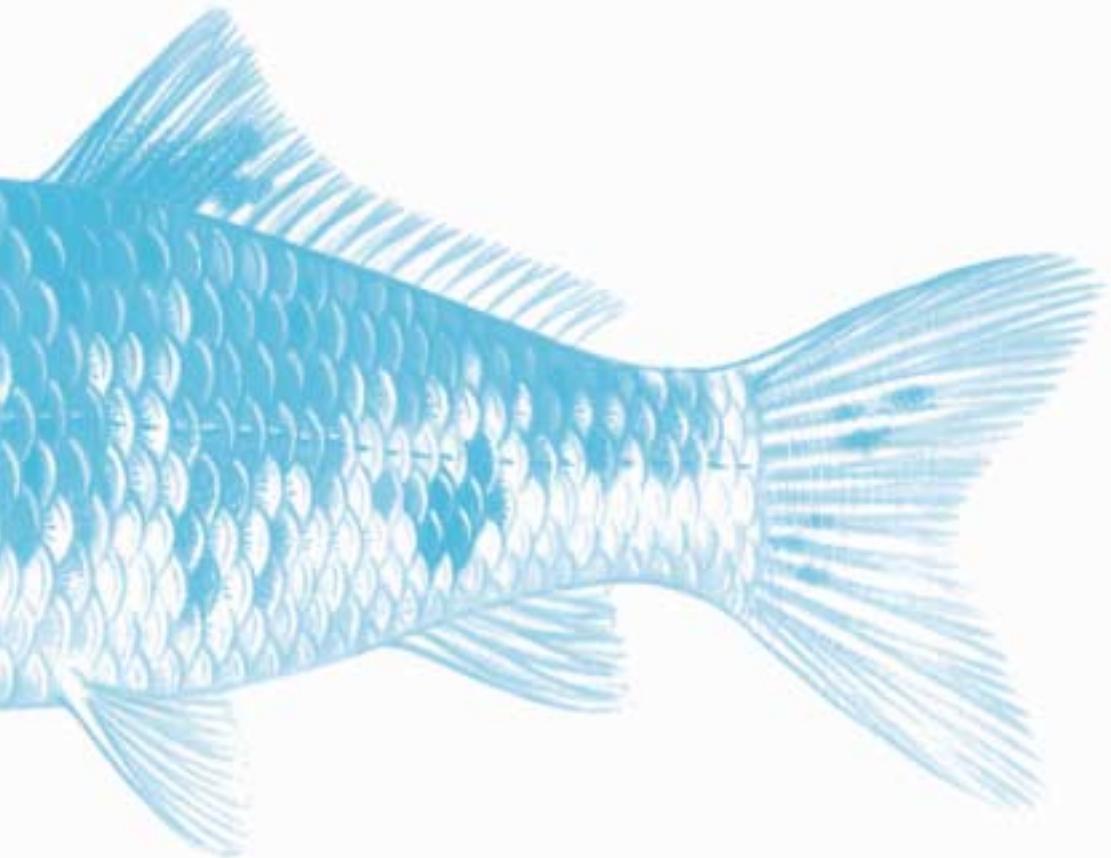


***FISH
VETERINARY
JOURNAL***

The Journal of the Fish Veterinary Society

Issue Number 2 • May 1998



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The FISH VETERINARY SOCIETY was formed in July 1990, with the intention of bringing together veterinarians with an interest in fish, so that they may benefit from mutual experiences and discussions, and help to advance the veterinary care and welfare of fish.



The society provides:

- two scientific meetings, held annually
- publication of the *Fish Veterinary Journal*
- publication of policy documents on fish health and welfare
- political lobbying and representation on behalf of the members' interests

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Notes for contributors

The Fish Veterinary Journal invites contributions from members and other professional colleagues and is keen to publish original research, review articles and clinical case histories on all aspects of fish health. Letters, book reviews and other comment on relevant topics are also welcomed.

Scientific articles submitted to, or published in, other refereed journals will not be considered for publication. Papers and short communications submitted for publication are subject to peer review. The editor has the final decision on publication and if accepted, the copyright becomes the property of the Fish Veterinary Society.

Manuscripts and all communications should be sent to W.H. Wildgoose, 655 High Road, Leyton, London E10 6RA. Manuscripts should be submitted in duplicate, typewritten using a Times or Roman font (double line spaced) on one side of A4 paper with wide margins. Scientific articles may also be submitted as an ASCII file on a 3½" diskette (MS-DOS format). The journal cannot accept responsibility for loss or damage of manuscripts.

Format:

Papers should be headed with the full title, which should describe accurately the subject matter. The initials and surnames of the authors, full postal addresses should follow. Each paper should have a self contained summary (maximum of 150 words) which embodies the main conclusions.

Abbreviations should be avoided. Where they must be used, the word or phrase must be given on the first occasion, *eg* infectious pancreatic necrosis (IPN). All units of measurement should be given in the metric system and temperatures in °C. Blood biochemistry values should be expressed in standard SI units. Medicinal products should be referred to by their generic name, followed by proprietary name and manufacturer in brackets when first mentioned, *eg* amoxycillin (Vetremox®, Vetrepharm). The full Latin name for each species should appear at least once when mentioned in the text.

Length of papers:

Papers should be concise. As a guide, the maximum length for scientific articles is 3000 words; for review articles up to 4000 words; for short communications and clinical case reports 1500 words.

Tables and illustrations:

The minimum number of figures necessary to clarify the text should be included and should contain only essential data. Tables must be typewritten on separate sheets and numbered. Illustrations should be drawn in black ink on white paper and should be suitable for direct photographic reproduction.

Legends should be typed on a separate sheet. Photographs should be clear and sharp, and in colour where possible (transparencies should be accompanied by one set of prints.) Photomicrographs must state magnification and stain technique. Each illustration or photograph should bear the author's name and figure number in pencil (or on a label) on the back and an arrow used to identify the top edge. All photographs will be printed in black & white but may be reproduced in colour at the author(s) expense.

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Morrison, C.M., Cornick, J.W., Shum, G. & Zwicker, B. (1984) Histopathology of atypical *Aeromonas salmonicida* infection in Atlantic cod, *Gadus morhua* L. *Journal of Fish Diseases* **7**, 477–494

Roberts, R.J. (1993) Motile aeromonad septicemia. In: *Bacterial Diseases of Fish*. (eds V. Inglis, R.J. Roberts & N.R. Bromage). Blackwell Scientific Publications, Oxford. pp143–155

If three or more authors are quoted, then all must be listed in the references and should be written as 'Morrison and others 1984' in the body of the text.

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Miscellaneous:

A brief personal profile of academic achievements and the current position of the author(s) is also required as a foot-note (maximum of 100 words) for scientific articles.

Offprints may be purchased if ordered and paid for in advance of publication.

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President's Address

Andrew N. Grant

Marine Harvest McConnell, Farms Office, Blar Mhor Industrial Estate,
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This year marks the re-launch of the Fish Veterinary Journal in new livery and style under the capable editorship of Willie Wildgoose. We hope that the Journal will become a respected source of information on fish veterinary matters and would encourage contributions of all sorts from society members.

The November 1997 scientific meeting marked the formation of a new committee and we wish to record our gratitude to the outgoing officers for their hard work. The new committee is detailed on page *iii* and we look forward to building on the foundations of the Society which were laid eight years ago.

Much has changed in those years and the profession as a whole faces many challenges. Livestock production will never be the same post-BSE and this is reflected in the detailed attention paid by major customers to production methods and demand for reliable traceback to the farm of origin. This is no less true of farmed fish than of terrestrial species. Particular attention focuses on welfare, medicines use and residues, and the potential environmental consequences of fish farming. These are all areas where the veterinarian can play a key role in educating the customer and consumer and help to dispel some of the misinformation which exists. The Fish Veterinary Society (FVS) will continue to play its part in this process.

Medicines availability is of great concern for fish veterinarians and the situation is unlikely to improve in the future. This places particular demands on us to conserve such medicines as are available by judicious use according to clinical need. At the same time we have to contend with strict environmental legislation relating to the discharge of medicines from fish farms. The Society will continue to lobby on behalf of the 'minor species' to ensure that effective control of disease is not compromised.

The FVS has been invited to make a submission to the RCVS on the Veterinary Surgeons Act. As things stand, fish are neither included in, nor excluded from the Act and this has significant consequences. The Society's

position has always been that fish should be included in any future revision of the Act. There will be a consultation exercise with members to ensure that the breadth of opinion is sought before a final submission is made.

Pet fish ownership continues to grow and many practitioners find themselves presented with a moribund or more usually dead and decomposed fish with a demand for diagnosis and treatment. This can be difficult to say the least ! There is however help readily available from more experienced members and you will see in this issue of the Journal that we intend to set up an FVS web site with links to other sites containing useful information. We also hope to compile a database of members' interests and expertise to serve as an information source.

Finally the new committee intends to take full advantage of electronic communication to keep members better informed on fish veterinary matters. There is, however, no substitute for old fashioned conversation and we hope that all meetings will be well attended to foster the original aims of the FVS namely, to stimulate the exchange of information.

Editor's Comments

William H. Wildgoose

655 High Road, Leyton, London E10 6RA

I never thought that one day I would be doing this job, but it is strange what directions we take and where we end up in life. What started as a passing interest in parrots in small animal practice led to other exotic pets and finally to ornamental fish. As time went by, I suddenly found myself as scientific editor to a koi health magazine and later giving presentations on pet fish to hobbyists and vets. As a member of the board that established the RCVS Certificate in Fish Health & Production, I felt it was important to prove that this post-graduate qualification was achievable, and successfully passed the exam last year.

At the root of all this has been my fascination with fish diseases and how we can make a significant contribution to their health and welfare. I learned the slow way, much by trial and error, and I am always grateful to colleagues who have been willing to help and have patiently tolerated me over the years. During all my time as a member of the Fish Veterinary Society I have been impressed by the willingness of colleagues to share their experiences and offer advice in this developing field of veterinary medicine.

Producing this Journal has been an interesting challenge and I am very grateful to all the contributors and reviewers who responded promptly to my persistent phonecalls, letters, faxes and e-mails. In particular, the meticulous and speedy efforts by Keith Treves Brown was greatly appreciated. Valuable background help was given by the staff at the RCVS Library and *The Pig Journal*, Margaret Melling and the printer, Tony Tolver. I would like to pay special thanks to my mentor and editor of the *Koi Health Quarterly*, John Redgrove, who has guided me through four years of writing articles and slowly improved my grammar and style. And finally, the financial support from all our sponsors and advertisers was an important element and has helped make the Journal a more professional product.

In this issue I hope to have presented a wide range of topics with something to interest most readers, and a balance between scientific papers, proceedings and topical contributions. I would also like to encourage colleagues to submit papers, short communications, case histories, letters and other relevant articles for publication. It is you, the reader, that makes this Journal what it is; I am just the caretaker.

Management and control of proliferative kidney disease (PKD) in a freshwater Atlantic salmon (*Salmo salar* L.) farm in Ireland: a case history

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Abstract

*During July 1992, an acute clinical outbreak of proliferative kidney disease (PKD) was experienced in two strains ('Irish' and 'Norwegian') of juvenile (age 0+) Atlantic salmon (*Salmo salar* L.) held at two adjacent freshwater sites on the River Lee in southern Ireland. Various management strategies (including reduced stocking densities, handling, feeding rates and increased oxygenation), and treatment regimes (involving malachite green and fumagillin DCH) were used to control the disease. A total of 1.3 million juveniles died during the PKD outbreak, representing 61.6% and 54.6% of the Norwegian stock at the two farms respectively. The Irish stock appeared to be more resistant to the disease and only 15.6% died. The weekly prevalence of PKD fluctuated throughout the summer but seemed to disappear by mid-August.*

Although PKD was detected again during 1993, no clinical outbreak occurred. In conjunction with the management strategies adopted in 1992, seven consecutive weekly prophylactic bath treatments with malachite green (1.6 ppm for 40 minutes) administered prior to mid-July appeared to control the disease. During August 1993, a ten day course of fumagillin (6 mg/kg bodyweight per day) reduced the prevalence of the PKD parasite in a trial batch of juveniles from 24% to zero. The results of this study demonstrated the effectiveness of various management strategies and treatment regimes in controlling PKD.

Introduction

Proliferative kidney disease (PKD) is considered to be one of the most economically important diseases affecting both wild and commercially reared salmonids in freshwater (Hedrick and others 1993). The disease has been recorded in Canada, the U.S.A. and several European countries including Ireland (O'Brien and others 1977, O'Flynn and Mulcahy 1995).

PKD was once considered an untreatable disease. However, at least two chemotherapeutants (malachite green and fumagillin DCH) are now known to be effective in controlling infections (Alderman and Clifton-Hadley 1988, Hedrick and others 1988). Various management and husbandry strategies can also be applied in order to help reduce the risks and severity of the disease.

Under field conditions, repeated bath applications of malachite green gave good control over PKD (Alderman and Clifton-Hadley 1988). However, there are several problems with malachite green: it is toxic to fish, particularly at high temperatures (Alderman 1985); it accumulates in fish tissues (Gerundo and others 1991); and there is growing concern about the effect of malachite green discharges on the environment and on consumer safety (Hedrick and others 1993).

More recently, orally administered fumagillin was shown to be effective in protecting chinook salmon (*Oncorhynchus tshawytscha* Walbaum) and rainbow trout (*Oncorhynchus mykiss* Walbaum) against PKD (Hedrick and others 1988, Wishkovsky and others 1990, Le Gouvello and others 1993a&b). The antibiotic was also demonstrated to be effective against renal sphaerosporosis (*Sphaerospora renicola*) in common carp (*Cyprinus carpio* L.), and *Loma salmonae* in chinook salmon (Kent and Dawe 1994). Dosing, however, must be critically controlled to avoid toxic side-effects (Wishkovsky and others 1990, Lauren and others 1989). Studies on its effectiveness in controlling PKD in Atlantic salmon (*Salmo salar* L.) have not been published.

Materials and methods

During mid-July 1992, an acute outbreak of PKD was experienced in two strains of juvenile Atlantic salmon (age 0+) held at two freshwater farms on the River Lee, Co Cork, in the south of Ireland: Carrigadrohid and Inniscarra (Fig 1). Carrigadrohid is a conventional land-based gravity-fed hatchery and

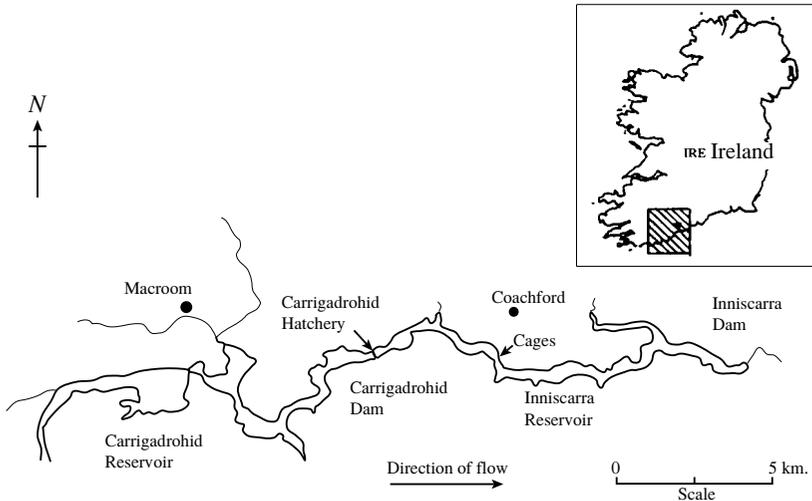


Figure 1: Carrigadrohid and Inniscarra Reservoirs on the River Lee system, showing the positions of the cages and the hatchery.

smolt-rearing farm. Inniscarra is a supplementary cage-based smolt-rearing farm located about 4 km downstream of Carrigadrohid. The largest grade fry are usually transferred from Carrigadrohid to Inniscarra during the summer months for on-rearing to the smolt stage. Both farms have been managed by the same company since 1970 and 1980 respectively. Water quality is typically alkaline (pH 7.19–7.46), soft (17.29–38.23 mg CaCO₃/litre), and eutrophic (0.53–12.82 µg/litre chlorophyll; 11–44 mg/m³ total phosphate). Prior to 1992, PKD had not been recorded at either farm. Two stocks of salmon are reared in both farms: a native Irish grilse stock which has been used for restocking the River Lee since 1971 ('Irish stock'), and a commercially farmed two-sea-winter stock of Norwegian origin which has been used by the Irish salmon farming industry for almost two decades ('Norwegian stock').

