply scientific reasoning for the better health and management of fishes. Through his veterinary career, he has appeared on TV (Creature Features, Stateline, Catalyst, ABC news), been interviewed on radio (Curtin FM), appeared in newspapers (The Sunday Times UK, Herald Sun, The Examiner, Sunday Tasmanian, The Cairns Post, Canning Times), magazines (Australian Aquarium Magazine, Aquarium Keeper Australia, TIME Australia Magazine, Your Pet Magazine, Woman's Day, Pets – Taking Care of Your Family's Best Friend, Animals' Voice) and appears on several local and international websites (ABC Online).

He is the consultant veterinarian to AQWA (the Aquarium of WA), is an adjunct lecturer at Murdoch University and provides advice on animal welfare as it pertains to fishes to several universities and the RSPCA. His clients are diverse and range from individual pet fish owners, to retailers, farmers and exporters, locally, interstate and in Singapore.

Dr Matt Landos

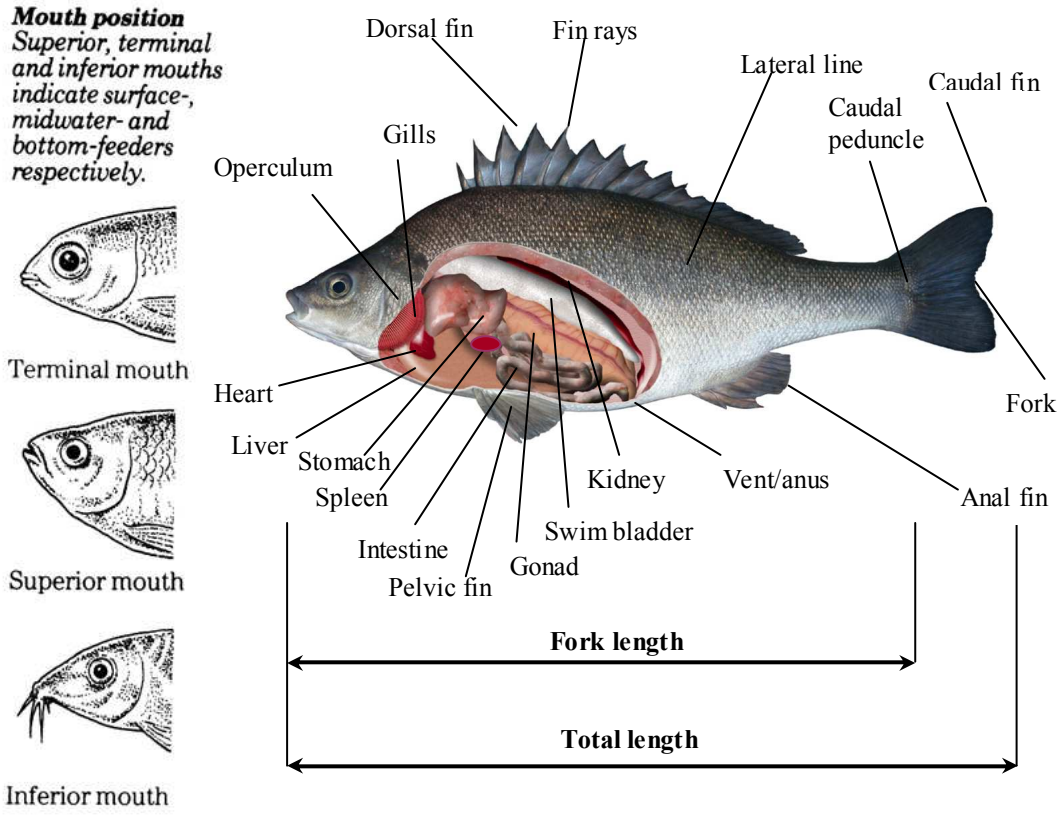
Dr Landos is the Founding Director of Future Fisheries Veterinary Service, is an honorary lecturer in aquatic animal health and associate researcher at the University of Sydney, Faculty of Veterinary Science and was the 2011 president of the Aquatic Animal Health Chapter of the Australian College of Veterinary Scientists.

Dr Landos commenced his consultancy practice in aquatic animals in 2005 after a 5 year stint with the NSW DPI as the Veterinary Officer in Aquatic Animal Health. The client base is located throughout Australia, and it ranges from small native fish hatcheries to 3,000 tonne sea cage operations. He works with all aquatic species including molluscs, crustacea and finfish. He reviews emergency disease preparedness plans and develops health management plans for aquaculture industries. He has had a prominent media profile in recent years associated with investigation of the impacts of environmental pollutants on fisheries in relation to the notorious two-headed Australian bass larvae case from the Noosa River.

CONTENTS

ANATOMY AND FUNCTION

Below is a diagram of various important anatomical features of a fish, using a silver perch as the model.



Fishes vary in size (from a few millimetres to a few meters), shape, dietary preferences, water quality parameters and more. To appreciate these differences, we will be taking a dive into the watery wonders of fish folk. This section aims to explore just how much you can tell about a fish by merely looking at it.

