FISH VETERINARY JOURNAL

The *Fish Veterinary Journal* is published by the Fish Veterinary Society and provided free to the Society's members. Additional copies can be obtained from the editorial department for £15 (UK) and £25 (overseas).

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.

FISH
veterinary
SOCIETY

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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MEMBERSHIP of the Fish Veterinary Society is open to members of the Royal College of Veterinary Surgeons, although the Society will consider applications from overseas veterinarians and those with an appropriate interest/ degree as set out in the Constitution. Currently membership costs £50 per annum. Veterinary students may become associate (non-voting) members of the Society; they pay no fee until they graduate, at which point they will become full members if they so wish. Enquiries regarding membership of the Fish Veterinary Society should be addressed to the treasurer — see the application form at the back of this Journal.

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, with 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* amoxicillin (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 3,000 words; for review articles up to 4,000 words; for short communications and clinical case reports up to 1,500 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 expense of the author(s).

References:

Only papers closely related to the author's work should be mentioned. These should be stated in chronological order in the body of the text and should be listed in alphabetical order and include the full title thus:

Hanson, L.A. & Grizzle, J.M. (1985) Nitrite-induced predisposition of channel catfish to bacterial diseases. *Progressive Fish-Culturist* **47**, 98–101

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 septicaemia. 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.

Personal communications should be cited as such.

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.

The *Fish Veterinary Journal* is covered by the CAB abstracts database.

President's Reflections

Marian F. McLoughlin
35 Cherryvalley Park, Belfast BT5 6PN

As the Fish Veterinary Society approaches it's tenth birthday and a new millennium I am honoured to have been elected as its President and look forward to steering it through the challenges of the next two years. During my term I wish ensure that the 3Cs; Communication, Co-operation and Continuous Professional Development (CPD) are the basis of our overall strategy of continuous improvement.

The FVS was originally set up as a forum for debate and exchange of information and ideas between vets who have an interest in fish. This objective remains at the heart of the society's activities and the *Fish Veterinary Journal* is a key element of our communication strategy. The internet also provides us with an unlimited opportunity to find and disseminate information rapidly. The FVS recognises the importance of this medium and has recently set up a dedicated web site which we hope will become an important link for promoting FVS and facilitating improved communication and contacts world-wide: **www.fishvetsociety.org.uk**

Our membership numbers have been steady at around 70 for a number of years, but in order to ensure vitality and continuity of our organisation we need new members and new ideas. It was therefore decided at the last AGM that we should open our membership to non-vets, who can bring a wealth of experience and knowledge to the FVS (see page 93). We hope that this strategy of increased co-operation and contact with other interested parties will enhance and advance the aims of the FVS and build important bridges with our non-veterinary colleagues. If every member could introduce a new member we would double our membership. *Your Society needs You*!

Infectious salmon anaemia (ISA) is still a major cloud over the Scottish salmon industry and while moves to allow a more realistic slaughter policy and limited use of vaccines are in progress, there is a serious risk that in the absence of adequate compensation that the disease will go underground. It is essential that lessons are learnt from other species and countries, and that the FVS help the authorities to recognise the impossibility of eliminating

ISA virus from the aquatic environment. We must develop disease control strategies that protect all our fish stocks and not eradicate them and their dependent rural communities. FVS continues to inform decision-makers in this area.

The availability of licensed veterinary medicines continues to cause concern for aquaculture and ornamental fish alike. While it essential to have tight controls on our inputs into food producing animals, the cost, duration and variation of the current licensing processes mean that many useful products will never get marketing authorisation, while the impact of new vaccines and medicines may be diminished because of delays. Despite the problems, new sea lice treatments, antibiotics and vaccines are coming onto the market and vets must continue to play an important role in the development and monitoring of their use to ensure that our limited armoury is protected from abuse and over-use.

The availability of suitable CPD events for fish vets is very limited within the British Isles and we often have to travel overseas in order to find appropriate meetings to fulfil our CPD requirements. The FVS scientific meetings help towards this objective, but due to lack of funds we are unable to bring in overseas contributors on a regular basis. We therefore need to generate more income through sponsorship, introducing meeting fees and attracting new members so that we can maintain and improve our contribution to your CPD.

We also have the RCVS Certificate and Diploma in Fish Health and Production, which if we don't support, we will lose as a standard for a post-graduate qualification for vets interested in fish. It is an excellent way of updating and consolidating your knowledge and very rewarding. *GO for it*! Ask our editor or read the articles in the *Fish Veterinary Journal (2 & 3)*.

Finally, I would like to thank our hard working committees past and present for their dedication and determination. Your committee are only caretakers of YOUR society, do your bit by paying your fees, seeking out funding and attending our meetings. We always welcome new ideas and suggestions and we are used to being bombarded with e-mails so get on-line and let us know what we can do for YOU. The next meeting will be in Belfast in early October 2000 and we promise you an enlightening scientific programme and lots of craic. So I look forward to welcoming you all to Belfast.