The purpose of this report is to describe the progress of the disease throughout the summer of 1992 and 1993 and to discuss the efficacy of management strategies and control measures, including the use of malachite green and fumagillin.

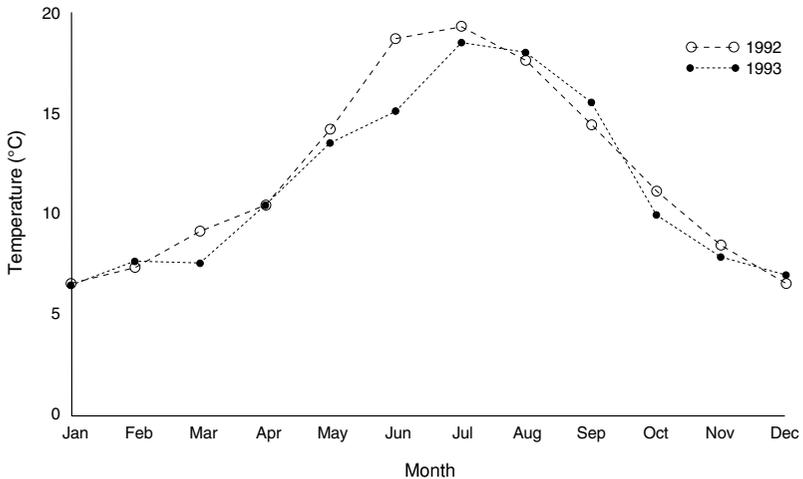


Figure 2: Mean monthly water temperatures (°C) at Carrigadrohid in 1992 and 1993.

Clinical history in 1992

PKD was recorded for the first time at Carrigadrohid and Inniscarra following a significant increase in mortalities in 0+ age Norwegian stock (average weight 5 gram) on 7 July 1992; 100% prevalence was confirmed in a sample of 30 moribund fry on 9 July 1992. Diagnosis of PKD was based on overt clinical signs (*eg* swollen abdomen and kidneys, pale gills and exophthalmos) and was confirmed by histological examination of several organs including kidney, liver, spleen and pancreatic tissue (Roberts 1989). Samples were examined histologically on a regular basis up to the beginning of September in order to monitor the progress of the outbreak and the efficacy of treatments. The occurrence of bacterial gill disease during the same period compounded the PKD problem.

Although water temperatures were high (19–20°C) (Fig 2), it was decided to dose the fish initially with a 2 ppm flush of malachite green. This was followed by two one-hour bath treatments at seven day intervals; various dose rates were used on individual tanks and cages (0.5–2 ppm). During the last

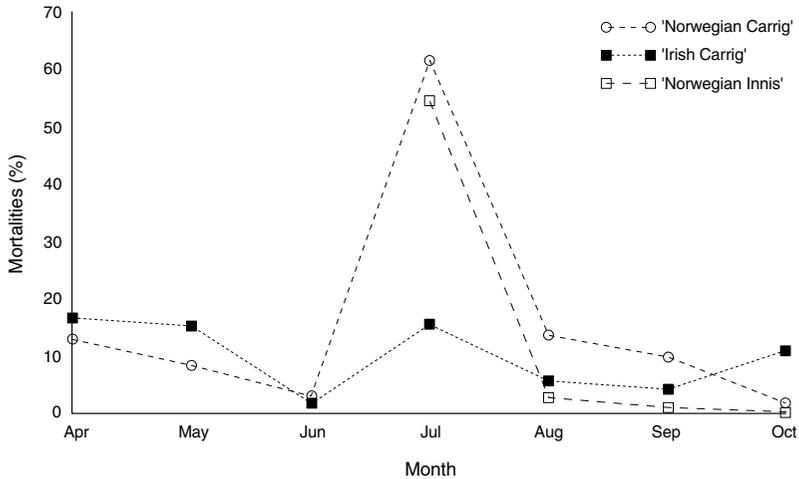


Figure 3: Monthly mortalities (%) of Norwegian and Irish salmon parr at Carrigadrohid and Inniscarra during 1992.

three weeks of the outbreak, a dose rate of 1.6 ppm for 40 minutes was used throughout both farms (Alderman and Clifton-Hadley 1988).

Dose rates were calculated according to individual tank and cage volumes. The depth of the cage was reduced to 1 metre and the net was surrounded by a tarpaulin bag prior to treatment. The required concentration of malachite green for tanks and cages was pre-diluted in 10 and 300 litres of water respectively. The pre-diluted dose was applied evenly over the surface of the tank and cage with watering cans and pumps. Supplemental oxygen was supplied to each tank and cage during the treatment period.

Although initial stocking densities were relatively high (10 kg/m³), it was decided not to handle the fish in any way (particularly by grading) lest this should aggravate the problem. Feeding rates were reduced to 1% bodyweight per day and the feeding period was also reduced to two 4-hour periods per day (6–10am and 4–8pm). Supplemental oxygen was continuously added to the hatchery water supply using the on-site oxygen generation system.

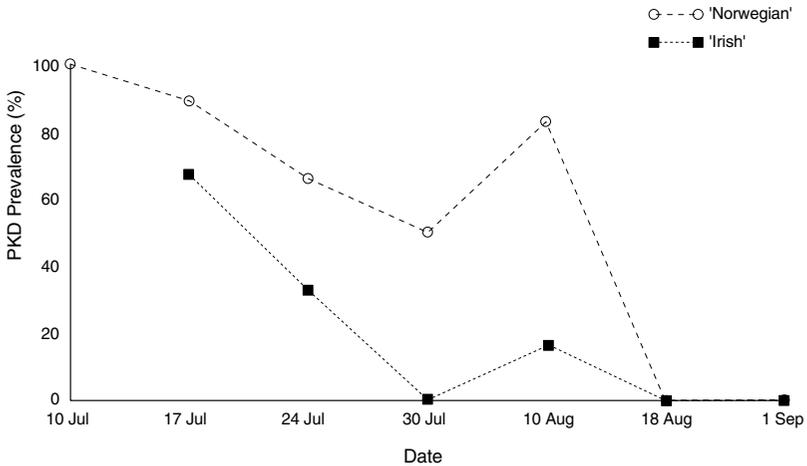


Figure 4: Weekly prevalence (%) of PKD in Norwegian and Irish salmon parr at Carrigadrohid during 1992.

Mortalities increased dramatically during the second week of July and then decreased gradually during the second half of the month (Fig 3). A total of 1.3 million fry died during the PKD outbreak, representing 61.6% of the Norwegian stock at Carrigadrohid and 54.6% of the same stock at Inniscarra. The Irish stock at Carrigadrohid was affected to a much lesser extent (15.6%). Weekly samples (30 fish from each stock) showed that the prevalence of the PKD parasite in both stocks fluctuated throughout the summer but was histologically undetectable by mid-August (Fig 4).

Clinical history in 1993

Based on the experience gained during 1992, the following management strategies were adopted during 1993: stocking densities were kept at a lower level (less than 5 kg/m³); no grading was carried out between July and October; the water supply was continuously oxygenated throughout the summer; feeding rates and feeding periods were reduced as in 1992; seven consecutive weekly prophylactic bath treatments with malachite green (1.6 ppm for 40 minutes) were administered prior to mid-July (between 13

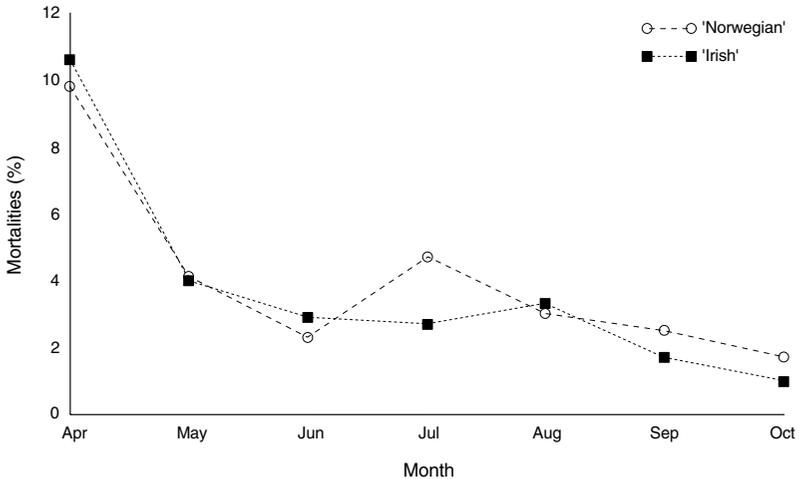


Figure 5: Monthly mortalities (%) of Norwegian and Irish salmon parr at Carrigadrohid during 1993.

May and 9 July); and trials were carried out using fumagillin. No stock was held at Inniscarra throughout the summer of 1993. Samples (30 fish) of the Norwegian stock were analysed on a regular basis for PKD parasites from early April until late October 1993.

Compared with 1992, water temperatures were lower in June and July 1993 but slightly higher in August and September (Fig 2). Cumulative mortalities in the Norwegian and Irish stocks between July and September 1993 (3.8% and 3.3% respectively) (Fig 5) were substantially lower in comparison with deaths experienced during the same period in 1992.

Although some evidence of PKD was found during mid-July, mortalities remained at a relatively low level. However, during the first week of August up to 16% PKD prevalence was found, and although this increased to 50% by mid-August and 66% by the end of the month, no significant mortalities occurred (Fig 6). Although the prevalence of PKD appeared to fluctuate throughout the summer months (as in 1992), the parasite persisted for a longer period during 1993 (up to mid-October).

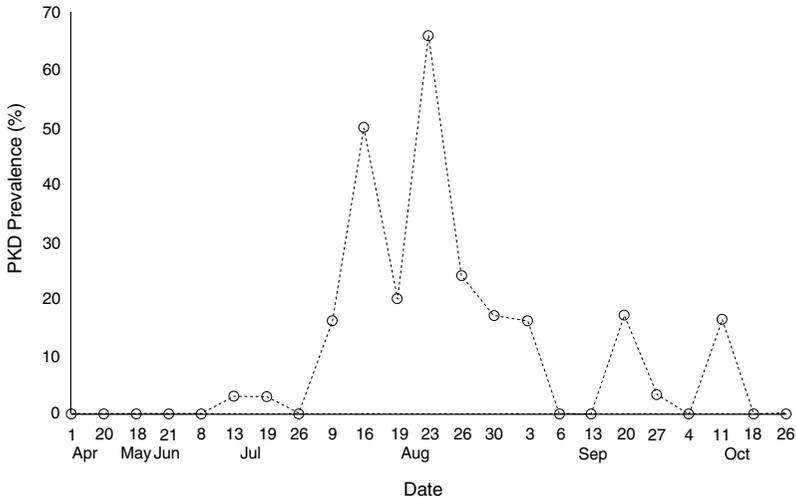


Figure 6: Weekly prevalence (%) of PKD in Norwegian salmon parr at Carrigadrohid during 1993.

Fumagillin trial

During late August 1993 a trial batch of 0+ Norwegian parr (average weight 10 gram) which were exhibiting a 24% PKD prevalence were fed with fumagillin at a dose rate of 6 mg/kg bodyweight per day for 10 days. No PKD parasites were found in the trial batch at the end of the treatment period (early September), but PKD was still present (at 16% prevalence) in the untreated control group. There were no apparent adverse reactions, such as loss of appetite, to the fumagillin treatment.

Discussion

A combination of high water temperatures (over 19°C) and high stocking densities (over 10 kg/m³) would appear to have precipitated the clinical outbreak of PKD during early July 1992. Although the PKD parasite appeared around the same time in both years (early and mid-July respectively), its prevalence, albeit higher, was of much shorter duration in 1992 (6 weeks)

than in 1993 (12 weeks). Lower water temperatures in June and July 1993 may have delayed the development of the PKD parasite by about one week, but higher temperatures in August and September may have accounted for its persistence until mid-October. Hedrick and others (1993) pointed out that while water temperature is known to greatly effect the initiation and progression of PKD infections, outbreaks can vary markedly in their severity.

Although malachite green did not appear to be as effective in controlling PKD once the disease was established in 1992, it seemed to be more effective when it was applied prophylactically as a bath treatment (at a dose rate of 1.6 ppm for 40 minutes) for seven consecutive weeks prior to the expected appearance of the disease in 1993. Similar results were found by Alderman and Clifton-Hadley (1988) during field trials with rainbow trout.

While the single field trial with fumagillin during 1993 showed that the antibiotic was very effective in eliminating PKD parasites from an asymptotically infected stock, the results were somewhat inconclusive in the absence of a clinical outbreak in untreated stocks. Further studies on the effectiveness of fumagillin against PKD under Irish farming conditions are clearly required.

Although treatment with malachite green and fumagillin has shown some promise, the use of these compounds has given rise to concerns about toxicity, tissue residues, discharge to the environment and consumer safety. Indeed, because of European Union legislation there are problems with the use of malachite green in European fish farms and the chemical has already been banned in the U.S.A. (Schnick and Meyer 1978). However, an encouraging line of research involving the production of monoclonal antibodies could eventually lead to the production of an effective vaccine against PKD (de Mateo and others 1993).

The Irish stock appeared to have a higher resistance to PKD than the Norwegian stock. Ellis and others (1982) reported similar findings in Atlantic salmon parr in Scotland; they found that a Norwegian stock was more susceptible to PKD than Scottish stocks. The apparent differential susceptibility of various salmon stocks to PKD should be taken into account in the design and comparison of PKD trials. Furthermore, the greater resistance to PKD exhibited by some native salmon stocks could be utilised in selective breeding programmes for commercial aquaculture. The possibilities for

genetic improvement of disease resistance in fish was recently reviewed by Fjalestad and others (1993).

The fact that Irish salmon stocks appear to be more resistant to PKD than foreign stocks would seem to suggest that the PKD parasite has been in Irish waters for a longer period of time than previously thought. Indeed, a recent examination of archival histological material indicates that PKD has been present in Ireland since 1964 (McArdle, unpublished data).

Changes in husbandry and management strategies, including a reduction in stocking densities, feeding rates and handling, delayed grading, increased oxygenation, together with prophylactic treatments with malachite green, reduced the impact of PKD during 1993. Although the overall effect of PKD preventative measures are a reduction in the output and efficiency of the hatchery in terms of growth, food conversion efficiency, parr condition and smolt yield, these negative effects have to be balanced against the opportunity of reducing potentially high mortalities.

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Twelve month study of ulcer disease in a pond of koi carp (*Cyprinus carpio*)

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Abstract

This study documents the events of ulcer disease in a koi pond and records the changes in antibiotic sensitivity of the bacterial isolates during a twelve month period. Various antimicrobial drugs including a novel antibiotic, thiamphenicol, and immunostimulants were used. Details of the bacteriological investigation are given and the various aspects of the disease in koi are discussed.

Introduction

Ulcer disease is one of the commonest and most problematic diseases in koi, *Cyprinus carpio*, causing large ulcers to develop on the head and body of the fish. These lesions initially appear as a 'bruise' in the skin and over a period of several days progress to the loss of scales and dermal tissue often extending to expose the underlying body muscle or cartilage. Secondary invasion of the ulcer by bacteria and fungi is common. The severity of the disease varies but significant mortality may occur.

Bacterial isolates from these ulcers often produce a mixed culture of Gram-negative micro-organisms including *Aeromonas* and *Pseudomonas* spp. Physiological stress from poor water quality and overcrowding are considered to be major factors in the development of the disease. Various methods of treatment have been used but these generally involve debridement of the lesion and use of antibiotic medication (Scott 1992). Bacterial resistance to antimicrobial drugs is now becoming a major factor in the success of therapy. Despite the fact that this is a common disease in koi, detailed information is sparse and primarily originates from studies of the disease in other species of fish such as farmed carp and salmonids. As a result, interpretation of similarities have resulted in conclusions about the nature of the disease in ornamental carp, the significance of which is questionable and often confusing.

Carp Erythrodermatitis (CE) is a frequent, ulcerative skin disease in farmed carp caused by atypical *Aeromonas salmonicida* (Bootsma and others 1977). This organism has also been isolated in cases of ulcer disease in goldfish, *Carassius auratus*, (Shotts and others 1980). Skin ulcers become secondarily infected by other aeromonads and pseudomonads (Austin and Austin 1993a). The lesions have a 'punched out' appearance with a red centre, surrounded by a white rim and outer erythematous area. Spring viraemia of carp (SVC) virus is also considered an important factor in the development of CE in countries where this virus is present (Fijan 1973).