Skin & scales

The mucus, skin and scales serve to protect fish from its external environment, functions as a sensory organ (tactile and lateral line) and have excretory, respiratory, osmoregulatory and immune functions.

The most superficial layer of the skin comprises of a mucopolysaccharide layer that contains immunoglobulins, lysozyme and free fatty acids; and together they have anti-pathogenic activities. Excessive use of astringent medications (e.g. copper sulphate, formalin, benzalkonium chloride) or excessively acidic conditions will damage the protective layer, predisposing fish to secondary infections (e.g. epizootic ulcerative syndrome). The epidermis comprises of (non-keratinised) squamous epithelial cells (still capable of replication at all levels), mucus cells, fright cells, melanocytes and various leukocytes (macrophages, lymphocytes, eosinophilic granular cells).[2] The dermis is divided into the stratum spongiosum (composed of loose collage and

CATEGORIES OF FISH

This section does not intend to classify fish into their absolute phylogenetic tree, but to familiarise the reader with the differences and commonalities between the vast variety of species available to the aquarist.[3]

Cyprinids

Origin: Europe, Asia, Africa, and North America.
Aquarium system: tropical freshwater.
Characteristics: no teeth in their jaws (possess pharyngeal teeth); egg scatterer; forked tail.
General types: Barb (Tiger, Rosy, and Golden); Danio (Pearl, Zebra, and Giant); Rasbora.

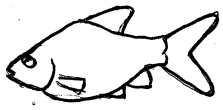
Goldfish

No barbels.
Body more rotund than common carp.
Various body morphs and colours been selected – twin tailed, telescope, celestials, oranda, pearl scale, shubunkin, etc.
Males develop white, 1mm nodules on their opercula and leading edge of their fins during breeding season (spring time - ~ Aug/Sept).
Common disease issues: swimbladder disorder (fancy varieties), mycobacteriosis, flukes.

Carp

E.g. koi carp/Japanese carp.
Have barbels;
Multi-coloured, elongated bodies.
Males are more slender than females and develop "rough" opercula and flanks during breeding season (spring time - ~ Aug/Sept).
Common disease issues: flukes, bacterial septicaemia, fish lice.

Barb



Most have barbels;
Mid to bottom dwellers;
Prefer still waters;
Tendency to nip the fins of tank mates.

Danio

Prefer fast, well-oxygenated cooler waters;
Surface dwellers and feed from the surface;
Thrive in schools of 5 or more fish.

Rasbora

Top to mid-water.
Thrive in schools of 7 or more fish.

NITROGEN CYCLE & BIO-FILTERS

The conversion of ammonia (the major excretory product of fish) to nitrite (by *Nitrosomonas* and *Nitrosococcus*) and from nitrite to nitrate (by *Nitrobacter, Nitrococcus* and *Nitrospina*) is termed the "Nitrogen Cycle" and follows these equations (after ammonia is ionised):

$NH_3 + H_2O \Leftrightarrow NH_4^+ + OH^-$

$2NH_4^+ + 3O_2 + 4HCO_3^- \Rightarrow 2NO_2^- + 4H_2CO_3 + 2H_2O$

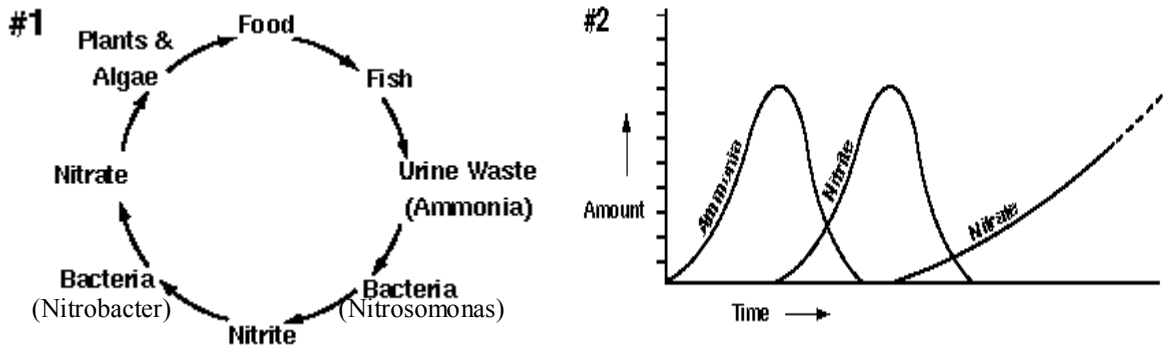
$2NO_2^- + 2O_2 \Rightarrow 2NO_3^- + 2H_2O$

This is an oxygen-dependent process and will cease if dissolved oxygen (DO) levels fall below 2mg/L. The biological filter operates most efficiently at 28-36^{o}C where every 4^{o}C increase in the temperature will make the filter 50% more efficient. The temperature tolerance range for biofilters is 12 - 58^{o}C.[4]

Nitrifying bacteria grow slowly and may take between 2-6 weeks in freshwater to develop sufficient numbers to adequately filter the water. This time is called the "conditioning period". An established, well-balanced aquarium should have no ammonia or nitrite.