Editor's Comments

William H. Wildgoose
655 High Road, Leyton, London E10 6RA

Sadly, this will be the last issue of the *Fish Veterinary Journal* that I will edit at present. I have volunteered to be the editor for the second edition of the *BSAVA Manual of Ornamental Fish* and now realise that it is not possible to continue with both projects — I still have a day job in general practice. Consequently, I have allowed myself the luxury of two pages so that I can pass some comments on my involvement in the last four issues.

Editing the Journal has been an interesting and challenging experience, and it has evolved significantly in the three years since I took on the role of publications officer. This is partly through my own desire to provide a useful platform for informal papers and articles for veterinarians with an interest in fish health. While our standards may not be as heady as some other highly respected Journals, we have had the benefit of a short interval from receipt to publication, with a lead time as short as six weeks. This minor point is of great importance to some authors and enables the prompt publication of important new findings. However, this does not mean that we have lowered our standards since all articles are reviewed and commented on by at least two, and occasionally four, reviewers. Consequently, I often need to urge colleagues to fit an urgent review of a paper into their already busy schedule. Despite this, I am always impressed by the fact that no-one has ever declined the task and as I have said repeatedly, I am immensely grateful for all their efforts.

Each issue has taken over 100 hours to edit and another 50 hours to carry out the routine tasks of appealing for sponsorship, coercing authors, preparing drafts and sending out the finished item to our readers. A similar amount of time is spent mulling over plans and thinking about various aspects of layout and publication. The quality of the Journal does not just materialise or come about by accident or compromise: it is the result of much effort by many individuals.

Presentations given at our biannual scientific meetings are often chosen because they are topical or review important subjects. These form the

backbone of the Journal and keep readers informed of progress in both the scientific and commercial world of fish health. They are a great benefit to our members since on average only 20% are able to attend meetings — they are also an excellent reminder for those who did attend. Some authors have had to be coerced in the past and I apologise for my tenacity, but I was only doing my job. Despite that, most have produced impressive papers: I know only too well how much time and effort must be put into even the smallest article.

I am grateful to have been in a position where I could continue to publish my own articles on ornamental fish. I hope that my marathon reviews of some subjects have been of value to some readers. They have forced me to investigate my cases more thoroughly and expand my own knowledge. Although this may appear to be a perk of the job, I did not allow myself to escape critical comment from reviewers and I always asked for honest opinions. As a result, I have learned important lessons and feel that I have improved my technique.

There is a vast wealth of experience and unusual case material in general practice which is of great interest to many readers. Despite the complexity of some subjects, we have striven for clarity and simplicity where possible, so that readers can understand all articles. When necessary, we have also taken considerable time to edit articles and encourage novice authors to finally achieve publication. Therefore, I would urge you all to put pen to paper and contribute articles to this Journal — *your Journal*.

I am indebted to the sub-editors, Keith Treves Brown and Howard Thresher, for their valued assistance with editing and proof-reading: these are roles which should never be underestimated in maintaining our high standards. Mike Williams at Akalat Publishing has always ensured that the finished product reflects the efforts of all our hard work. And finally, I would like to thank all our commercial sponsors and advertisers for their continued financial support: without this, the Journal would not exist.

The *Fish Veterinary Journal* has established its own reputation as a source of authoritative, up-to-date information in many areas of fish health. I look forward to watching it continue to grow and develop into a more active forum in the future.

Dental overgrowth and trimming in a pufferfish

R. Rees Davies

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Case history

A tropical freshwater pufferfish (*Tetraodon palembangensis*) was presented with overgrowth of the upper teeth which appeared to interfere with prehension. It was part of a mixed collection of eight fish, comprising this species and *T somphongsi*. All the fish were around four centimetres long and kept in a 120 litre tank with filtration, aeration and sand substrate. The fish were fed mainly daphnia and bloodworm with no coral, clams or other hard molluscs in their diet. All the other fish appeared healthy.

The affected fish was anaesthetised by immersion in a dilute solution of benzocaine. This was added to the water at an initial dose of 25 mg/litre and then incrementally to a final concentration of 75 mg/litre. Satisfactory anaesthesia allowed the teeth to be clipped to approximately the correct length with iris scissors, followed by smoothing and contouring with a dental burr. Post-operative recovery was uneventful.

Discussion

Biology

Pufferfishes belong to the family Tetraodontidae, a name given because of the presence of four teeth at the front of the mouth. Their skin is scaleless but lined with small bony plates, or spines in some species. The oesophagus of these fish contain a number of sacs which can be inflated with water or air, causing the body to swell to an enormous size, thus either deterring predators or becoming physically too large to be attacked. Their flesh contains various neurotoxins, including tetrodotoxin, and these are secreted when the fish is stressed. The toxins produce fatal cardio-respiratory effects on nearby fish (Malpezzi and others 1997), and in aquaria these can be fatal to both the pufferfish and the other inhabitants of the tank. Members of the genus Tetraodon live in tropical seas, estuaries and rivers of Africa and Asia. They will eat almost anything that can be removed from rock with

their sharp teeth, including seaweed, coral, sponges and invertebrates (Frank 1970).