Motile aeromonad septicaemia due to *A. hydrophila* (formerly called bacterial haemorrhagic septicaemia) is characterized by a haemorrhagic septicaemia and ulcerations. This disease is a particular feature of cultured carp and catfish (Roberts 1993). *A. hydrophila* is essentially an opportunist pathogen which invades the tissues of a host rendered susceptible by stress or other disease processes (Frerichs and Roberts 1989).

This report details the events of ulcer disease in one pond of koi over a period of 12 months in the south-east of England. The bacteriological samples in this case formed part of a project being carried out at the CEFAS Laboratory in Weymouth. The trial of a novel antibiotic and an immuno-stimulant was also carried out under the guidance of Vetrepharm Ltd., Fordingbridge.

Case history

The koi were kept in an indoor pond containing 6,400 litres approximately 70 cm deep and heated to an average of 15°C (60°F). The pond and filters had been set up for 2 months and water was pumped into two external biological filter units. Each consisted of a single chamber containing two layers of foam matting and pieces of corrugated plastic tubing (Flocor®). All the fish had been introduced over the previous 4 weeks and had received a bath of formalin and malachite green to remove ectoparasites. At the start of the study the owner had 25 adult koi measuring from 30–50 cm, which had been bought from several different local koi dealers.

8 March 94

The first case was presented at the surgery with an ulcer above the left eye which had invaded the local tissues and caused necrosis of the cartilage.

There was no intra-ocular damage but a large swelling on the left maxillary area extended into the roof of the mouth. Due to the extensive nature of the lesion and uncertain outcome, the owner requested euthanasia of the fish. Several other fish were reported to have shallow body ulcers, but no samples were taken for bacteriological examination at this time.

Postmortem examination revealed a cystic lesion containing serosanguineous fluid with local destruction of the cartilages of the skull. There were no abnormalities within the body cavity. Microscopic examination of body mucus revealed several trichodinids and a few skin flukes, *Gyrodactylus* sp. Water chemistry kits (Aquamerck simple tests, Merck) revealed high levels of nitrites (over 1.0 ppm) in the pond water.

The owner had added 15 kg of salt to the pond which produced a salinity of 2.3 ppt. He was advised to change half of the pond water every 3 days, and add salt to maintain the current salinity. The fish were starved for 4 days to reduce ammonia excretion and water quality was monitored daily by checking pH, ammonia, nitrite and nitrate levels. Commercially prepared medicated food pellets containing 0.1% oxolinic acid (King British) was to be fed to all fish for 14 days.

Ten days later the fish were stable but high nitrite levels remained. One fish had died but was unavailable for postmortem examination.

1 April 1994

Two fish were presented with ulcerations on the head which had been cleaned with povidone-iodine (Tamodine®, Vetark). The owner ran out of medicated food after seven days but failed to request more. Both fish were anaesthetised with tricaine methane sulphonate (MS222®, Thomson & Joseph) by immersion, the wounds debrided, dressed with povidone-iodine (Pevidine® Surgical Scrub, C-Vet) and then packed with a waterproof protective paste (Orabase®, Convatec). Antibiotic injections containing trimethoprim and sulfadoxine (Borgal® 7.5%, Hoechst) were given into the body muscle at a dose of 75 mg/kg. A further 14 day course of oxolinic acid medicated food pellets was dispensed.

Fourteen days later, water conditions were now acceptable (about 0.15 ppm nitrite). One fish was improving but the other had died.

5 May 1994

Water quality had improved and the nitrite level was now 0.05 ppm. However, body ulcerations were now affecting most of the remaining koi. The pond was visited to allow a better appreciation of the problem. Six badly affected fish were examined and revealed ulcers measuring up to 50 mm in diameter. These fish were anaesthetised, the ulcers debrided and treated as before and oxolinic acid medicated pellet food was fed for 10 days. Bacterial swabs were taken from three fish and a sample of pond water. They were sent for culture and sensitivity tests at two laboratories; CEFAS Laboratory, Weymouth and Institute of Aquaculture, Stirling. Skin scraping examination revealed many live trichodinids and a commercial medication containing malachite green, acriflavine and quinine sulphate (WS3®, King British) was recommended as a bath treatment. Salinity was maintained at 3 ppt.

Four days later one of the fish had died but the others appeared stable. A further four days later, two more fish had deteriorated and were euthanased by the owner.

Two weeks after the visit, bacteriology results arrived and are shown on Tables 1 and 2. *Aeromonas hydrophila* was the most frequently isolated organism and showed resistance to oxolinic acid, amoxycillin and oxytetracycline. In addition an isolate of *A. hydrophila* with resistance to potentiated sulphonamides was found in the pond water sample. Other bacteria identified were *Pseudomonas fluorescens* and other unspecified *Aeromonas* spp. On the basis of these results, an in-feed course of trimethoprim and sulphadiazine (Tribrissen® 40% powder, Pitman-Moore Ltd.) was added at the rate of 15 gram/kg of food to achieve the recommended dose rate (Scott 1992) of 30 mg active ingredient/kg of bodyweight. The total bodyweight of the remaining 19 fish was approximately 20 kg and 1 kg of food fed at a rate of 0.5% would last 10 days. Borgal® injection was supplied for the owner to inject into anorexic fish.

Twelve days later four more fish were euthanased due to the severity of their lesions. All remaining fish had ulcers of varying degrees but in the owner's opinion, some lesions were showing signs of healing.

31 May 1994

Six koi were caught, anaesthetised and their lesions debrided and treated as before using povidone-iodine, but no fish received antibiotics by injection.

Skin scrapings failed to reveal any ectoparasites and further bacterial swabs were taken. Due to the poor progress with potentiated sulphonamide and the moderate sensitivity to chloramphenicol on both laboratory results, thiamphenicol was used as an in-feed medication. In the absence of a suitable product containing chloramphenicol suitable for surface-coating, this novel antibiotic was used as part of a drug trial for a veterinary pharmaceutical company. The suggested dose rate based on trials in salmonids was 50 mg/kg bodyweight fed for 10 days (F. Macdonald, personal communication). The water temperature had been increased to 21°C (70°F) to assist recovery.

Two days later, a 15 cm koi with a 10 mm ulcer on one side and a deep 20 mm ulcer on the other flank was sacrificed and killed with an anaesthetic overdose. Bacterial swabs were taken from a skin ulcer and the kidney. Routine tissue samples (gill, spleen, liver, kidney, heart and skin) were fixed in formal saline for histological examination at the Institute of Aquaculture, Stirling. The section of the skin was typical of a longstanding ulcer with chronic inflammation, fibrosis and melanisation. Some small granulomas were found in the liver and kidney, and a low level of focal endocarditis and cardiomyopathy in the heart. The pancreas showed slight focal necrosis and some inflammatory cells were present on the peritoneal surface of some organs. Gram and Ziehl-Neelsen staining failed to reveal any bacterial pathogens. Bacteriology results are shown on Tables 1 and 2.

21st June 1994

All fish were examined and all were found to have skin ulcers. Some fish were making slow progress but others had developed fin and mouth rot due to *Flexibacter columnaris* which was identified from microscopic examination of a fresh skin scraping. Most ulcers were healing and exhibited less inflammation, a clean wound surface or fine epithelial covering. A few fish with large ulcers showed no improvement and some had healed ulcers but were now developing new ulcers elsewhere on the body.

The worst affected fish were anaesthetised, the wounds vigorously debrided and treated topically as before with povidone-iodine. Further bacterial swabs were taken. These fish were injected with gentamicin (Cidomycin®, Roussel Labs) at 10 mg/kg. Against my advice, the owner introduced three new fish during the previous week, one of which had developed a superficial ulcer. To reduce the bacterial load in the water, 30% water changes every three days were performed and salt added to maintain salinity at 3 ppt. Thiamphenicol

was continued for a further 10 days and a glucan (Macrogard®, Vetrepharm) was added to food at 1.25 gram/kg of food for three weeks.

Two weeks later, the bacteriology reports revealed a widespread resistance to most antibiotics. However, the owner reported that most were improving clinically although one fish had to be euthanased. Many fish were showing visible signs of wound healing and all were now eating well.

Two weeks later, most fish were recovering. Wounds were covered with a white epithelium and pigmentation in some lesions indicated a full recovery. A few fish had ulcers which were not inflamed but a further two fish had been euthanased due to deterioration. Water quality was good and no further treatment was required.

In September 1994, two fish developed ulcers which were treated with Borgal® 7.5% injections.

Four months later (February 1995) another two koi developed ulcers and more Borgal® was dispensed.

17 March 1995

Two fish developed shallow ulcers and were examined. One fish also had several small blister-like lesions on the tail. Bacterial swabs were taken and the ulcers treated as before and followed by injection with gentamicin which was given every three days on three further occasions. All fish were fed with a pellet surface-coated with glucan and mannan oligosaccharides (Vetreguard®, Vetrepharm) at a rate of 1 gram/kg of food. Salinity was maintained at 3 ppt and the water temperature at 14°C (55°F).

Three weeks later one fish was better, the other remained the same. The bacteriology report confirmed that the dominant flora of the ulcers and pond water was *A. hydrophila* with a wide range of resistance (Table 1).

Another week later, the second fish was making poor progress and treatment was changed to enrofloxacin (Baytril® 5% Injection, Bayer) at 10 mg/kg bodyweight every two days for five occasions. The fish improved over the following three weeks.

Laboratory methods and results

Bacteriological samples were taken using standard swabs and sent in charcoal transport agar by first class post to the laboratory. These were inoculated on to various agar plates for single colony isolation and incubated as follows:

Tryptone soya agar (TSA)	at 30 °C for 12–18 hours
<i>Aeromonas</i> sp. agar	at 30 °C for 12–18 hours
Columbia agar with sheep blood	at 17 °C for 5 days
Coomassie Brilliant Blue agar (CBB)	at 17 °C for 5 days

Except with the samples from 1995, duplicate plates were incubated as a control measure. When a pure culture was obtained, the organism was identified by production of a characteristic biochemical profile with the use of the API 20NE (API, Basingstoke) identification system.

Antibiotic sensitivity tests were carried out with a suspension of the isolate inoculated on to four Mueller Hinton Agar (MHA) plates to obtain an even lawn of bacterial growth and the following antibiotic discs used:

Oxytetracycline	30 µg	Erythromycin	10 µg
Oxolinic acid	2 µg	Flumequine	30 µg
Amoxycillin	10 µg	Ciprofloxacin	5 µg
Potentiated Sulphonamide	25 µg	Ofloxacin	5 µg
Chloramphenicol	30 µg	Gentamicin	30 µg
Furazolidone	15 µg		

Although thiamphenicol was used in the course of treatment, sensitivity discs were unavailable at the time of testing. Interest in the fluoroquinolones (flumequine, ciprofloxacin, ofloxacin) was part of a study of *A. hydrophila* infection in man.

Drug inhibition zones were measured visually and the result of both duplicate plates recorded. For this report an average figure was taken by rounding down to the nearest whole millimetre. The average difference between zone measurements in the duplicate plates was 1.47 mm (n=297).

The relative numbers of species isolated do not relate to the frequency of occurrence in the samples but represent the range of different organisms present. A written comment with each report gave an indication of the dominant organism based on a visual assessment. Although the laboratory

Table 1 Drug inhibition zones (in millimetres) of *Aeromonas* sp. isolates

Antimicrobial	OTC	OA	AMX	TS	CHL	FUR	E	FLM	CIP	OFX	GENT
Disc Concentration µg	30	2	10	25	30	15	10	30	5	5	30
Visit Date—Treatment + medication											
Report date											
8/Mar/94—Oxolinic acid medicated pellets											
11/April/94 — Borgal® injections + Oxolinic acid medicated pellets											
5/May/94—Oxolinic acid medicated pellets											
12/May/94 MAFF											
fish 1 <i>Aeromonas hydrophila</i>	r	r	r	27	29	r	r	r	21	20	27
fish 2 <i>Aeromonas hydrophila</i>	r	r	r	22	28	r	r	r	22	r	26
fish 2 <i>Aeromonas hydrophila</i>	r	r	r	21	r	27	r	r	20	r	24
fish 3 <i>Aeromonas</i> sp.	r	r	r	26	29	r	r	21	23	21	23
pond <i>Aeromonas hydrophila</i>	20	r	20	r	r	r	r	23	22	20	23
23/May/94 Stirling											
<i>Aeromonas hydrophila</i>	r	r	r	S	S	S	S				S
<i>Aeromonas hydrophila</i>	r	r	r	S	S	S	S				S
19/May/94 — Borgal® injections + Tribissen® 40% surface-coating											
31/May/94 — Thiamphenicol surface-coating											
16/June/94											
fish 4 <i>Aeromonas</i> sp.	r	r	r	r	r	r	23	r	27	26	28
fish 8 <i>Aeromonas hydrophila</i>	r	r	r	r	32	r	r	r	23	21	29
16/June/94 (sacrificed fish)											
fish 9 no bacteria in kidney											
fish 9 <i>Aeromonas</i> sp.	r	r	r	r	r	r	r	r	r	22	27
fish 9 <i>Aeromonas</i> sp.	r	r	r	r	r	r	r	r	20	21	26
21/June/94 — Gentamicin injection + Macrogard® + Thiamphenicol surface-coating											
5/July/94											
fish 10 <i>Aeromonas hydrophila</i>	r	r	r	r	r	r	24	r	21	20	25
fish 10 <i>Aeromonas hydrophila</i>	r	r	r	r	r	r	24	r	r	r	22
fish 11 <i>Aeromonas hydrophila</i>	r	r	r	r	r	r	23	r	r	r	21
fish 11 <i>Aeromonas hydrophila</i>	r	r	r	r	r	r	r	r	r	r	22
fish 12 <i>Aeromonas hydrophila</i>	r	r	r	r	r	r	r	r	r	r	23
fish 12 <i>Aeromonas hydrophila</i>	r	r	r	r	r	r	24	r	r	r	23
7/Sept/94 — Borgal® injection											
27/Feb/95 — Borgal® injection											
17/Mar/95 — Gentamicin injection + Vetreguard® surface-coating											
3/April/95											
fish 13 <i>Aeromonas hydrophila</i>	r	r	r	r	r	r	25	r	24	23	26
fish 13 <i>Aeromonas hydrophila</i>	r	r	r	r	r	r	28	r	25	23	27
pond <i>Aeromonas</i> sp.	r	r	r	r	35	30	r	r	31	28	24
pond <i>Aeromonas hydrophila</i>	r	r	26	r	r	r	22	r	25	21	26
pond <i>Aeromonas hydrophila</i>	r	r	28	r	29	25	23	r	27	21	26
pond <i>Aeromonas hydrophila</i>	r	r	r	r	r	r	27	r	25	23	27
11/April/95 — Baytril® injections											

Table 2 Drug inhibition zones (in millimetres) of other bacterial isolates

Antimicrobial	OTC	OA	AMX	TS	CHL	FUR	E	FLM	CIP	OFX	GENT
Disc Concentration µg	30	2	10	25	30	15	10	30	5	5	30
Visit Date - Treatment + medication											
Report date											
5/May/94											
12/May/94 MAFF											
fish 2 <i>Pseudomonas fluorescens</i>	21	22	r	20	r	r	r	27	24	23	24
23/May/94 Stirling											
<i>Pseudomonas</i> sp.	S	r	r	S	r	r	S				S
<i>Pseudomonas</i> sp.	S	S	r	S	r	r	r				S
31/May/94											
16/Jun/94 MAFF											
fish 4 <i>Pseudomonas fluorescens</i>	21	r	r	r	r	r	r	r	30	21	28
fish 5 <i>Pseudomonas</i> sp.	25	23	r	26	27	30	r	r	30	27	29
fish 5 <i>Pseudomonas</i> sp.	r	r	r	22	r	r	r	r	r	r	30
fish 6 <i>Pseudomonas fluorescens</i>	r	r	21	r	r	20	r	r	35	24	30
fish 6 <i>Pseudomonas</i> sp.	r	r	25	r	r	r	r	r	r	r	28
fish 7 <i>Pseudomonas fluorescens</i>	23	22	r	25	27	30	r	r	30	28	30
fish 8 <i>Pseudomonas fluorescens</i>	r	r	r	r	31	r	r	r	24	23	28
pond <i>Pseudomonas fluorescens</i>	r	r	r	r	r	20	r	r	34	24	28
pond <i>Pseudomonas</i> sp.	22	r	r	r	r	r	r	r	31	21	27
16/June/95 (sacrificed fish)											
fish 9 <i>Pseudomonas</i> sp.	r	r	23	r	27	r	r	r	r	r	28
fish 9 <i>Pseudomonas fluorescens</i>	r	r	r	r	31	r	r	r	26	25	27
21/June/94											
5/July/94											
All <i>A. hydrophila</i>											
17/Mar/95											
3/April/95											
All <i>A. hydrophila</i> or sp.											