In the natural environment, the end product (nitrate) will be incorporated into plants/algae. However, in the aquarium, this will accumulate in fish tanks unless it is removed by partial water changes or by using a denitrification unit.

Figure. The Nitrogen cycle.



This "Nitrogen Cycle" takes significantly longer to establish in the marine aquaria. In fact, the time it takes may be up to double. A way to speed up the process is to set up the tank as a freshwater system (without fish or plants) and seed with ammonium chloride. Once the filter is conditioned, convert this set-up to marine by increasing the salinity by 10g/L/day until the desired SG is reached.

Some people condition the filter using fish and others use meat to supply the system with nitrogen. However, this may have unfavourable consequences if the fish carry

pathogens or the decaying meat breeds other non-desirables such as fungi and other bacteria. In a large freshwater set-up, a biological filter may be "seeded" by adding 46mg/L of NH_4Cl and 73mg/L of $NaNO_2$ for 20 days, maintaining the water temperature at 20-28^oC.[4] Test the ammonia and nitrite levels prior to adding fish. Once the aquarium is set-up, do not increase the biomass by more than 3-4% at any one time. If need be (e.g. in a fish shop situation where you have a large order in), supplementary chemical filtration (e.g. zeolite) may be necessary.

NB: Bio-filters will also consume Ca^{+2}, Mg^{+2}, HCO_3^- and CO_3^{-2} (the latter two contribute to decrease in pH) and so partial water changes to replace the "lost" ions or buffering may be necessary from time to time.

Bio-filter requirements vary depending on species, water temperature, pH, fish size, stocking density, oxygen consumption, ammonia production, substrate type and surface area to volume ratio, filter efficiency, clogging by solids, water flow rate and feed amount, frequency and type. As a rule of thumb, the bio-filter volume should be 10-20% of tank volume.

If the bio-filter requirements need to be calculated. The following equations will be helpful[4]:

Daily feed (dry weight) may be calculated by the following equation.

$$\text{Daily feed} = (\text{body weight}) \times 0.02$$

Ammonia production rate (APR) may be calculated by the following equation.

$$\text{APR} = (\text{daily feed}) \times (\text{total fish biomass}) \div 100 \times 0.025\text{kg}$$

Bio-filter surface area requirement (BSA).

$$\text{BSA} = \text{APR} \div (\text{nitrification rate})$$

Volume of substrate required (SV).

$$\text{SV} = \text{BSA} \div (\text{specific surface area/unit volume}) \times 1.2$$

If ammonia and/or nitrite are allowed to build up considerably, fish deaths result in a condition called, "new tank syndrome" – see under Water Quality – Ammonia and Nitrite.

Some chemotherapeutants may affect the biofilter. These are tabulated below.

WATER QUALITY PARAMETERS FOR POPULAR GROUPS OF AQUATIC ANIMALS

Freshwater

Tropical community

Ammonia	<0.2mg/L
Nitrite	<0.2mg/L
Nitrate	<50mg/L (<110mg/L tolerance)
pH	6.5 - 7.5 (oviparous fish towards acidic end, viviparous fish towards basic pH)
GH	100 - 200mg/L
Specific gravity	1.000
Temperature	24 - 28^{o}C

Amazonian (soft, acid)

Ammonia	<0.2mg/L
Nitrite	<0.2mg/L
Nitrate	<50mg/L (<110mg/L tolerance)
pH	6.8
GH	80 - 140mg/L
KH	70 - 100mg/L
Temperature	25^{o}C

Special considerations:

- Some fish may need cover (planting, caves, and bogwood).

Africans (hard, alkaline)

Ammonia	<0.1mg/L
Nitrite	<0.2mg/L
Nitrate	<50mg/L (<110mg/L tolerance)
pH	8.0
GH	300mg/L
Temperature	25 - 29^{o}C

Special considerations:

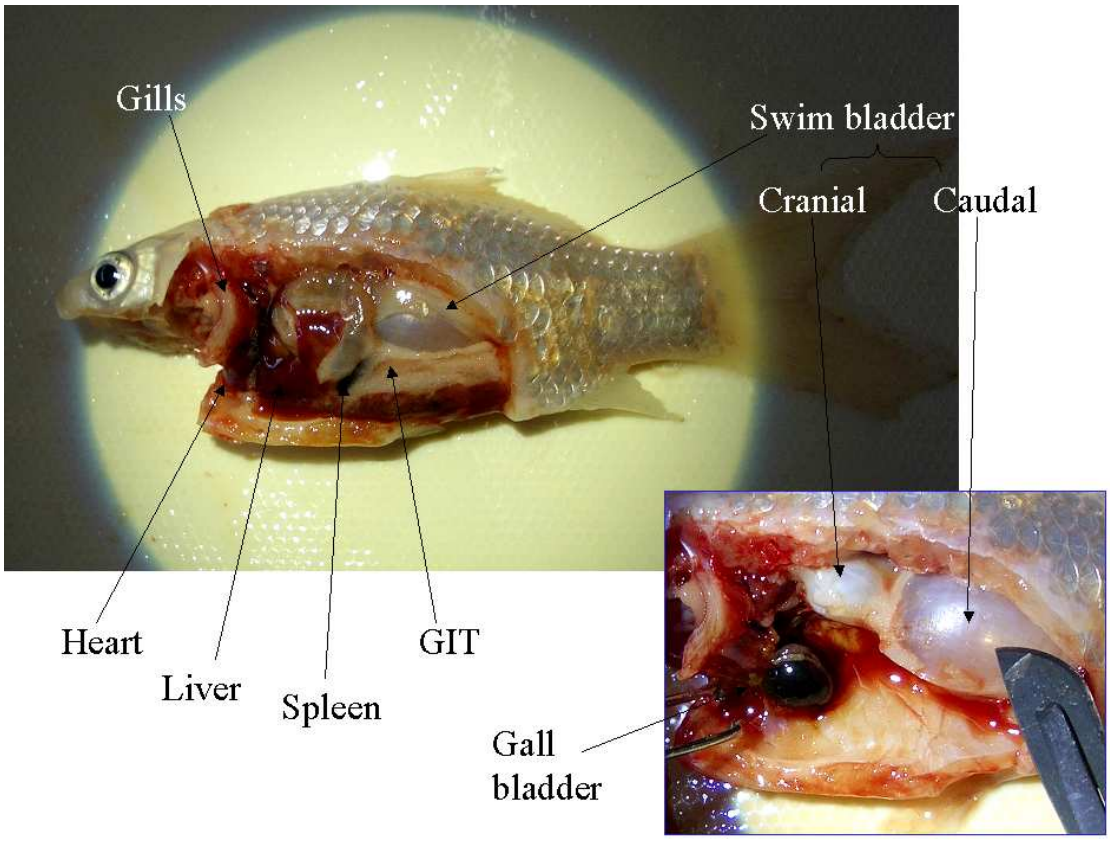
- Allow 10-12x cichlid lengths in diameter - A large majority of these cichlids are highly territorial (especially towards fish with similar appearance).

Salmonids (Salmon & Trout) – Freshwater phase[13]

Ammonia	<0.0125mg/L
Nitrite	<0.02mg/L
pH	5.5 - 8.5
Nitrogen	<105%
Dissolved oxygen	>6mg/L
Carbon dioxide	<10mg/L
Temperature	12 - 15^{o}C for Atlantic salmon 14 - 18^{o}C for rainbow trout (tolerance range: -1 - 25 ^{o}C)
Suspended solids	<80mg/L
Iron (total)	<1mg/L

Internal gross pathology
Look for
-haemorrhage (petechiae-ecchymoses);
-ascites (exudate/transudate; clear-bloody);
-nodules/granulomas (especially in kidney, spleen and liver);
-parasites (nematodes, trematodes);
-presence of feed in gut;
-size of organs (spleen and kidney enlarge in systemic infections).

Sites for microbiology swabs: kidney, spleen, liver, heart, brain. Sterile technique must be used to access these organs to avoid contamination of the swabs.



Histology
On many occasions it is necessary to utilise more powerful diagnostic techniques. Post mortem on typically affected, freshly euthanased fish (fish that have been dead for even a few hours are often of no diagnostic value) will allow sample preservation for histopathology (using 10% formalin buffered formalin) and microbiology (transport swabs or direct plate inoculation). Wear gloves to minimise zoonotic potential of some fish pathogens (e.g. Atypical mycobacteria).

Tissues to collect for histopathology: anterior kidney, posterior kidney, swim bladder, pancreas, liver, spleen, heart, gut, brain, gill (keep entire on arch), skin (include any skin lesions with margin of "normal" tissue; take care to avoid scale detachment). It is important to use sufficient volume of formalin (1:10 tissue to formalin) to adequately preserve specimens for the lab.

Fixatives

10% Neutral Buffered Formalin (fish, freshwater invertebrates)

- Formalin (40% w/v formaldehyde) 100mL
- Sodium phosphate, monobasic monohydrate 4g
- Sodium phosphate, dibasic, anhydrous 6.5g
- Distilled water to 1L

This solution is stable for many months at room temperature. Fix small blocks of tissue (10x10x3mm) for up to 24 hours.

Seawater Formalin (marine shellfish and crustaceans)

As for 10% NBF, but fill with seawater to 1L.

Davidson's Fixative (marine shellfish and crustaceans)

- 600ml Seawater
- 600ml 95% ethanol
- 400ml 37% formaldehyde
- Add 200ml glacial acetic acid prior to use.