KEY

r = resistant = diameter of 19 mm or less.

Samples sent to Stirling were interpreted as either sensitive (S) or resistant (r).

'fish No.' does not represent different fish but is used to differentiate between samples on each visit.

OTC = oxytetracycline

OA = oxolinic acid

AMX = amoxicillin

TS = potentiated sulphonamide

CHL = chloramphenicol

FUR = furazolidone

E = erythromycin

FLM = flumequine

CIP = ciprofloxacin

OFX = ofloxacin

GENT = gentamicin

study was aimed at isolating and identifying *A. hydrophila*, only aeromonads and pseudomonads were isolated from the samples. The use of the API test kits limited identification of some species and as a result non-speciated aeromonads were either *A. sobria*, *A. caviae* or *A. hydrophila* which did not fit the classic biochemical profile. Non-speciated pseudomonads were likely to be those other than *P. fluorescens* or *P. aeruginosa*.

Discussion

Despite the financial value of some koi, owners are often unwilling to pay for laboratory tests and in this case keeping costs to a minimum was emphasised. The distance to the practice made it difficult to have cadavers brought for routine postmortem examinations and the owner was reluctant to sacrifice any fish. These elements hindered initial investigations.

Epidemiology

Disease in koi may be influenced by various factors among which overcrowding, trauma, transport and temperature fluctuation are the most common environmental stresses. The water is recycled through a biological filtration system and high organic loads may develop.

• Transmission

The koi came from several different sources with no quarantine measures being taken and a minimal treatment given to avoid introducing ectoparasites. Although the fish appeared healthy at the time, it is inevitable that some pathogens will have been introduced with the fish.

• Temperature

Koi are kept in ponds where water temperatures may range from 3–20 °C (37–68 °F) throughout the year. Infection with *A. hydrophila* in salmonids is said to occur above 9.4 °C (46 °F) (Groberg and others 1978). From the author's experience, there is a seasonal incidence with most cases of ulcer disease occurring between May and July. The incidence of this disease varies from year to year and seasonal temperatures may be a significant factor. The number of cases seen in 1995 (5) was considerably lower than in the previous year (over 30) when the mild winter weather was followed by hot summer months in 1995.

• **Species**

From other cases, some aspects of ulcer disease in koi suggest that the bacterial infection is species specific, or that koi have a predisposition for this disease. Despite its severity, it is rare for other species eg goldfish, *Carassius auratus*, and orfe, *Leuciscus idus*, in the same pond to be affected. The pathology of infection with atypical *A. salmonicida* in some fish such as Atlantic cod, *Gadus morhua*, is different and a marked leucocyte response and cystic lesions are produced (Morrison and others 1984).

• **Management aspects**

Many diseases in ornamental fish have an underlying management component where poor water quality or other stresses precipitate disease. Although the stocking rate was not excessive, infrequent tests were carried out to monitor water quality. The rapid stocking of the pond gave insufficient time for the bacteria in the biological filter to become established. As a result, high nitrite levels developed which may have triggered the outbreak of the disease. A previous report has linked high environmental nitrite levels with bacterial ulceration in channel catfish, *Ictalurus punctatus* (Hanson and Grizzle 1985).

Treatment of nitrite toxicity requires immediate and substantial water changes to dilute the levels in the water, but not so much as to stress the fish. The addition of sodium chloride reduces the toxicity of nitrite by competing for uptake by the gills. Koi tolerate low levels of salinity well, and concentrations up to 6 ppt are safe (Scott 1992). Filter media from an established pond filter can also be used to introduce an active bacterial flora of *Nitrobacter* sp. etc to help reduce nitrite levels, but no suitable material was available in this case. Starving the fish reduces ammonia production and the burden on the bacterial filter, and thus indirectly reduces nitrite levels in the water.

Clinical signs

Apart from varying degrees of ulceration, there were few other clinical signs. Some fish had a generalised erythema of the skin suggesting systemic infection. The small cystic lesions found on the tail of one fish have only been seen in one other similar case in which the koi also had a skin ulcer (the author, unpublished data). The cause of these vesicles is unknown but may represent a localised epithelial response (spongiosis) to bacteria. In both cases no ulceration developed and the lesions disappeared following antibiotic treatment.

Laboratory results

• Postmortem

Despite the death or euthanasia of ten ulcerated fish, postmortem examination was only possible in two cases. In the sacrificed fish (fish 1 and 9) there were no internal haemorrhages as described in carp erythrodermatitis (CE) (McCarthy and Roberts 1980, Roberts 1993) suggesting that the superficial ulcers are a primary feature of the present disease in koi and that any internal lesions are a secondary effect following bacterial invasion. Dropsy with abdominal enlargement from ascitic fluid is also associated with CE but was not seen in any of these cases. Although histopathology revealed small granulomas in the kidney of fish 9, no bacteria were recovered from a swab or found using Gram and Ziehl-Neelsen stains on further tissue sections suggesting that there was no systemic spread of infection.

• Bacteriology

Interpretation of bacteriological tests depends on the experience of the laboratory technician. The use of selective growth media, the choice of the dominant colony and a sufficient number of colonies is required to give a true impression of the bacterial flora of the lesion. Identification of the bacterial species was performed using the API test kits, however it is well reported that these have limitations when used with fish pathogens (Austin and Austin 1993b). Since the project at Weymouth was aimed at the study of *A. hydrophila*, particular effort was made to identify the dominant organisms. *Pseudomonas fluorescens* was isolated from half of the cases and although it is ubiquitous, it is generally considered to be an opportunist pathogen and found as a result of secondary invasion of ulcers.

A. hydrophila was isolated from most swabs except fish 5, 6 and 7. Despite its ubiquitous presence in the environment as seen in the pond samples it is difficult to assess if it is a primary or secondary invader (Stoskopf 1993, Roberts 1993). This species is not considered an obligate pathogen but will cause disease in stressed fish. Inevitably some species may be more virulent than others and this may be related to the involvement of endo- and exotoxins. It is now considered that there may be several different subspecies concealed under the same name of *A. hydrophila* (Austin and Austin 1993b). Koch's postulates have not yet been tested to assess the involvement of *A. hydrophila* in ulcer disease in koi but further investigation and challenge studies are continuing at Weymouth.

Atypical *A. salmonicida*, the causative agent of CE, is an obligate pathogen. It is easily overgrown on many media since it takes 3 to 4 days to produce visible colonies. Austin (1993) recovered a fastidious form of atypical *A. salmonicida* from ulcerated carp in England but the lesions also showed evidence of secondary invasion by *A. hydrophila*. However, despite all efforts using selective media such as Coomassie Brilliant Blue agar (CBB), atypical *A. salmonicida* was not found in any of the current 23 samples.

• Sensitivity

There is uncertainty about the significance of antibiotic sensitivity tests which are influenced by the ability of the drug to diffuse through the agar and the concentrations of drug in the discs. MHA plates were used to optimise the diffusion of potentiated sulphonamides and measurements of the zone of bacterial inhibition was given rather than an interpretation of the drug sensitivity. Bacteriological tests were not performed from the outset and limit the interpretation of the first reports. However, in the isolates of *A. hydrophila* it can be seen that resistance developed to potentiated sulphonamides after using Tribissen® and to chloramphenicol following the use of thiamphenicol. Although there were no thiamphenicol discs available, bacterial resistance was suspected. However thiamphenicol continued to be used since it was the most practical in-feed drug and there was some clinical improvement. Resistance to gentamicin did not develop but this drug was only available as an injectable preparation.

The prophylactic use of antibiotics in exporting countries in the Far East is considered to be a factor in the development of bacterial resistance to antimicrobial drugs. This has been recorded in *A. hydrophila* (formerly called *A. liquifaciens*) isolated from freshwater fishes (Aoki and Egusa 1971). Resistance is generally related to plasmid transfer but samples on 21 June 94 show resistance to the quinolones which is usually regarded as a mutational or genetic resistance. However, further samples on 17 March 95 show a recovery of sensitivity to ciprofloxacin, ofloxacin and erythromycin. Resistance of *A. hydrophila* to a wide range of antimicrobial compounds including ampicillin, erythromycin, nitrofurantoin, novobiocin, streptomycin, sulphonamides and tetracycline have been recorded (Austin & Austin 1993b).

This case revealed a range of bacteria with different sensitivities confirming that there is often more than one pattern of resistance present in bacteria in a given population of fish (Branson and Southgate 1992).

Treatment

Various methods of wound treatment have been described and include debridement (Scott 1992), cryosurgery (Reynolds 1993) and cauterization with liquid phenol (Lance Jepson, personal communication). Since the removal of dead tissue is required for early epithelialisation of wounds (Frerichs and Roberts 1989), simple but vigorous debridement is important. Ulcers should only be debrided once since repeat treatments may delay epithelialisation (Scott 1992).

The choice of antibiotic should be based on bacterial sensitivity test results but in practice this can take up to 14 days and it is unrealistic to wait that long without antimicrobial treatment. The use of antibiotic baths or dips is considered ineffective (Scott 1992) and these may have adverse environmental effects. An initial approach using an injectable drug followed by an in-feed medication is the most practical method of treatment in koi. However, there may be limitations in the availability of a suitable formulation, palatability and bio-availability of the active ingredient. Efficacy depends on the stage of the disease, bacterial resistance and overall condition of the fish (Scott 1992). In this case several fish were euthanased due to the severity of their lesions.

Despite bacterial resistance to several antibiotics, many fish recovered, suggesting that antimicrobials have a limited effect and that good wound care and water management play a more important role in ulcer disease.

The circumstances of this case precluded controlled assessment of effects of the immune stimulants. However, the results of the treatment used on 17 March 1995 and lack of further cases suggests that Vetreguard® may have provided some benefit to the in-contact fish.

Adding sodium chloride salt to the water at 3 gram/litre reduces nitrite toxicity and assists wound healing by reducing osmotic stress at the site of ulceration.

Control

The control of *A. hydrophila* infection is ideally linked to the control of underlying factors which have caused the fish to become susceptible to the bacterial infection and include:

1. Regular monitoring of water quality
2. The use of disinfectants for nets
3. Reducing the organic and bacterial load by substantial water changes
4. Segregation of diseased fish. In this case the isolation facility was so small that it was better to keep all the fish in the main pond.
5. The use of vaccines is at an early stage of development. The only vaccine currently licensed for use in koi is AquaVac® Cyprivac CE (Aquaculture Vaccines Ltd.), a dip vaccine containing three strains of *A. salmonicida* for protection against carp erythrodermatitis. This has not been proven to have any cross-protection against *A. hydrophila* and had limited benefit in ulcer disease of koi (the author, unpublished data). The antigenic diversity of *A. hydrophila* has inherent problems in the production of a vaccine but Austin and Austin (1993) conclude that there is every possibility that a vaccine should work.
6. The longterm use of immune stimulants may prove beneficial. Evidence of its success in the control of furunculosis in salmonids suggests that there is potential for use in koi.

Zoonotic aspects

Despite the ubiquitous nature of *Aeromonas* and *Pseudomonas* spp. only a small number of human infections occur. Humans infected with *Aeromonas* spp. may show a variety of clinical signs but the commonest syndromes are gastroenteritis and localised wound infections from contamination of skin lesions. Although this is a minor zoonotic hazard to fish keepers, there is likely to be a greater risk to individuals with immune deficiency as a result of AIDS and other diseases. Although there is a potential for pseudomonads to cause human infection, no documented cases related to fish exposure have been reported (Nemetz and Shotts 1993).

Conclusion

There is a need for a better understanding of ulcer disease in koi and Koch's postulates must be tested to assess the involvement of *A. hydrophila*. The fact that atypical *A. salmonicida* could not be isolated suggests that it may not be the causal agent in koi. Failure to culture bacteria from the kidney in the sacrificed fish implies that this is not a systemic disease but that systemic spread may be a secondary effect. Therefore ulcer disease in koi would appear to be a result of superficial infection of the skin with *A. hydrophila* and epithelial damage from the production of various toxins or enzymes.

Further studies are required to investigate the bio-availability and tissue levels of drugs in ornamental fish since current dosages are based on those currently recommended for use in farmed salmonids in the UK. Investigation of the antigenic relationships of the different strains of *A. hydrophila* and challenge studies are also required to reveal the cause of ulcer disease in koi.

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Water quality and rainbow trout farming

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Abstract

The following paper seeks to review current knowledge regarding water quality and fish health in aquaculture, by first examining what we understand by the term 'water quality'. Known laboratory data is reviewed, along with a discussion of how this may be interpreted in the farm situation, and why it is often difficult to make firm assertions regarding the impact of water quality on fish health. Finally, some practical requirements for preventing problems and investigating suspected water quality incidents at sites are outlined.

Introduction

'Water quality' is a term often used in aquaculture to describe a single concept. However, for a trout farmer, three main water quality-related questions exist; firstly, 'Is the water supply I use secure and wholesome for fish culture?' secondly, 'Is the water quality within the ponds suitable to maintain low stress, but good fish growth?' and finally, 'Is the quality of my outflow water maintained within the constraints of my discharge licence?'. Whilst the answer to the first question relies, for the most part, on processes which occur outside and upstream of the fish farm, the second and third questions are to a large extent determined by the actions of the farm operator alone. In broad terms therefore it may be said that water quality at a farm site is defined by a combination of the processes which influence planning (eg the siting of the farm) and management decisions (eg stocking, feeding regimes). When many rainbow trout farms were established in England during the 1950s through to the 1970s, perceptions of both fish farmers and water companies were very different to those which now prevail. Fish farming was not seen as a significant source of pollution, and outflow water quality was not regarded as a potential point of disagreement by either regulator or farmer. Water was viewed as an abundant and pure resource, and the use of water for rearing of fish considered to be a suitable and appropriate activity. However, increasing restrictions on quality of discharge, our expanding

knowledge of the effects of water quality upon fish growth and health, together with a general presumption for maximising efficiency of water usage by existing and future industrial and agricultural users have changed this perception.

In the following review, the water quality conditions which affect rainbow trout health will be discussed. The elements of water quality most likely to cause ill health, poor growth or mortality are considered, together with their mode of action. Aspects of fish farming which are likely to result in poor water quality, and thus impinge upon fish health are also discussed. In conclusion, the remedial action which may be required, together with the means to identify, and reduce exposure to, water quality problems are considered.

The known environmental limits of rainbow trout (*Oncorhynchus mykiss*)

It is worth pausing to consider what is known about the capacity of rainbow trout to survive in the aquatic environment. Much data is derived from experimental, rather than production conditions, relating to the determination of the toxicity of various compounds to fish and other aquatic lifeforms. However, whilst the toxicity of individual pollutants, mixtures of pollutants and poorly defined pollution sources have been investigated over many years (Brown and others 1969, McLeay and Gordon 1978, Cameron and Koch 1980), the impact of stress and the physical nature of the tests employed may not be fully transferable to the fish farm environment. Toxicity and mortality data from waterborne toxicants are largely derived from acute 96 hour LC₅₀ (lethal concentration to 50% of exposed organisms) tests, and thus need to be interpreted, in respect of the fish culture environment, with some care. The nature of the culture environment for fish is similar to that of the test animals, in that natural avoidance strategies cannot be employed and the fish therefore must remain exposed to the toxicant, but two difficulties remain. Firstly, feeding regime, stocking densities, range of fish sizes and handling methods encountered at a fish farm differ substantially from experimental conditions. As a result, the stresses experienced by a fish population on a farm cannot be replicated adequately, or be expected likely to occur uniformly throughout a site; therefore the response of the fish in culture to the toxicant(s) are unlikely to match exactly results obtained under test conditions. Secondly, acute exposure tests, which form the bulk of toxicity investigations undertaken,

often use concentrations which are far greater than may be reasonably experienced in nature. The response of the fish to such exposures may not, as a result, generate results directly relevant to the fish farm environment; the objective in the culture environment is to maintain optimum, rather than non-toxic levels of potentially harmful substances, and little experimental data exists for that purpose.

In Table 1 below, the data reported sets out some of the most basic requirements of rainbow trout in terms of water quality. Where data exceeds these ranges, it is likely that some level of stress or discomfort will occur within the population.

Table 1:

Reported environmental ranges of rainbow trout (*O. mykiss*) survival.

Parameter	Range	Source
Water temperature (°C)	< 24.9–26.3 < 25.0	Alabaster and Lloyd (1980) Shepherd and Bromage (1988)
Dissolved oxygen (mg/litre)	>5.0–saturation	Alabaster and Lloyd (1980)
pH	5.0–8.4	Various authors

As a consequence, most water quality limits presented in the literature (eg Alabaster and Lloyd 1980) provide values which err on the side of caution (so called 'safe' concentrations), rather than define absolute limits of tolerance by trout.

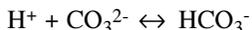
The role of pH, water hardness and water temperature

Three key elements in defining water quality have a substantial influence upon the aquatic environment, these are pH, water hardness and water temperature. This section briefly reflects their role in controlling the fitness of water quality and the toxicity of some selected parameters.

pH is the value assigned to define the presence of hydrogen ions (H⁺) in water, and this value varies, depending upon local conditions such as underlying geology, soil type and rainfall regime. The pH scale expresses the

number of H⁺ ions present as a negative logarithmic value, therefore, when pH falls from 8.0 to 6.0, the number of H⁺ ions in solution increases by a factor of 100, and thus becomes more acidic. In this respect, reported pH levels are often incorrectly calculated as arithmetic mean values; often of more interest in questions of fish health are the ranges of pH values to which the fish have been exposed. So why is pH important? Firstly, pH in itself is an important measure; when pH falls outside the range pH 4.5-8.5, most freshwater fish species begin to be affected directly by the acidity/alkalinity of the water (Evans and others 1988; Freda and others 1991). Secondly, water pH indirectly controls the solubility of metals and the ionised status of many of the major elements which may impact upon fish health, some of which are discussed below.

Water hardness is an expression of the concentration of calcium (Ca) and magnesium (Mg) present in solution. These two elements are derived from rock weathering, and are extremely important to freshwater chemistry, as they are usually present in carbonate (or bicarbonate) form. The carbonate/bicarbonate interaction is fundamental to the maintenance of H⁺ concentrations, and therefore pH levels in a solution, through the following relationship:



Therefore, the concentration of carbonate/bicarbonate complexes are controlled to a large extent by the presence or absence of Ca and Mg, and these in turn help moderate, or 'buffer' pH. Waters which are deficient in water hardness (termed 'soft water'), occur in areas such as the Highlands of Scotland, Dumfries and Galloway, and some upland areas of England and Wales to the North of the Tees-Exe Line. In such areas, natural control of pH is limited, due to the lack of carbonate and bicarbonate supply from the local geology. Persistent low pH rainfall in these areas have exhausted this buffering system, and as a result, these areas have experienced the greatest problems with acid rain. Many parts of the UK are subject to acid rain deposition, but it is the combination of geology and rainfall chemistry which contribute to producing low pH freshwaters (UNEP 1994, Baird 1995). A thorough review of the effects of acid precipitation on fish, and particularly the effects upon aquaculture operations, are presented in Exley and Phillips (1988).

Water temperature plays a unique role in the variability of water quality, affecting the aquatic environment in three distinct ways in terms of fish health. Firstly, there is the consideration of temperature regulation; being

poikilothermic (cold-blooded), fish must make metabolic sacrifices in order to remain in equilibrium with their surroundings, and in doing so, become stressed. Secondly, water temperature controls the amount of dissolved gases present in solution, and thus determines the fundamental fitness of the water for fish culture (eg as water temperature increases, the capacity for oxygen transport is decreased). Thirdly, water temperature exerts an effect upon the chemical composition of elements within the water column, and as we shall see below, the temperature of water may be critical in determining the effect of certain compounds on fish health. Finally, temperature changes can occur rapidly (as can pH), and even without exceeding critical values (Table 1), may induce stress.

The effects of dissolved metal elements upon fish health

It is useful to understand how the measurement of metal elements is undertaken in the laboratory prior to discussing the effects which such elements have upon fish health. Typically, when a total metal concentration is reported, it is the product of an unfiltered water sample being acidified, and then analysed for metal content by spectrophotometric analysis. In terms of the biology and action of metal toxicity, this 'total' value may have little or no meaning. The process of analysis may include parts of the metal incorporated into small solid particles or rendered inert through chelation (combined with Ca, Mg, organic acids and humic substances that are present in the water), but unlikely to have any impact upon the health of the fish (Vanderborgh and others 1990, Witters and others 1992). Usually, the more significant part of an element, in terms of fish health, is the soluble component of any metal present. Whilst considerable difficulties still arise in determining the potential of soluble metal elements to affect fish health, most toxicity data reported is expressed in this manner (Table 2).

It is unlikely that the source of a metal pollution incident will be internally generated within a fish farm site. Such impacts are more usually the result of industrial discharges (both deliberate and accidental) or a function of unusual background geology. Ingestion of contaminated sediments and sediment dwelling organisms by fish can result in harmful exposures to metal elements (Woodward and others 1993), and historically, levels of lead (Pb) in feeds caused some fish health problems, however, it is more typical to expect the toxic impact of metal elements to come from the dissolved ionised components of metal elements. Whilst a number of chemical equilibrium

considerations still play a vital role in controlling toxicity of any given metal element (Exley and others 1996), the most likely route to poor fish health in an aquaculture system, where the predominant food source is artificial diet, will instead be through surface contact with the element in question. Given that the gills represent a large reaction surface area (approximately 90% of the total fish body surface (Lock and others 1994)), and they contain active ion transport cells, it is not surprising to find that they are a focus for the action of most contaminants, particularly metal elements.

Jobling (1995) contains an excellent review of the functioning of gill structures in both marine and freshwater fish which will not be revisited here. However, the action of various toxic metal elements on gill tissue is similar, with the disruption, blockage and impairment of gills at the cellular level to such an extent that hypertrophy is induced, and both ion exchange functions and the osmotic balance of the fish are compromised. Deposition of metal elements occurs both on the surface and within the branchial cells — iron and zinc (Cameron and Koch 1980), aluminium (Youson and Neville 1987). The active transport in both directions across the gill membrane of important electrolyte components such as chloride (Cl^-), sodium (Na^+) and calcium (Ca^{2+}) suffer interference in the presence of elevated concentrations of soluble metals. For example:

- zinc (Zn) and copper (Cu) as competing elements with Ca^{2+} for positions within and on the surface of cell structures (Lloyd 1992)
- aluminium (Al^{3+}) affecting Cl^- , Na^+ and Ca^{2+} uptake (Playle and Wood 1991)

Table 2:

Ranges of limits and expected water quality conditions for toxic effects of some metal elements to fish (reported by various authors in Howells 1994)

Element	Soluble Concentration (mg/litre)	Conditions
Al	<0.03–<1.00	range up from $\text{pH} >4.5$ range up from hardness >2.0 mg/litre
Cu	0.001–0.028	10-500 mg/litre hardness
Cr	<0.025	'most natural waters'
Ni	<0.01	20 mg/litre hardness
Zn	0.03–0.5	10-500 mg/litre hardness

Metal poisoning of fish populations can also lead to failure or damage to organs other than the gills. Other likely centres of chronic and acute metal poisoning include the liver, pancreas and distal renal tubules. Neurological dysfunction and accumulation of metal within the cerebral membranes has also been demonstrated in rainbow trout (Exley 1996). The products of metal poisoning of fish can be expressed in a number of ways; accumulation in the kidneys and liver *eg* aluminium (Handy 1993, Exley 1996) and elevated blood levels of a toxic element *eg* lead (Hodson and others 1978).

The focus of research regarding the effects of aluminium upon fish populations is due to three main driving forces: firstly, the ubiquity of aluminium in the natural environment (it is the third most common element in the earth's crust), secondly, the impacts of acid rain on aquatic aluminium chemistry in many parts of the world, and finally, concerns with human health in relation to aluminium exposure. It can also be added that the use of fish, and particularly rainbow trout, as a target organism for laboratory studies of aluminium toxicology has further contributed to the creation of a vast published literature on this subject area.

Water quality effects of fish metabolism and aquaculture production practice

A primary part of fish metabolism is performed through the breakdown of proteins, and as a result, compounds of nitrogen are released in the form of respiratory products, urine, and faecal matter. In aquaculture operations, these are the primary sources of nitrogen waste in the production process. Ammonia and nitrite are the two inorganic forms of nitrogen compound considered to be deleterious to fish health, and are considered below. Nitrate (NO_3^-) is considered to be non-toxic to salmonids, however, it is worth noting that dependent on other water quality conditions, dissolved inorganic nitrogen can be rapidly transformed to any of the three oxidation states, thus where possible, it is desirable in aquaculture operations to maintain low nitrogen levels in the culture water at all times.

Ammonium (NH_4^+)

The total ammoniacal nitrogen (TAN) present in an aquatic system comprises both ammonium (NH_4^+) and ammonia (NH_3). TAN is a byproduct of fish metabolism and whilst ammonium is largely non-toxic to fish, the unionised form, ammonia (NH_3 — also referred to as unionised ammonia) is considered

to be highly toxic. The presence or absence of ammonia is controlled primarily by ambient water temperature and pH (Table 3). At higher temperatures and pH levels, ammonia concentrations and therefore its toxicity to fish increases; unfortunately, production of TAN in aquaculture systems also increases with temperature (Kelly and others 1994).

Table 3:

Variation of ammonia levels with pH and temperature

Temperature (°C)	pH = 6.0	pH = 7.0	pH = 8.0	pH = 9.0
	% of TAN present as ammonia			
5	0.01	0.13	1.24	11.18
10	0.02	0.19	1.83	15.70
15	0.03	0.27	2.68	21.59
20	0.04	0.40	3.83	28.47
25	0.06	0.57	5.44	36.53
30	0.08	0.81	7.52	44.84

An absolute value of ammonia is usually given to delimit its toxic threshold to fish (Table 4); this must, however, be placed in context with the above data. For example, at pH 6.0 and 20°C, a TAN value of 10 mg/litre will have an ammonia concentration of 0.004 mg/litre, whilst the same sample raised to pH 7.0 and 25°C has an ammonia concentration of 0.06 mg/litre. Whilst a high TAN value in the water of a fish farm does not per se mean that there will be health implications for the stock, Twitchen and Eddy (1994) suggest that at lower pH conditions, the presence of NH_4^+ in excessive concentration may still result in competition with Na^+ cells of the gill tissue and lead to increased ion efflux across the gills.

Ruffier and others (1981) defined three ways in which high levels of ammonia may cause fish mortality:

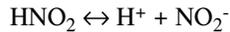
- (i) the presence of high NH_3 leads to increased water absorption across the gill, with subsequent stress on the kidneys. Once water intake exceeds the maximum urine production rate, death due to kidney failure follows.
- (ii) the presence of NH_3 prevents normal ammonia excretion occurring across the gill, leading in turn to high ammonia levels in the bloodstream and neuro/cytological failure.

- (iii) damage to the gill structure occurs to such a great extent that suffocation occurs, due to impaired gas exchange across the epithelium.

In the cases above, however, the actual situation is more complex, as lower pH levels are thought to exist close to the gill surface, due to the presence of CO₂, and also the production of mucus. This in turn will reduce the anticipated toxicity of NH₃ to fish for a given water pH.

Nitrite (NO₂⁻)

Like ammonia, the relationship between total nitrite and unionised forms such as nitrous acid (HNO₂) is again strongly, but in this case inversely, pH-dependent:



Wedemeyer and Yasutake (1978) report that at pH 6.0 and 8.0 respectively, 0.29% and 0.0029% of the total NO₂⁻ is in the form of HNO₂. The specific mode of toxicity for NO₂⁻ in fish, as with other animals and mammals, is through the promotion of methaemoglobinaemia; fish become unable to transport oxygen in response to the presence of high levels of NO₂⁻ in the water (Eddy and Williams, 1994). NO₂⁻ passes into the chloride cells of fish gills, and the presence of Cl⁻ in the water has an ameliorating effect upon the toxicity of NO₂⁻, reducing the potential for penetration of the gill cells (Table 4).

In pond systems, it is unlikely that nitrite poisoning would be a substantial risk to fish health; it is a very unstable compound in freshwaters and is rapidly oxidised to NO₃⁻ or reduced to NH₄⁺; fish farm facilities using large-scale water recirculation have the greatest potential risk from unwanted levels of NO₂⁻, and thus usually incorporate nitrifying bacteria filters to assist in its elimination from the water column.

Table 4:

Suggested safe limits of dissolved nitrogen compounds to rainbow trout (from Alabaster and Lloyd 1980, and Eddy and Williams 1994)

Parameter	'Safe' concentration
unionised ammonia	<0.025 mg NH ₃ /litre
Nitrite	<0.050 mg NO ₂ -N /litre (in 25 mg/litre Cl ⁻) <0.010 mg NO ₂ -N /litre (in 1 mg/litre Cl ⁻)

Other pollutants and sources of water quality degradation

Other pollutants which might affect fish health are often the result of one-off accidental spillage or leakage, although on occasion, deliberate and malicious discharges can occur. In this respect, a large number of potential sources and types of pollutants exist, including discharges of agricultural slurries, fuel oil leakages and herbicide/pesticide spillages. In addition, self-generated problems, such as algal blooms, can occur within fish farms. Algal blooms are most likely to affect pond systems, as tank systems are generally more turbulent. Turbulence prevents the build up of algal numbers in the water surface, and thus lowers the opportunity for a bloom to develop. Whilst there has been much discussion on the impact of toxins derived from blue-green algae on fish in freshwaters (Rodger and others 1994), a more likely concern at a fish farm relates to the effect of high algal biomass on dissolved oxygen levels. The oxygen demand of the algae themselves can, at critical periods of the day such as early morning, create a drop in oxygen levels sufficient to be lethal to fish stocks.

Factors influencing the effects of poor water quality on fish

It is also important to consider the effect of size and lifestage of the fish on its response to poor water quality conditions (Chapman 1978, Beattie and Pascoe 1978, Chapman and Stevens 1978). In addition, the history of exposures of fish populations to a pollutant can also influence their response to later water quality 'events' (Pascoe and Beattie 1979). Fish are known to be able produce metallothionein in order to resist metal intoxication; however, this may only be successful against the presence of copper, zinc, cadmium and mercury (Haux and Förlin, 1988).

The discussion above has centred mainly upon acute exposures of fish to certain toxic substances, but in chronic exposures, non-specific signs of stress upon fish are evidenced, such as loss of appetite (Waiwood and Beamish 1978) and lower growth rates (Woodward and others 1993). Whilst the gills are the foci for effects of pollutant exposure, the effects on fish of chronic exposures to pollutants are also usually expressed by degeneration of internal organs such as the kidney and liver; in all cases, such diagnoses are likely to be confirmed only through histological analyses in conjunction with relevant water quality data.

Preventing the effects of poor water quality

Only a limited amount of planning at a fish farm can be undertaken which might prevent, at source, upstream pollution. In terms of man-made pollution problems, often the only solution for a fish farmer is to maintain good relations with relevant upstream users. Thus, if incidents occur upstream which may impact upon fish health, a telephone call may be sufficient warning to allow the fish farmer to implement a contingency plan, and prevent entry of contaminated water into the site. Lack of consultation or co-operation can easily lead to significant impacts; two personally observed examples include the disturbance by a contractor of a pipeline supplying a hatchery in Eire, leading to ingress of fine particulates and gill damage to salmon smolts, whilst a farmer ploughing close to the banks of the intake stream of a trout farm in Scotland resulted in large volumes of silt being released into the ponds, and fish halting or reducing feed intake for the two weeks following the incident.

In Scotland, some salmon hatcheries and trout production sites operate pH monitors linked to automated liming systems. The addition of powdered lime (calcium carbonate) is automatically metered in acid-sensitive sites to alleviate the acute effects of low pH inflow waters, and thus prevent stress created by low pH and pH-mediated metal toxicity. Few fish farming areas in England suffer from such acute problems, for reasons previously discussed.