Virology

Preservation of tissues for viral isolation: best to send tissues fresh on ice on express courier to laboratory. Your local laboratory may be able to help with making transport media (containing antibiotics and anti-fungals). Matching samples of formalin, glutaraldehyde and 70% ethanol fixed tissues should be sent separately, to allow processing for histology, electron microscopy and molecular assays.

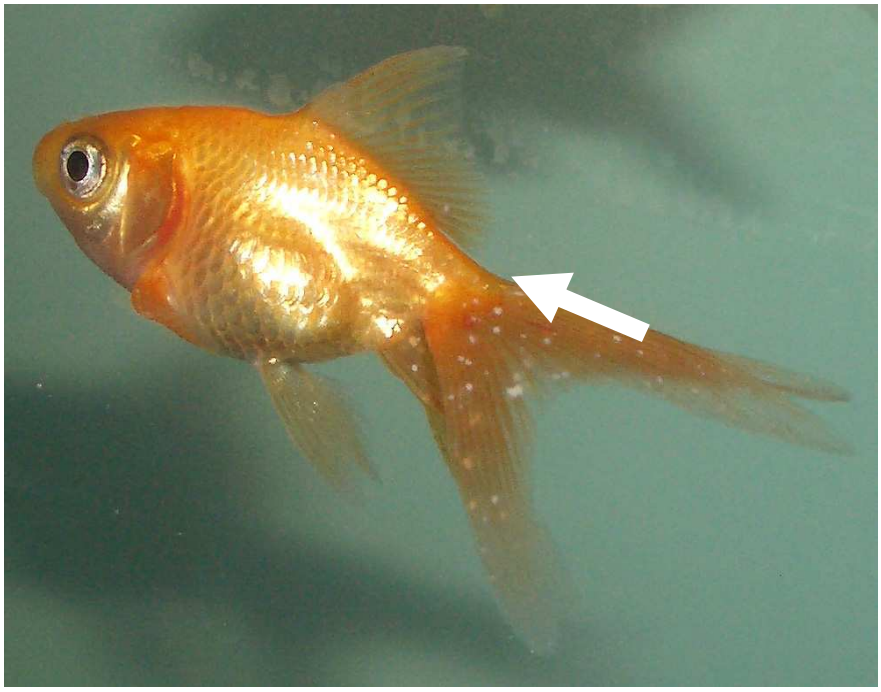
Molecular Biology

Material for PCR testing need to be preserved in 95% ethanol. There is also a product called "RNA Later" that can preserve genetic material, including RNA material, for long periods. It is important not to cross-contaminate samples. Remember that PCR tests only provides you with evidence for the presence or absence of that organism's DNA. A positive result does not necessarily mean there is disease per se. And a negative result could mean that you may have not selected your primers properly. Results should be interpreted in light of clinical signs and histopathology findings.

White Spot Disease ('Ich')
– *Ichthyophthirius multifilis* (freshwater) & *Cryptocaryon irritans* (marine).

'Ich' is a very common protozoan disease of both marine and freshwater fish. The organism is a small, round, ciliate protozoan with a distinctive horse-shoe shaped nucleus that lives on the skin and gills of the fish where it produces lesions that take on the form of tiny (~1mm) white cystic nodules.

Clinical Signs
The parasitic cysts are so large that they are visible to the naked eye – affected fish will look as though they have been sprinkled with sugar and will often demonstrate irritation; scratching and rubbing against the rocks and tank. However, this may not always be present if only the "swarmer" stages are present. Thickening of the skin epithelium and increased mucus production on the skin may occur, but these may only be appreciated microscopically. The fins may also become ragged.



Since the gills are also exposed to the external aqueous environment, the organism will also parasitise the gills, causing increased mucus production as well as gill epithelial hyperplasia. This leads to respiratory embarrassment and fish will present with increased respiratory rate and flared opercula. In heavy infestations, fish will become lethargic and depressed, its respiratory rate slows and becomes shallow. Ulcers form when the encysted stages break out of the skin and the gills. In heavy infections, mortality can be high which may be in part due to electrolyte loss (in freshwater fishes) or dehydration (marine fishes) through these ulcers. In those that survive the initial insult, the ulcers would provide portals of entry for secondary infection with bacterial and fungal opportunists.

Transmission
'Ich' is a very common infection of fish in both freshwater and marine systems and that, in low numbers, it may produce little or no clinical signs. It is important therefore to always look for other pathogens (bacteria, fungi, other protozoa etc.)