Within fish farm sites, much greater control of water quality is possible, due to the managed nature of the culture environment. Most visitors to fish farms will be familiar with oxygen monitoring and alarm systems, linked to some form of supplemental oxygen supply. Aeration and oxygenation also have direct benefits in reducing levels of ammonia and nitrite, as in well aerated conditions both tend to be converted to nitrate. The ameliorative effect of Cl⁻ in the water on nitrite concentrations has been noted in the above discussion; however, such salt solutions should only be applied very carefully to avoid further stress to the fish.

With regard to nitrogen compounds, however, it is also of importance to reduce waste feed wherever possible. Wasted feed results from excessive feed supply, which also carries direct (increased recurrent expenditure) and potential indirect (fines for failure to meet discharge quality limits) financial penalties to the farm. Pond systems are especially prone to water quality

problems resulting from wasted feed, as such solid waste is often retained as sediment. This in turn becomes a reservoir of nitrogen which is released to the water above under favourable conditions, and thus creates or sustains poor water quality conditions within a pond.

Getting samples which mean something

Regardless of the type of incident which may have occurred, often the least satisfactory aspect of the investigation of an incidence of poor fish health is the identification of the culprit element. Identification of the element is important for two reasons, first, it may allow for the instigation of a remediation plan, and perhaps restore fish health, but secondly, may identify and hopefully isolate the source of pollution for the future. However, the time lag between which a pollution incident occurs and the observation of erratic fish performance/mortality is often too great to obtain a sample of the suspect water, and it is also understandably low on the priority list of the farm operator. Often the sampling of water and its analysis is relegated to the status of a 'fire brigade' service, *ie* only required after an incident has occurred.

When advising at a site regarding fish health, there remain certain basic items of information which should always be obtained, including as full a list as possible of the following water quality parameters:

Parameter	Value
pH	range, rather than mean
Temperature	mean and range
Dissolved oxygen	daily mean and daily range
Hardness:	mean and range (also alkalinity if possible)
Ammonia:	mean and range
Nitrite:	mean and range

These data should relate not only to the inflow to the sites, but also to the outflow from production enclosures, in order to obtain a picture of water quality within the ponds or tanks, assuming that the water is reasonably well mixed. Records of discharge water quality collected by the Environment Agency (or SEPA in Scotland) offices should also be considered as a potential source of data.

Before going out onto the site, what resources are required to obtain a sample? Firstly, a good working relationship and advice from the laboratory with which you deal. Typically, they should be able to direct you as to where, when, what volume and how many samples to take, if they cannot themselves send staff to undertake the sampling. Secondly, they should provide the correct container, properly prepared. This is absolutely essential for any analysis *eg* glass or Pyrex containers cannot be used for analyses involving aluminium, whilst plastic bottles are of little use when assessing suspected hydrocarbon/organic chemical pollution incidents. Under no circumstances should other containers, such as household plastic or glass bottles be used, as more often than not they are themselves a source of contamination for the sample.

Once the sample has been taken, the final question is: can the laboratory undertake the sample analysis to an adequate standard? Two subsidiary questions arise from this question; first, is the technique used suitable, and second, is it possible to draw conclusions with any certainty from the results obtained? In terms of the first point, the laboratories should be using recognised methods, such as the various officially published techniques from HMSO, and be actively involved in quality assurance schemes such as NAMAS or recognised by relevant authorities. With regard to the second point, unfortunately few fish farms possess adequate data, but if available, such sampling is more likely to be of assistance when combined with evidence from tissue analysis of the fish population itself.

Conclusions

In fish farms, both internal and external sources of poor water quality can affect fish health, often with very similar results. Many of the effects of poor water quality in aquaculture are seen in the disruption and destruction of gill function, and can be caused both by metal elements and nitrogenous compounds alike. In general, fish are affected primarily by specific dissolved species of compounds, such as dissolved metal ions, and ammonia, rather than complexed forms; therefore analysis of water quality carried out to assist in fish health diagnosis must be undertaken with this in mind. Chronic effects of poor water quality can be to some extent survived by fish, although usually with reduced rates of growth and impairment of organ functions. However, it is not often possible to make explicit assertions concerning the impact of water quality on fish health, due to the nature of the data record.

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Notes from the winter scientific meeting held at the Royal College of Veterinary Surgeons, London on 28 November 1997.

The first speaker was **Tony Owen**, Head of Fisheries in England and Wales. His abstract follows this report and a few additional notes are added here.

This is part of the Environmental Agency and has approximately 9,000 staff and an annual budget of £565m. The Agency is split into eight regions and sub-divided into 26 units.

Of the total budget, £260m is spent on flood defence, £170m on pollution control and £20m on fisheries. The fisheries section has a total of 520 staff and these are concerned with:

- fisheries legislation and regulations,
- enforcing legislation,
- monitoring and improving fisheries,
- promotion and advisory functions,
- control and movement of fish (6,000 applications per year)
- the use of emergency powers under the Diseases of Fish Act.

At present approximately £4.5m per year is spent combating poaching. It was highlighted that poachers have now become more aggressive and violent in their activity.

The Agency's involvement with the upkeep of the rivers is very important. Agriculture is still having an impact on gravel rivers. This is preventing fish from spawning and reducing egg survival simply due to the encroachment of the river banks by grazing animals. There are about 135,000 km of main river systems in England and Wales. The monitoring programme to examine all of these rivers is on a rolling five-year theme. One of the current problems is the legal interpretation of a fish farm with respect to the Salmon and Freshwater Fisheries Act and European legislation.

The speaker also highlighted the fact that 'exotic' predatory fish which are introduced into the UK can decimate natural populations of fish. The Wels catfish (*Silurus glanis*) is a valuable fish for fisheries and anglers, and has been a particular problem in this respect. Predation could represent up to £70 per day for the average fishery. In their own right, fisheries are a potential problem because they tend to stock fish at around 1 kg/m³ to assist anglers whereas their natural stocking rates would be closer to 200–300 gram/m³.

A few comments on specific diseases related to fisheries were made. Spring

Carp Syndrome causes a high mortality and at the present very little is known about this disease. Carp pox is a common infection. Spring Viraemia of Carp (SVC) is associated with the illegal movement of fish between fisheries. There are now cases occurring every year and some of these result in 100% mortality.

The risk of introducing disease with 'exotic' species of fish is very real. For example, the nematode *Anguillicola crassus*, infects the swim-bladder of eels and has been introduced into the country. This parasite grows rapidly inside the host, causing a reduction in growth and an increase in mortality rates following rupture of the swim bladder. Prior to 1987, it was unreported but now there is no river in England or Wales that isn't infected with this parasite.

Another example is the carp tapeworm, *Bothriocephalus acheilognathi*, sometimes called the Chinese tapeworm. This was introduced in grass carp from Eastern Europe and is now commonly found in carp that are losing condition and dying.

The monogenean, *Gyrodactylus salaris*, is relatively easily treated in farmed salmonids in an enclosed system. However it is almost impossible to eradicate from fish in a river system. The parasite is now found extensively in France where Rotenone (a piscicide) has been used to kill all fish in a river before being restocked with disease-free fish. One major problem is the difficulty of identifying it from other less pathogenic Gyrodactylids and there is scope for a gene probe which can be specific, accurate and quick. Research in this area is currently under way. Routine screening for this parasite is complicated by the fact that it is also thought to infect eels, for which it is non-pathogenic, and at present there are no effective control measures.

The introduction of 'exotic' strains of the bacteria, *Aeromonas* sp, may cause far more severe disease in native fish than in exotic species.

The speaker also drew attention to the ornamental trade and the fact that in excess of 100 million live fish are imported each year. At the present time there is no monitoring of the effluent from garden centres which also sell fish and there is no need to have a discharge consent if the effluent is disposed down sewers before it goes into the rivers. These were areas of concern which may become important in the future. A relevant piece of legislation, the Import of Live Fish Act was passed in 1980 but still needs to be completed.

Verity Blackwell gave a presentation on zoonotic tuberculosis. Her abstract follows this report and a few additional notes are added here.

Mycobacterial infection in fish causes significant morbidity and mortality. On fish farms in Thailand the mortality rate can be up to 20%. As a human pathogen, the Public Health laboratories suggest that there are approximately 20 cases each year in the UK. The disease often affects swimmers, fishermen (particularly in the Chesapeake Bay area) and tropical fish enthusiasts. The latter account for up to 80% of human cases. There has been one report associated with a dolphin trainer and so far, there has been no direct spread between people. Occasionally infection is found in people that have had no contact with fish.

Generally infection is associated with abrasions or puncture wounds of the upper extremities. It results in nodules in the skin which increase in size over two to four weeks and these may rupture or ulcerate later. It may also spread up the lymphatic system (sporothricoid spread). In chronic cases, it can take 10 years to resolve and it has been associated with disseminating and fatal infections in immuno-compromised patients.

Diagnosis requires both culture and histopathology. However organisms are difficult to isolate and in addition to low culture temperatures, may take up to 12 weeks to grow. Histologically there may be giant cell formation but it is rare to see the bacterium in sections. PCR methods have been developed which can detect small amounts of DNA and as few as four to five bacteria.

Treatment requires several months of antibiotic therapy and occasionally excision is performed.

The next talk was about flatfish farming by **Richard Slaski** of Mannan Seafarms. His abstract follows this report and a few additional notes are added here.

Currently there is approximately £2–£3 per kilogram profit on flatfish but despite this, specialised hatchery and growing farms are required for successful farming of these high-value fish. Turbot take four months to grow to 10 gram fish in the hatchery and then another two years to grow to 1–2 kg. Atlantic halibut grow faster in cold waters but a hatchery has a nine-month cycle and require a further two years on-growing in the sea. The Japanese flounder grows relatively fast and can be sold for £8–£15 per kilogram.

Turbot eggs are about 1 mm in diameter and hatch after five days. The larvae are 3 mm long and don't feed for the first three days since they are living off the yolk sac. However their first feed must be live organisms, such as rotifers and artemia (live brine shrimp). This is not their normal diet but the

microbiology of the rotifer helps to establish the larval gut flora. More research into the bacteriology involved and the possible use of probiotics is needed to fully understand this essential early feeding. Young turbot can develop nutritional problems in the first two to three weeks of life which results in non-pigmentation of the skin and various deformities. The same has been seen in farmed halibut.

Turbot grow faster at temperatures between 9–17°C and consequently fish farms off the west coast of Scotland have less than ideal temperatures. The best turbot growing areas are in north west Spain (Galicia).

Ulcerations on the belly of large farmed halibut appears to be due to the very smooth surfaces of the rearing tanks. It is thought that the excessive contact with the surface prevents adequate water circulation under the fish and results in skin necrosis. This has been prevented by using gravel on the tank floor.

Edward Branson gave an update of rainbow trout fry syndrome (RTFS) with slides loaned by Rachel Rangdale (CEFAS, Weymouth) who was unable to attend the meeting. Unfortunately there were problems with the slide projector but it was an entertaining and muddled half-hour.

Peter Scott concluded the day with a presentation drawing on his experience of the problems and perspectives of commercial aquaria. He discussed the various requirements of design, husbandry, disease and commercial interests. The need for adequate facilities to trap large fish such as sharks is often overlooked and isolation tanks are often limited. The practical problems of transporting and treating some fish were highlighted using examples from Peter's career. This included the surgical repair of a rectal prolapse in a shark using a nylon purse-string suture. Disease problems and nutritional deficiencies are common problems which can be treated successfully. In addition to zoonotic infections, hazards from dangerous species also need to be considered when carrying out a health and safety risk assessment.

The meeting was generously sponsored by Vetrepharm Ltd, Fordingbridge.

D.G. Parsons
W.H. Wildgoose

The Environment Agency and fish health: an overview

A.G. Owen

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Under the Environment Act 1995, the Environment Agency has a general duty to maintain, improve and develop salmon, trout, freshwater fish and eel fisheries under its jurisdiction. The Agency's responsibilities extend to most inland waters, including rivers, streams, lakes, canals and reservoirs in England and Wales. The Agency is also responsible for salmon, migratory trout and eel fisheries in coastal waters to a distance of 6 nautical miles.

The Agency was formed on the 1st April 1996 and inherited the duties, powers and responsibilities of the National Rivers Authority (NRA), Her Majesty's Inspectorate of Pollution (HMIP) and the Waste Regulatory Authorities (WRA's). It employs approximately 9000 staff of which 430 are engaged in fisheries work. The operating fisheries budget is about £22 million.

The Agency's vision for fisheries is that all waters of England and Wales will be capable of sustaining healthy and thriving fish populations and everyone will have the opportunity to experience a diverse range of good quality fishing.

Legal duties

Of specific relevance to fish health, is the duty under the Diseases of Fish Acts 1937 and 1983 to notify the Ministry of Agriculture, Fisheries and Food (MAFF) of the occurrence of fish diseases in waters which do not comprise a fish farm. MAFF can impose a range of requirements on the Agency if an area is designated under these Acts.

Principal powers

Of the range of powers available to the Agency, the most notable in respect of fish health is the power to control the movement and introduction of fish into inland waters, which are not fish farms. The principal mechanism for application of this power, is Section 30 of the Salmon and Freshwater Fisheries Act 1975. Under Section 30 before introducing (stocking) any fish (or spawn) into inland waters, you must obtain the written consent of the

Environment Agency. Failure to meet this obligation is a criminal offence. Alongside Section 30, the Agency is a statutory consultee under the Wildlife and Countryside Act 1981 for any applications to MAFF for a licence to introduce non-native species of fish into inland waters.

Section 30 policy

The Agency has a clear policy regarding the introduction of fish which is set within the context of and is consistent with broader legislation, notably the Fish Health Directive EC 91/67. Under the Agency's policy mandatory health checks under Section 30 will be required where fish are to be moved into rivers, streams, drains or canals, where the risk of fish movement into or from the water is likely. Detailed documentation on the policy and the requirements for fish health checks are available from the fisheries department at any Agency office.

The Agency has also published its 'Buyer Beware' code, which includes advice on good practice designed to reduce the risks associated with stocking. The 10 key points of the code are:

1. Ask the question, do you need to stock?
2. Make sure all relevant paperwork is in order.
3. Beware that no supplier of fish is Environment Agency or MAFF recommended.
4. Only buy from reputable farms or dealers.
5. Be a careful buyer.
6. If possible, attend the removal of fish.
7. Be there when the fish are stocked.
8. Never accept fish unless you are satisfied that they are healthy.
9. Make sure you are in control and you get what you want.
10. If in doubt, ask a reputable body for help and advice.

Recent changes to the legislation

There is no question that the EC Directive 91/67 has had an impact on our ability to safe-guard native fish stocks from the threat of diseases being introduced from countries outside the UK. Recent legal cases have demonstrated, that there is concern that a large number of fish have been illegally imported into the UK, since trading restrictions with the EU have been relaxed. This statement is further backed by evidence relating to the increased incidence of exotic fish species occurring in inland waters, when no paper work exists to verify that they were legally introduced.

The potential threats to the UK's fish stocks are significant. Of note, the severe disease of farmed salmonids, Viral Haemorrhagic Septicaemia (VHS), is known to be carried by the Wels catfish, an exotic and popular sport fish. There is considerable concern relating to the monogenetic trematode parasite of salmon, *Gyrodactylus salaris* of which the host range is poorly understood. Further evidence exists from the increased prevalence of a range of fish parasites, which hitherto had not been recorded in the UK.

The Environment Agency is pressing very strongly for the Import of Live Fish Act (1980) to be invoked to close a loop-hole in the law which makes prosecutions for illegal introductions of exotic fish species very difficult. The Agency is optimistic that an Order will be made which will make the keeping of an illegally introduced exotic fish illegal. This change will shift the burden of proof to the keeper of the fish, to demonstrate that the fish were legally introduced: if not, then action can be taken.

Conclusions

The Environment Agency issues over 5,000 consents a year to stock fish into inland waters. We have great concerns regarding the health of wild fish populations in England and Wales, and are keen to ensure that the necessary legislative infrastructure is in place to protect our fish stocks. The consequences of the introduction of a serious disease to the wild, could have a consequentially serious impact to the fish farming industry: VHS is given as a potential example. The Agency is keen to work together with the veterinary profession to secure a safe future for the nations fish stocks.

Dr Tony Owen graduated from the University of Wales Institute of Science and Technology (UWIST) in 1981 and holds an M.Sc. from the University of London in 1983 and a Ph.D. from the University of Essex (1988). He has worked in a variety of posts within the water industry including with Anglian Water, the National Rivers Authority and the Environment Agency. Dr Owen held the post of Head of Fisheries with the Agency for two years before moving into general management. He is currently Area Manager for the North Wessex Area of the Environment Agency at Bridgwater, Somerset.