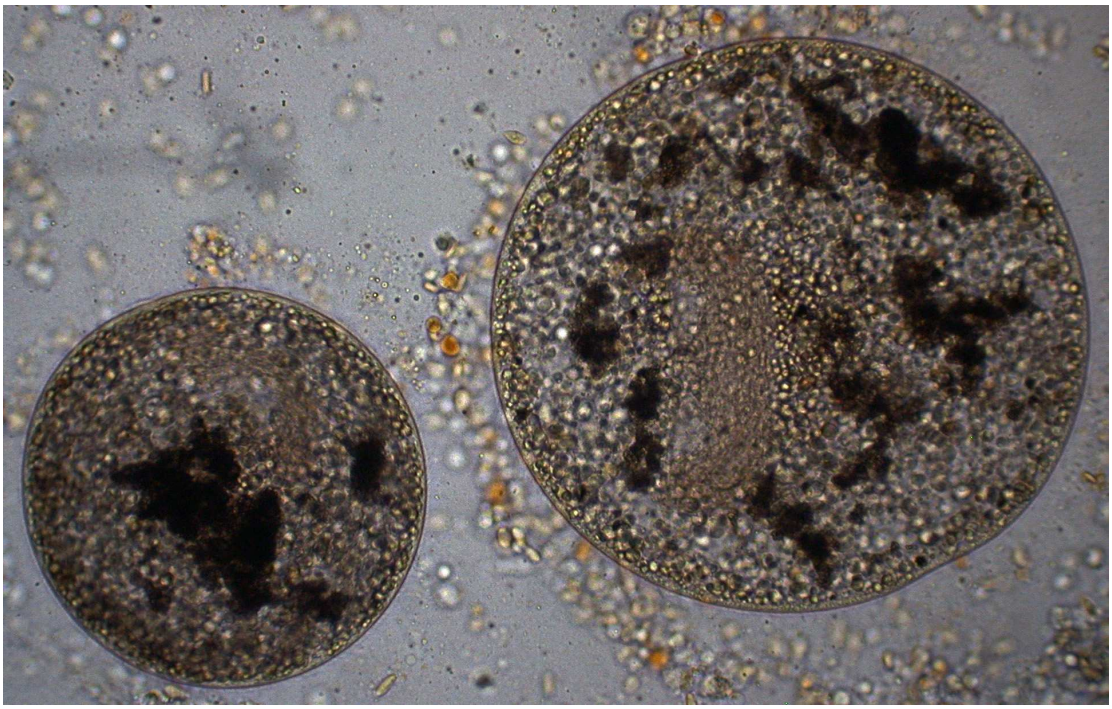
when presented with a sick fish with only a small burden of Ich, as this may be an incidental problem.

'Ich' replicates quickly and so a minor burden in one fish tank can rapidly become a serious burden to a tank full of fishes if left untreated. Mature Ich parasites that have been feeding on the host eventually fall off the host to the bottom of the tank. There they secrete a protective cyst around themselves and begin to divide, producing many hundreds of infective stages (swarmers) which swim off in search of a host.

The transmission of 'ich' is facilitated by high stocking densities of fish. Individual encysted stages on fish may go unnoticed by fish-owners and yet be a major source of infection for other fish. Temperature also plays a big role in disease transmission with the lifecycle being completed more rapidly at higher temperatures (3-4 days at 21^{o}C vs 5 weeks at 10^{o}C). This factor is particularly important when dealing with Cryptocaryon outbreaks as it is more temperature-governed than Ichthyophthirius.

Diagnosis

Diagnosis is made by performing a skin or gill scraping in the region of one of the white lesions and by identification of the spherical ciliate organisms, with their characteristic slow spinning motion, in a fresh wet preparation. Notice also that the parasites can be of various sizes, which is pathognomonic for "Ich" (other ciliated parasites are of uniform size and shape).



Treatment

Formalin may be used, however, because it displaces dissolved oxygen, it is not recommended if fish exhibit severe respiratory embarrassment. Malachite green + formalin combination is the most effective treatment since the mixture has a "synergistic effect" and a smaller concentration of each ingredient is used. Dip treatments and osmotic challenges will only be effective against the non-encysted stages of the parasite. Thus, this will need to be repeated every 2-3 days for 10 days.

Thermal challenge by raising the water temperature to at least 32^{o}C for a few hours every 3 to 5 days (provided the water is well-aerated and that the fishes will tolerate it) is another method used. The high temperature interferes with the reproduction of the parasite. Since the organism is an obligate parasite, allowing the aquarium or pond to be left fish-free for at least 7 days at >20^{o}C usually eliminates the white spot parasite.

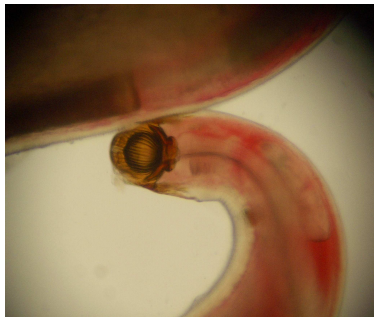
It has been reported that some fishes that recover from the infection will develop immunity to the disease.

See the medication section under "*Protozoa – General"*.

<><

Nematodes - Camallanus

These are large roundworms (pictured below) that infects the gastrointestinal tract of fish, most commonly seen in livebearing fish, cichlids and other freshwater species. *Camallanus* species have indirect life cycles and are ovoviviparous - the female worms incubate the eggs which hatch into larvae within their bodies and the egg sacs containing these larvae (pictured below right) are excreted into the water with the fish's faeces. The larvae are ingested by a crustacean intermediate host in order to develop into L3s. When fish eat the infected crustacean, the larval worms mature in the gastrointestinal tract of the fish.


Clinical Signs
The worms can cause intestinal obstruction, weight loss and eventual death of the fish. The first evidence of infection is a red, worm animal protruding from the anus of the fish. They do so in order to lay their eggs (which contain larvae) into the environment.

Diagnosis
Camallanus species can be identified by their blood red colour; their location further toward the posterior of the intestinal tract and often protruding from the anus of the fish (hence "-*anus*"); the presence of a buccal capsule (mouth structure) that is divided into two lateral valves, giving the mouth a slit like appearance (pictured above middle - hence "*Camel*"); and, if gravid females are present, the presence of both eggs and larvae within their bodies.

Treatment
Because *Camallanus* nematodes are located within the intestinal tract, common dewormers should be effective (e.g. levamisole).

Prevention
As a copepod or other crustacean host is required to complete the life cycle, avoiding the use of copepods or related crustaceans as food items will help reduce potential for infection. Evaluation of brood stock for the presence of parasites prior to use will also aid in identifying carriers.

Routes of administration

Topical

This usually involves the application of a paste/gel onto an external lesion (e.g. applying Clonea over a fungal skin infection).

Parenteral

Intramuscular (IM)

Aim for the muscle block just ventral and caudal to the dorsal fin. Needle should be positioned between the scales and aimed to come in from a caudo-rostral angle. The most commonly used method for antibiotic injection. The medication is rapidly dispersed by the rich blood supply. Inject slowly to prevent reflux. This technique is recommended for gravid females and not recommended for small fish and fish with little muscle bulk. Additionally, the disadvantage of this method is that the injection site may discolour (aim for dark areas on the fish).

Intraperitoneal (IP)

Place the fish on its side or on its back to allow the peritoneal organs to gravitate away from the body wall. Insert needle into this space at a shallow angle to avoid penetrating the internal organs. This is a common route of administrating vaccines as it is done on small fish and will not harm the flesh. It is also good for administrating "painful" medications such as enrofloxacin. The disadvantage of this method is damaging peritoneal organs or injecting into the ovary where the drug will not redistribute to the rest of the body.

Intravenous (IV)

Fish must be anaesthetised. Insert needle midline and at the angle shown, just caudal to the ventral fin. Stop just short of the spine. This technique is used more commonly for drawing blood than for administering drugs.

Per os

This is a common delivery method for antibiotics on large farms for ease of medicating large numbers of fish and also minimises handling stress. However, the delivery of the correct doses to each individual fish is difficult (sick fish are usually inappetent and it is these that should actually be receiving medication). Medication is either sprayed onto the food, impregnated into the food, or prepared with gelatine with food. Depending on the drug, this medicated food may sometimes be less palatable. It is thus recommended to reduce the amount of food fed per day by 25-50%.

Skin injury protocol

1. Anaesthetise fish (*Aqui-S*, *Alfaxan*).
2. Debride/excise (only done once since repeat treatments may delay epithelialisation).
3. Make a wet prep & an impression smear (diff quick stain) to assess presence of infection.
4. Topical iodine (use *Betadine* diluted 1:10 or *F-10* diluted 1:100).
5. Topical *Panalog* cream ointment for bacterial infection; or Clonea cream for fungal infection (leave on skin for 30-60 seconds to allow local absorption).
6. Seal wound with dental gel (*Orabase*).
7. Anti-inflammatory (*Metacam*) – 1 drop PO.
8. Injectable antibiotic intramuscular if indicated (*enrofloxacin*).
9. Increase water temperature and aeration.
10. 3-5mg/L *NaCl* (reduce osmotic stress) and some *Vitamin C* (promote wound healing).
11. Immunostimulants may be beneficial.
12. In bath medication for 7-14 days or very clean water.
13. *Determine the primary cause of the lesions and act accordingly.
    - Check water parameters.
    - Anything causing mechanical damage?
    - Attacked by predator, other fish?
    - Rough surfaces?
    - Rough handling?
    - Any other stressors?
    - Over crowding?
    - High organic loads?

Fungal infection

Potassium permanganate

Bath: 10mg/L as a 30 minute.

PI: ~2mg/L (to maintain pink colouration for >4 hours).

Malachite green

*May be toxic to tetras, catfish, loaches & other scaleless fish.

*Not to be used on food fish.

Topical: 20mg/L (keep away from eyes & gills).

Bath: 1-2mg/L for 30-60 minutes.

Repeat alternate days for a maximum of 4 treatments.

Povidone iodine

Topical: Debride wound and topical application diluted 1:10.

F-10

(54g/L benzalkonium chloride, 4g/L polyhexamethylene biguanide hydrochloride)

Topical: Debride wound and topical application diluted 1:100 daily until lesion resolves.