This article is based on a presentation given at the winter meeting of the Fish Veterinary Society at the Royal College of Veterinary Surgeons in London, 28 November 1997 and submitted for publication in March 1998.

Zoonotic Tuberculosis

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Many atypical mycobacteria can cause infection of the skin and soft tissues, and collectively do so more frequently than *Mycobacterium tuberculosis*. *Mycobacterium marinum* is one of the more common atypical mycobacteria causing cutaneous disease in the UK.

M. marinum is a photochromic bacterium that can be isolated from fresh, brackish or salt water including swimming pools and tropical fish tanks. It was first described in 1926 by Aronson in a cod and is now a recognised pathogen in over 150 types of fish, both fresh and salt water. It was first reported as a human pathogen in the 1950's after an outbreak of skin lesions in patients from a small town in Sweden that was eventually traced to infected water in the local open-air swimming pool (Linnell and Norden 1954). More frequently infection in man is acquired through abrasions or puncture wounds in an aquatic environment. It is a well documented hazard for fishermen, tropical fishkeepers and workers in the sea food industry and is variably known as 'aquarium or fish-tank granuloma' or 'fish-fancier's finger'.

Disseminated and systemic infections with *M. marinum* have been reported in immuno-compromised individuals such as those with HIV infection or solid-organ transplants (Gombert and others 1981, Tchornobay and others 1992, Hanau and others 1994). The most common clinical picture however, is that of single or multiple cutaneous nodules in normal, otherwise healthy, individuals (Wolinsky 1979, Collins and others 1985). The upper limb is most commonly affected. The lesions may occasionally ulcerate. Lymphatic spread may give rise to several lesions in a sporotrichoid picture. Corneal lesions, synovitis and osteomyelitis have all also been reported due to *M. marinum* infection (Wolinsky 1979, Collins and others 1985). Whilst superficial lesions may occasionally resolve, treatment with systemic antibiotics is generally necessary. The antibiotics most commonly used are minocycline, doxycycline and co-trimoxazole although rifampicin is sometimes beneficial. Treatment is often prolonged lasting several months and recurrence on cessation of treatment is not uncommon (Jolly and Seabury 1972, Prevost and others 1982, Savin 1992).

Cutaneous mycobacterioses may demonstrate a wide variation both in their clinical and histological features. The differential diagnosis can be wide, encompassing a variety of infections including sporotrichosis, blastomycosis, malignancy and Leishmaniasis. Currently, diagnosis largely relies on conventional histopathology and culture. Histopathological features, whilst suggestive, are not diagnostic. In *M. marinum* infection typical tuberculoid granulomas are not invariably seen and more often a diffuse mixed lymphohistiocytic infiltrate is present. The overlying epidermis may be hyperkeratotic and show pseudoepithelial hyperplasia. These features are not diagnostic and unfortunately the lesions are often paucibacillary (Jolly and Seabury 1972, Travis and others 1985).

Prolonged culture is necessary and may be unsuccessful as the bacterium has strict temperature requirements for growth of 30–33°C. Given this situation the use of either species-specific monoclonal antibodies (MAbs) in conjunction with immuno-histochemical staining, or the application of polymerase chain reaction (PCR) technology would facilitate the accurate identification of *M. marinum* in paucibacillary cutaneous lesions.

We have produced a specific 56kDa MAb against *M. marinum* using the cyclophosphamide B-cell ablation technique (Hamilton and others 1990). This MAb has been shown both by enzyme-linked immunosorbent assay (ELISA) and Western blotting to be specific to *M. marinum* when assayed with antigens from other *Mycobacterium* spp., diamorphic fungi and *Leishmania*. We are currently evaluating its use in the diagnosis of cutaneous infections on both routine histopathological specimens and frozen sections.

PCR has been successfully employed in the detection of *M. marinum* in the European sea-bass *Dicentrarchus labrax* by Knibb and others (1993) using amplification of the 16srDNA sequence and subsequent digestion with restriction endonucleases. PCR is being widely used in the diagnosis of cutaneous mycobacterioses (Degitz 1996) and we are currently adapting this method for use in detecting *M. marinum* from cutaneous infections in man.

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Verity Blackwell graduated in 1991 from Nottingham University Medical School. She currently works as a research registrar in dermatology at Guy's Hospital where she is investigating the use of diagnostic tests for 'fish tank granuloma' in humans.

This article is based on a presentation given at the winter meeting of the Fish Veterinary Society in London on 28 November 1997. It was submitted for publication in January 1998.

Current overview of flatfish farming

R.J. Slaski

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The farming of true marine finfish around the world has concentrated on species with a high market value. This has been essential due to the substantial costs of development for the industry up to this point.

Species: Several species of flatfish have proved highly suitable for farming:

Japanese flounder *Paralichthys olivaceus* - hirame:

Production in Japan, Korea, China

— estimated 10,000 tonnes per annum.

Turbot *Scophthalmus maximus*:

Production in Spain, France, United Kingdom, Ireland and Portugal

— 3,500 tonnes per annum.

Halibut *Hippoglossus hippoglossus*:

Still at the research and development stage in Scotland, Norway, Iceland and Canada.

Production techniques: The industry has largely developed a two-stage production strategy. The delicate larval stages are reared in sophisticated marine hatcheries, with eggs obtained from captive broodstocks. The juvenile flatfish are strong enough to be transferred to the on-growing farms once they have reached a weight of 5–10 grams.

Flatfish species present some unique technical and economic challenges for the farmer due to their shape and behavioural characteristics. The requirement to provide a relatively rigid bottom upon which the fish can rest has resulted in the dominance of land-based on-growing units for these species. Such an on-growing system offers several advantages to the farmer in terms of security and improved stock control, but tends to be more expensive than production systems which are based upon floating cages in the sea.

Health and disease issues: Flatfish, although relatively hardy in terms of survival through the on-growing cycle, are susceptible to a range of diseases which are potentially economically significant to the farmer. These include:

Bacteria : *Aeromonas salmonicida*, *Vibrio anguillarum*,
Pseudomonas and *Enterococcus* spp.

- Viruses : Viral haemorrhagic septicaemia (VHS), infectious pancreatic necrosis (IPN), nodavirus (? significance)
- Parasites : *Trichodina* spp., tapeworm, *Tetramicra brevifilum* (a microsporidian)
- Non infectious : Vitamin C deficiency, liver damage due to unprotected or high levels of fats in the diet, ultraviolet (UV) radiation damage

Modern vaccines, together with improved diets and husbandry techniques, have ensured that the impact of these health problems is minimised in the normal course of production. VHS virus is probably the greatest threat to the marine fish farming industry in Europe at the present time, and that threat is legislative rather than clinical: some modernisation of Directive 91/67/EEC is desperately needed.

The future of marine fish farming in the UK: There are three principle routes for the further development of marine fish farming in the UK which includes both flatfish and round-bodied fish species:

1. Intensive farming of high value species such as turbot, sea-bass *Dicentrarchus labrax* or even grouper (Serranidae) in temperature-controlled recirculation farms.
2. Cage farming of high value halibut, a species which suits the ambient seawater temperatures around the coast of the UK.
3. Cage farming of medium-value species *eg.* cod (Gadidae) and haddock *Melanogrammus aeglefinus*, which suit the ambient seawater temperatures around the UK.

Further development work is required before any of these options can be exploited fully. However there is tremendous market demand and a public perception that marine fish farming is a “good thing”, helping to preserve the dwindling wild stocks in our seas.

Richard J Slaski graduated in 1977 from Heriot-Watt University with a first class honours degree in marine biology. He has had extensive experience in several areas of fish farming and is now an independent marine farming consultant which includes strategy development for the British Halibut Assoc.

This article is based on a presentation given at the winter meeting of the Fish Veterinary Society in London on 28 November 1997. It was submitted for publication in January 1998.

Rainbow trout fry syndrome: an update

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Rainbow trout fry syndrome (RTFS) was first identified in the UK in 1984 and is seen in rainbow trout *Oncorhynchus mykiss* from 0.5 gram up to table-size, although it occurs mainly in the smaller sizes of fish. Losses of up to 70% have been attributed to the disease in some hatcheries. It has also been reported in France, Denmark, Spain, Germany, Italy, Finland and Chile.

The Disease

Affected fish are normally lethargic and anorexic, and hang at the water surface. In some cases dorsal skin erosions, usually behind the dorsal fin, have been reported. There is often ascites with bilateral exophthalmos. The gills are usually, but not invariably, very pale. The vent may be haemorrhagic, and there may be trailing mucoid casts. Concurrent problems, such as infestation with parasites, are very common. Corkscrew swimming has been reported, especially in recovering populations.

Internally the most obvious finding is marked splenomegaly: the spleen can be two to five times its normal size and is usually friable in nature. The surrounding peritoneum and fat is usually red in colour. The kidneys may be pale and slightly swollen. The intestine often contains a yellow or white mucoid discharge and the terminal gut may be congested.

On histological examination of tissues, the most obvious finding is damage to the spleen with a loss of capsular definition and replacement by loosely structured eosinophilic cellular and non-cellular material consisting of inflammatory cells, erythrocytes and strands of fibrin. Bacteria can be seen amongst this material and within macrophages. These changes tend to extend outwards from the spleen, and may envelop acinar cells and affect the peritoneum. The splenic stroma is more eosinophilic than normal, with minute holes appearing due to cellular damage. Haematopoietic tissue is depleted with pyknotic and karyorrhexic cells. Congestion is severe and the ellipsoidal sheaths are disrupted with vacuolation. The splenic changes are considered to be pathognomonic of the disease. Changes may be seen in other organs, for example the heart, gills and skin, but these are not consistent.

Not only do fish die as a direct consequence of this disease but there is some evidence to suggest that, because they become infected at such a small size, it can also interfere with vaccination against other diseases. As a consequence, there are increased losses associated with other diseases, such as ERM.

Aetiology

It is now almost certain that RTFS is primarily caused by the Gram-negative bacterium *Flavobacterium psychrophilum*, previously known as *Cytophaga psychrophila*. However other factors, such as handling and stress, can exacerbate the disease.

The bacterium is yellow-pigmented and rod-shaped. It will not grow on standard media and a specialised medium, RIV03, has been developed for the isolation of the organism by Rachel Rangdale at the CEFAS laboratory in Weymouth. However the presence of the bacterium in spleen squashes stained with Gram-Humberstone or haematoxylin and eosin can be used for rapid field diagnosis.

The causative bacterium has been found in the ovarian fluid of infected broodstock and this is considered to be a significant means of transmission of the disease to young fish. It has also been reported from America that the bacterium has been found within eggs but this has not been confirmed in UK.

Treatment

Of the drugs currently licensed in the UK for oral use in fish, oxytetracycline was originally the drug of choice for this condition. However, this drug has progressively become less effective in the field, even though it has remained effective in vitro in many cases. The reason for this difference in performance is not clear. Few farms in the UK now use oxytetracycline for this disease, and most treatment now involves the use of amoxicillin. However, even amoxicillin is now ineffective on some farms.

Work has been carried out at CEFAS to determine if any other drugs show potential for the treatment of RTFS. In vitro work was carried out to compare the MICs of 47 different isolates of the organism and a reference strain to ten different antibiotics: enrofloxacin, sarafloxacin, ciprofloxacin, oxolinic acid, doxycycline, oxytetracycline, amoxicillin, potentiated sulphonamide and florfenicol.

From the antibiotics tested, enrofloxacin, sarafloxacin and florfenicol showed potential for in vivo trials but no therapeutic effect has been found in subsequent trials with enrofloxacin.

Trials with florfenicol (Aquaflor® 50%, Schering-Plough) by oral administration, at a dose rate of 10 mg active ingredient/kg bodyweight/day for 10 days, have found it to be highly effective at controlling the disease, and more effective than amoxicillin which was used in a parallel control group. This drug has been used to good effect on some farms in the UK under Special Treatment Authorisations. In these cases the Aquaflor has been imported from Norway where it is licensed for use in fish.

Disinfection

Disinfection has been used in an attempt to control the spread of the disease.

The use of iodophors to remove the bacteria from eggs has been found to be ineffective at concentrations tolerated by the eggs. However, hydrogen peroxide at 100 ppm for 10 minutes, or glutaraldehyde at 200 ppm for 20 minutes, have been found to be effective against *F psychrophilum* on egg surfaces. A new product from Grampian Pharmaceuticals, GPRD02, has also been shown to be very effective when used at 100 ppm for 10 minutes.

For the disinfection of equipment, providing that it is clean and has been descaled, formalin (1–2% for 10 minutes), chlorine (0.5–1% free chlorine for 2 minutes) or exposure to alkaline conditions (pH 13 for 2 minutes) have been found to be effective. Cleanliness prior to disinfection is very important since dirt will deactivate the disinfectants and will also give shelter to the bacteria.

Vaccination

Non-specific immune responses in fish have been shown to be affected by RTFS. After an initial increase, there is a reduction in the levels of lysozyme and complement activity during infection. Although there is an increase in the level of anti-proteases in infected fish, these are ineffective at neutralising the extra-cellular products (ECP) of the bacterium.

Heat-inactivated strains of *F psychrophilum* have been shown to produce up to 60% relative percent survival (RPS) in fish challenged by intra-peritoneal injection. Bath-vaccinated fish in the same trial gained some protection, but at

a lower level. However, this protection was only achieved in larger fish (over 3 grams). More recent trials have shown improved protection but again only in the larger size of fish.

Work from the USA showed that an RPS of 100 could be achieved by using an adjuvanted vaccine injected into coho salmon *Oncorhynchus kisutch* of 0.5 gram in weight.

The results of these trials demonstrate the potential for vaccination against this disease, and work on vaccines continues in the UK, but whether it will be possible to effectively vaccinate fish at a size small enough to significantly reduce the impact of this disease, remains to be seen.

Edward Branson graduated from the Royal Veterinary College, London in 1979 and obtained his MSc in aquatic veterinary studies at the Institute of Aquaculture, Stirling in 1987. He is a self-employed aquatic veterinary pathologist and recognised by the RCVS as a specialist in fish health and production.

This is a summary of a presentation given to the Fish Veterinary Society at the Royal College of Veterinary Surgeons, 28 November 1997 and submitted for publication on 3 March 1998. These notes are an update of a previous article (*Fish Veterinary Journal*, No.1, 1996) and include new material supplied by Rachel Rangdale of the Institute of Aquaculture, Stirling and CEFAS, Weymouth.

RCVS Certificate in fish health and production

With the increasing level of specialisation that is now being encouraged in all aspects of veterinary medicine it was only a matter of time before more diverse subjects became available for examination at certificate and diploma level. The number of these specialities continues to expand and one of the latest additions is that of Fish Health and Production (FHP).

There is a growing importance in all aquatic species, and although they are not currently covered by the Veterinary Surgeons Act (1966), we have a commitment to the care of these animals beyond referring them to the fish farm or pet shop down the road. The aim of the certificate in fish health and production is to help give recognition to colleagues who hold a special interest in this expanding field. For the general practitioner where fish work constitutes a small but significant amount of their work, there is a need for an alternative to formal full-time courses. This certificate provides an opportunity for home study, offering an objective and sense of direction, and generating a focus of expertise in the veterinary profession. It is hoped that candidates will bring long-term benefits to this field by improving the knowledge base in aquatic animal medicine.

Candidates for the Certificate examination must be members of the RCVS or hold a similar qualification. Entry is restricted to those who have been qualified for at least three years, and who have spent at least two years at an approved practice or centre, with a minimum experience of 10% involvement in fish work.

This certificate can offer great variety in its curriculum and all fish species (both ornamental and farmed), shellfish, shrimp and aquatic invertebrates are included. The standard of certificate is geared to the equivalent of the well-informed new graduate with approximately two years of experience in fish medicine and covers the following key areas:

- Structure and economics of the UK aquaculture industry
- Water quality
- Reproduction and breeding
- Health programmes
- Nutrition
- Therapeutics
- Disease
- Legislation
- Welfare

Although a basic knowledge of the breadth of fish health and production is expected, the examinations allow a choice of questions to reflect specialist interests. International applicants should be aware that it is based on the UK fish industry, so legislation and 'support' systems may vary.

Advisors will be allocated to each candidate to assist in preparation for the examination and a reading list is also provided to help and encourage each candidate. Although various short courses already exist, it is hoped that in the future these can be tailored to reflect the requirements of the syllabus.