EUTHANASIA

Fish

1. Overdose with fish anaesthetic (Aqui-S or Alfaxan) or if these are inaccessible, clove-oil (eugenol) or benzocaine may be used.
2. If anaesthetic is not available, stun fish by percussion.
3. Then sever the spine, followed by pithing of the brain.

*NB: Step 3 may cause involuntary muscle twitching. Let client know this may occur and that the fish is unconscious and feels no pain.

Freezing fish or leaving fish to "suffocate" out of water are not acceptable methods of euthanasia.

Crustacea

Several methods are currently acceptable:

- Injection with xylazine (70mg/kg) or pentobarbitone (250mg/kg) before placing in boiling water (Oswald 1977).

- Clove oil 0.125ml/L for one hour (Gardner 1997).

- Cooled and then deep frozen.

- Salt/ice slurry for 20 minutes before sectioning to destroy ganglia or place in boiling water.

- For terrestrial invertebrates, dry ice (CO_2) or gaseous isofluorane may be introduced into a chamber holding the animal.

ANZCCART Guidelines (under revision)
http://www.adelaide.edu.au/ANZCCART/publications/Euthanasia.pdf

Cooper JE, 1990. Euthanasia of invertebrates. National Federation of Zoological gardens of Great Britain and Ireland, London.
Gardner C, 1997. Options for humane immobilizing and killing crabs. J Shellfish Res 16:219-224.
Oswald RL, 1977. Immobilization of decapod crustacean for experimental procedures. J Mar Biol Assoc, UK 57:715-721.

INDEX

REFERENCES

1. Stoskopf, M.K., *Fish Medicine*. 1993: Saunders Company.
2. Brown, L., *Aquaculture for Veterinarians: fish husbandry and medicine*. 1993, Oxford: Pergamon Press.
3. Mills, D., *You & Your Aquarium. The complete guide to collecting and keeping your aquarium fishes*. 1986: Colour Library Books.
4. Powell, M.D., *Technology for Aquaculture, Lecture Notes, KQA 214*. 2005: University of Tasmania.
5. Claude, E.B., *Water Quality in Ponds for Aquaculture*. 1996, Alabama: Auburn University.
6. Aquarium Industries, *Technical Notes for the keeping of freshwater fishes*. 2003.
7. Kelly, L.A., *Water quality and rainbow trout farming*. Fish Veterinary Journal., 1998. **May**(2): p. 31-47.
8. *Fish Diseases*. in *Refresher Course for Veterinarians*. 1988. Stephen Roberts Lecture Theatre University of Sydney: PGF Veterinary Science.
9. Annon., *Better Management Practices (BMP) Manual for Black Tiger Shrimp (Penaeus monodon) Hatcheries in Vietnam*. 2005, NACA.
10. Reddacliff, G.L., *Diseases of Aquarium Fishes. A practical guide for the Australian Veterinarian*. Vol. 25. 1985: University of Sydney.
11. Gadd, C., *CO2/KH/pH table*.
12. Lewbart, G.A., *Self-Assessment Colour Review of Ornamental Fish*. 1998, London: Manson Publishing.
13. Laird, L. and T. Needham, *Salmon and Trout Farming*. 1988, Chichester: Ellis Horwood Ltd.
14. Burke, C.M., et al., *Environmental requirements of abalone*. 2001, Launceston: University of Tasmania.
15. Lewbart, G.A., *Invertebrate Medicine*. 2006, Oxford: Blackwell Publishing.
16. PGF Veterinary Science, *Invertebrates in Aquaculture*. Vol. Proc No 117. 1989.
17. Chong, R.
18. Treves-Brown, K.M., *Applied Fish Pharmacology*. 2000, London: Kluwer Academic Publishers. 324.
19. Mylniczenko, N.D., et al., *Differences in hematocrit of blood samples obtained from two venipuncture sites in sharks*. American Journal of Veterinary Research, 2006. **67**(11): p. 1861-1864.
20. Botanic Gardens Trust, *Australian Freshwater Algae*, Department of Environment and Conservation (NSW).
21. Gratzek, J.B. and J.R. Matthews, eds. *Aquariology: The Science of Fish Health Management*. 1992, Tetra Press: Hong Kong.
22. Pyecroft, S.B.
23. Lewbart, G.A., *Koi Medicine & Management*. Antibicrobial Therapy in Exotics. Supplement to Compendium on Continuing Education for the Practicing Veterinarian., 1998. **20**(3(A)): p. 5-12.
24. Bayer, *Off label information sheet - Masoten for veterinary use*. 2005.
25. Bayer, *Antimicrobial Therapy in Exotics. Supplement to Compendium on Continuing Education for the Practicing Veterinarian*. 1998. **20**(3A).
26. Johnson, E.L., *Surgery in Koi*. Koi Health and Disease, 1997: p. 102-108.
27. PGF Veterinary Science, *Fin Fish Health*. Vol. Proceedings No. 347. 2002.
28. Ross, L.G. and B. Ross, *Anaesthetic & Sedative Techniques for Aquatic Animals*. Vol. 2nd Edition. 1999: Blackwell Science.