As with all certificate examinations, case books form an integral part of the course. These include three primary cases (2,000 to 3,000 words) and three secondary cases (up to 1,000 words). At least one case must involve food fish, and one ornamental or coarse fish. A case study could involve a small scale project in the field of importation, breeding or pharmaceuticals. It is hoped that by describing new methods and reporting unusual cases, candidates will contribute to the literature, and may submit case material for publication after completion of the Certificate.

A professional diary should be kept which lists attendance at relevant meetings and shows as well as presentations given, followed by a summary of up to 300 words. The aim of this is to improve professional standing and involvement in fish health and candidates should be seen to take an active role in the spread of information.

In total, the examinations consist of six case reports, two written papers and a clinical, oral and practical exam. Those who successfully pass the certificate level exams will be permitted to use the abbreviations CertFHP and may then proceed to the diploma level. Candidates should write to:

*The Royal College of Veterinary Surgeons,
62-64 Horseferry Road,
London SW1P 2AF*

for application forms and further information.

A personal note:

I sat this examination in 1997 and found it a challenging but rewarding experience. Although my work only involves ornamental fish, I found that by studying farmed fish methods it broadened my knowledge and helped to expand my approach to pet fish health.

*The casebook involved considerable effort and my 12-month case study of ulcer disease in koi is published in this journal. There are only scattered reports on ornamental fish disease in the scientific literature and a lot of unsubstantiated anecdotal information. I felt it important to investigate some of these cases and establish a rational and scientific approach. My other cases included a gastric foreign body in a red-tailed catfish, *Phractocephalus hemioliopterus*, brachycephaly in salmonids, a pigment cell tumour in goldfish, granulomatous lesions in koi and a survey of 'dropsy' in koi. Some of these may appear in future issues of this Journal.*

It is over twenty years since I was a student but I found 'seeing practice' to be a fascinating experience and concluded that we are never too old to go 'out on the rounds' with our colleagues. Forgetting my wellies on my first farm visit and showing a pathetic inability to catch a sea trout with a hand-net were more the trademarks of a student than a mature graduate.

I am indebted to all my professional colleagues for their help and patience in my journey through the Certificate. This took me to salmon farms in Scotland on cold wet winter days, to the impressive hi-tech fish disease lab in Weymouth, to the dusty bowels of the Natural History Museum looking for salmon skulls and to Billingsgate Fish Market at some ridiculously early hour in the morning. All memorable experiences. I am also very grateful for the support of my advisor, Lydia Brown, and my wife and daughter.

There are few other post-graduate qualifications in fish health for veterinary surgeons in the UK that do not involve full-time study. Much effort has been made by the RCVS board members to establish a syllabus for study. To support and provide for these qualifications is expensive, and there are now moves to make all subjects self-financing: enrolment for the certificate and exam currently costs £200 and £325 respectively. At present (May 1998) there are four candidates who have enrolled and only two that have successfully passed the exam. I feel that it is vital that more candidates enrol now for the Certificate and take the exam. Unless we are seen to be actively interested in this RCVS certificate and diploma, it may no longer be available.

The benefits of the qualification have been to improve my position in the field of fish health from a personal and professional point of view. More importantly, it has given me a greater degree of confidence in my approach to veterinary work and I would strongly encourage others to follow.

William H. Wildgoose CertFHP

Certificate of Fish Health & Production

REQUIREMENTS

be member of RCVS for 3 years
2 years experience at an approved practice or centre
minimum of 10% involvement in fish work
attend regular meetings & short courses, etc.

**APPLY for enrolment and initial approval of experience
before 1st. May**



**APPLY for final approval of experience
before 1st. May**



both above stages may be combined

APPLY for entry to examination before 1st October
**CASE BOOKS, PROFESSIONAL DIARY
and CPD STATEMENT to be submitted
before 1st November**



EXAMINATIONS held annually in May
two written papers
clinical, oral and practical exam

Fish vets in cyberspace

P.B. Green

Marine Harvest McConnell, Blar Mhor Industrial Estate, Fort William Highland PH33 7PT

The Society is putting a toe into technological waters with the launch of the Fish Veterinary Society Web Site. Aimed as an information point for current and potential members, the site will offer details of future events, an on-line calendar, reports on current issues, useful information and links to other Internet resources of interest to fish vets. Contact details and an on-line subscription form will also be provided. Initially the site will be run as a year long trial with the view to re-assessing its worth at the end of this period. There is plenty of scope for expansion to include other features. The site could present case histories, with full colour pictures and histological images, we have the option of starting an on-line discussion group if sufficient demand exists. Utilities such as drug inclusion-rate calculators and an on-line pharmacopoeia could be introduced.

All this may be of academic interest to members who have not even considered using the Internet, so I have collated some information on the available resources to give an overview of what the World Wide Web offers for fish vets and how to sample these resources with minimal outlay.

Aquaculture is quite well catered for on the web: Stirling University's Institute of Aquaculture web site:

(<http://www.stir.ac.uk/aqua/fishing/default.htm>)

is an excellent resource. It hosts a comprehensive list of hundreds of aquaculture-related sites including academic sources, commercial and trade organisations, government bodies and aquarist's home pages. In addition, the site lists many allied fields such as marine, freshwater and earth sciences plus environmental links. Using this resource you can quickly find and visit sites containing information relevant to your needs.

Aquaculture resources can also be tracked down using the free Internet search engines such as:

Webcrawler (<http://www.webcrawler.com>)

and Excite (<http://www.excite.com>).

These services catalogue web pages across the entire Internet and allow you

to search for pages containing a particular word or phrase. Typing the word 'koi' generates over 1,000 links to pages containing the word. Not all of these links will be worthwhile (the unregulated nature of the Internet means that literally anyone no matter how ill informed can publish their own pages about koi carp) but amongst these will be sites which contain valuable information. Searches can be refined; typing 'koi and parasites' lists about 10 pages containing both words.

For the more adventurous, the Internet offers additional resources beyond the web. Email list servers provide the electronic equivalent of mailing lists where groups sharing a common interest can exchange information on specialised subjects such as sea lice, killifish or squid biology. Internet news servers host discussion groups on topics such as aquaria and fisheries management. Bibliographic citations are also available, but differ from the preceding resources in that they generally charge for access. There are also many mainstream veterinary and general resources available on the Internet and with the size and content expanding rapidly, the Internet looks set to become the key information resource of the future. In the space of 30 minutes you might visit a site in Western Australia to read about the causes of plankton blooms, catch up on the latest effects of El Niño on South American fisheries stocks, look up the pharmacology of a novel antimicrobial agent, view the latest satellite image of weather patterns over the UK and participate in a discussion about winter care of koi carp in North America, all from the comfort of your own home.

For those who have not experienced accessing the Internet, actually trying it out is much better than relying on any description. For those without a computer or the inclination to invest in one, all is not lost. Internet Cafés are now fairly common sight in most cities (try 'Internet Services' in the Yellow Pages). These provide computer access to the Internet at a flat hourly rate plus help for the less technologically-minded. They are also a good place to sample the Internet before committing to any hardware purchases. Take this article along and try some of the addresses listed. Alternatively, find out if any friends or relatives have access and get them to give you a tour.

To access the Internet from home you need a computer and a modem (which connects your computer to the Internet using your phone line). Complete packages of all hardware are available from around £600 mail order (Computer magazines such as Computer Shopper are a good place to look) or

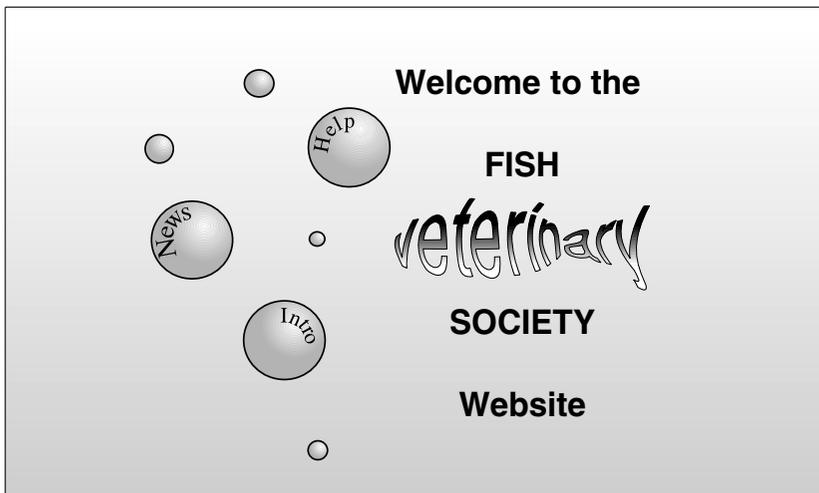
£800 from the high street. The second step is to install the software supplied by an Internet Service Provider or 'ISP'. These companies provide a local rate phone number for your modem to dial and connect to the Internet. They also supply the software to allow you to access the various Internet resources. Many of the larger ISP's such as AOL and CompuServe supply free software on the front of computer magazines. They also give you 'free' Internet access for a trial period so you can try before you buy (you still have to pay for the local phone call - about 60p an hour at weekend rates).

Once connected, you can begin to explore the many facets of the Internet. Of course, the best place to start is the Fish Veterinary Society web site. The address is:

<http://www.greens.net/fishvet>

When you visit, don't forget to sign the on-line guestbook. This will give us feedback on how many members are using the web. Suggestions for improvement are always welcome.

Happy surfing.



Diseases in Marine Aquarium Fish: causes-symptoms-treatment

Gerald Bassleer (English, 1996)

96 pages, hardback, £14.

Bassleer Biofish n.v., Stationsstraat 130, B-2235 Westmeerbeek, Belgium (tel: 00 32 16 69 6839 fax: 00 32 16 69 6831)

Information on diseases of marine aquarium fish is wafer-thin and often appears as a small chapter or appendix in most publications. As a vet in general practice, only 2% of my work in ornamental fish involves pet marine fish and my limited experience has been that these species are either healthy or dead. Any intermediate 'illness' is usually very brief, reflecting the difficulty of disease investigation in these fish.

The book is a robust, compact A5 publication written by a Belgian pathobiologist with extensive experience in the tropical fish trade. It is well laid out and illustrated with 74 colour photos (60 mm × 95 mm) and there are many useful comments based on the author's personal experience.

The text is in three sections. The first (13pp), entitled 'Diseases in marine fish: what factors play a role?' is a brief summary of environmental factors and provides a good refresher on the principles of filtration and water chemistry in marine aquaria. However, the use of chemical formulae rather than the full name is a disadvantage if, like me, you find it a challenge to identify some of the less common salts (eg NaBr, PBr, PHSO₄) — although I did spot one minor error, that 'rotten egg' gas is H₂S and not N₂. Quarantine of new arrivals is promoted logically and there is emphasis on maintaining good water quality. Examples of common environmental problems are discussed and advice on appropriate preventative and remedial measures is given. The book is dedicated to the 'Filipino fishermen and their families in the hope that cyanide poisoning is now a thing of the past' and the author discusses his personal views on this method of collecting wild fish.

The second section (53pp) is on 'Disease in marine fish: symptoms and treatment'. It starts with brief details of investigation methods used on dead fish but fails to emphasise that the specimens must be fresh (in my experience small tropical marine fish autolyse very rapidly). A certain degree of experience is assumed since tissue squash preparations are mentioned but there is no diagram showing the location of the internal organs. Diseases are grouped according to the pathogen: viral, bacterial, fungal and parasitic infections. Most are illustrated photographically and some line drawings are used to show the detail of parasites. Unfortunately all the photo-micrographs

have been taken with a condenser in position, restricting the field of view. Arrows highlighting the parasites would have been useful since protozoans are not demonstrated particularly well. There is useful information about manipulating the salinity to control protozoan parasites. It is good to see so many photos of the diseases described but it can be misleading to show non-specific clinical signs such as weight-loss or skin discoloration and suggest that these are due to one particular pathogen. Genetic anomalies and geriatric problems, such as neoplasia and overgrowth of teeth in puffer-fish, are not mentioned.

This section provides only limited technical information and unfortunately some of the terminology is rather dated with a few errors. 'Hole-in-the-head' or 'head-and-lateral-line' disease is probably the most common chronic disease of marine aquarium fish. Various explanations, including nutritional imbalance, toxins, viral and bacterial aetiologies, have been proposed but none has been proven. The author has suggested that *Hexamita/Spironucleus* sp. is the pathogen involved by drawing comparisons to a similar disease in freshwater fish but it has never been scientifically proven that the flagellates are even present in the lesions.

The final section (14pp), 'Treatment of diseases', provides basic details on a wide range of chemicals with notes on use, dosage and hazards. The subsection on antibacterial drugs lists 13 antibiotics, some of which are not available in the UK or in a suitable formulation. However there is no indication that the availability of these prescription drugs may be legally restricted in some countries. Most of these are quoted as in-water medications and some are suggested as a treatment for *Mycobacterial* infections. The author has also suggested various combinations of antibiotics which may be of questionable use in view of the bactericidal and bacteriostatic properties of the relevant drugs (eg neomycin with sulfathiazole/tetracycline). There are further notes on using 13 other chemicals and their synergistic combinations.

A small reading list is given at the end but most of these are dated and from the 1970s, possibly reflecting the fact that this book was originally published in German in 1991.

The author is to be congratulated on compiling a much needed book which fills a gap in the literature. It is written for hobbyists and dealers but contains useful information for veterinarians and other fish specialists. Despite its limitations it represents good value for money.

William H. Wildgoose

MEMBERSHIP APPLICATION

Eligibility

Membership of the Fish Veterinary Society is open to all members of the Royal College of Veterinary Surgeons, to those on the Supplementary Veterinary Register and to students studying for a degree entitling them to membership of the RCVS. The Society will also consider applications from overseas veterinarians.

I wish to become a member of the Fish Veterinary Society, subject to the conditions governing the same as set out in the Constitution of the Society.

I agree to pay my annual subscription in advance on 1st January each year, and if at any time I wish to resign from membership, undertake to send my resignation to the Honorary Treasurer by 1st December.

Name :

Address :

Telephone :

Fax :

e-mail :

University :

Degrees :

Date of graduation :

Fees : **Joining fee £50 + Annual fee £20**
(no charge to veterinary undergraduates)

*The sum of £70 is enclosed for full enrolment into the Fish Veterinary Society and membership for the current year /

*I am a veterinary undergraduate and wish to become an associate member of the Fish Veterinary Society **delete as appropriate*

— Details of paying by direct debit are available from the treasurer—

Signature :



MEMBERSHIP DATABASE

To help the Society provide a better service to its members we would be grateful if you could complete the following questionnaire by indicating your area of special interest.

Species of interest:

- | | | | |
|-----------------|--------------------------|-----------------------------|--------------------------|
| Salmon | <input type="checkbox"/> | Trout | <input type="checkbox"/> |
| Flatfish | <input type="checkbox"/> | Shellfish | <input type="checkbox"/> |
| Ornamental fish | <input type="checkbox"/> | Other (please specify)..... | |

Areas of interest:

- | | | | |
|-----------------------------|--------------------------|-----------------------|--------------------------|
| Pathology | <input type="checkbox"/> | <i>Histopathology</i> | <input type="checkbox"/> |
| <i>Bacteriology</i> | <input type="checkbox"/> | <i>Parasitology</i> | <input type="checkbox"/> |
| <i>Mycology</i> | <input type="checkbox"/> | <i>Virology</i> | <input type="checkbox"/> |
| Diagnostics | <input type="checkbox"/> | Genetics | <input type="checkbox"/> |
| Immunology | <input type="checkbox"/> | Legislation | <input type="checkbox"/> |
| Management | <input type="checkbox"/> | Nutrition | <input type="checkbox"/> |
| Pollution | <input type="checkbox"/> | Reproduction | <input type="checkbox"/> |
| Surgery | <input type="checkbox"/> | Therapeutics | <input type="checkbox"/> |
| Water Quality | <input type="checkbox"/> | Welfare | <input type="checkbox"/> |
| Other (please specify)..... | | | |

Other Membership:

- | | |
|---|--------------------------|
| British Veterinary Association | <input type="checkbox"/> |
| British Small Animal Veterinary Association | <input type="checkbox"/> |
| European Association of Fish Pathologists | <input type="checkbox"/> |
| British Trout Association | <input type="checkbox"/> |
| Scottish Salmon Growers Association | <input type="checkbox"/> |

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