Captive management
In general pufferfish are aggressive and best kept alone. The lack of aggression between members of this group may have been due to their small size and immaturity. They require a large tank and will destroy any plants or furniture and so a plain sandy substrate is advised. They thrive at a temperature of around 27°C. They require excellent water quality and often require weekly partial water changes in addition to filtration. They can be fed on a variety of foods including frozen brine shrimp, bloodworm, glass-worms, dried shrimp, dried krill, dried plankton, and when larger they will take tiger shrimp, lean beef and frozen smelt. Snails, mussels, clams and other molluscs provide one of the main sources of food and the action of breaking the shell is believed to keep the teeth to the appropriate length. Apple snails (*Ampullaria* species) should be avoided due to risk of spreading disease.

Dental overgrowth
In the wild, pufferfish feed on various invertebrates including corals and hard-shelled molluscs. However, in captivity, they are rarely given access to material that is hard enough to keep the teeth worn down. Consequently, dental overgrowth and difficulty with food capture and prehension develop. In the long-term, the provision of harder molluscs such as clams and snails, or corals should allow normal dental wear. The reason why only one in this group of eight fish developed the problem is unknown.

Anaesthesia
Anaesthesia in ornamental fish has been described using a number of agents (Sedgewick 1986, Potts 1987, Brown 1988, Brown 1992). The two anaesthetic agents recommended for use in fish are tricaine methane sulfonate (MS222, Thomson & Joseph) and benzocaine (Scott 1991, Brown 1992). Both are readily available, but only MS222 is currently licensed in the UK and US for fish anaesthesia. Both substances are acidic, especially MS222, and can act as an irritant and physiological stressor to the fish (Scott 1991, Brown 1992). Benzocaine has been reported by some to be more effective than MS222 (Potts 1987). MS222 dissolves directly in water whereas benzocaine must first be dissolved in acetone, methanol or ethanol (Scott 1991). The stock solution can be stored for up to three months if protected from light to prevent build-up of toxic breakdown products,

principally chlorine (Potts 1987, Brown 1992). Both agents are used by immersion.

In this case, anaesthesia was induced using benzocaine on the grounds of reduced pH alteration and also cost. A stock solution was prepared by dissolving four grams of benzocaine powder in 100 mls of acetone. The bag containing the fish was weighed to give an estimation of the volume of water, and an initial dose of 0·6 ml stock solution per litre added to produce a level of approximately 25 mg/litre of benzocaine (Scott 1991). The fish was gently encouraged to move around the bag to increase the flow of anaesthetic solution over the gills. Incremental doses of benzocaine/acetone solution were then added a few drops at a time until the desired depth of anaesthesia was reached. This finally required a dose of 2·7 mls of stock solution in 1·5 litres of water: an approximate level of 75 mg/litre giving surgical anaesthesia. This level of anaesthesia took 15 minutes to reach, due to the cautious incremental approach used. This contrasts to 50 mg/litre as quoted by Scott (1991) and an induction time of between 60 and 90 seconds described by Brown (1992).

As anaesthesia progressed, the fish was observed to reduce movement and reaction to stimuli, reduce respiration, lose balance control and then fail to respond to external stimuli. This closely follows stages one to three of the 'stages of anaesthesia' described by McFarland (1959). The fish was removed from the anaesthetic solution and placed on a paper towel moistened in the anaesthetic solution whilst the procedure was carried out.

Recovery from anaesthesia was achieved by returning the fish to a bag of fresh water from its own tank, with balance control returning in 30 seconds and full reactivity to stimuli restored within 5 minutes. To allow full recovery from the stress of surgery and to minimise risks of toxin release from the stressed fish, the owner isolated the fish for several days.

Procedure
Once anaesthetised, the upper teeth were cut back to slightly longer than their normal length using a pair of iris scissors. Contouring and smoothing of the teeth was attempted using a jeweller's file but stabilising the fish's head proved difficult. Intermittent filing with a dental burr overcame this problem and allowed a near normal finish with no appreciable thermal trauma to the tooth stubs.

One week later, the fish was successfully reintroduced to the rest of the group, was eating well and difficult to distinguish from its conspecifics. The inclusion of harder shellfish in the diet was advised. The owner began raising snails as live-food for the fish and had no further problems in the subsequent 12 months.

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Ron Rees Davies graduated from Liverpool in 1994. After working in various mixed animal practices, he spent two years in a 30% exotic pet practice in Somerset where he gained his RCVS Certificate in Zoological Medicine in 1998. He has now joined a four-person team at the department of avian and exotic species medicine at the Clockhouse Veterinary Hospital in Stroud where he sees a variety of first and second opinion exotic pet cases, including birds, reptiles and fish.

Mycobacteriosis: detection and identification of aquatic *Mycobacterium* species

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Abstract

The aim of this study was to develop methods for the detection of aquatic Mycobacterium species in fish, fish farm workers and the environment. Initially, a monoclonal antibody probe (Mab 8F7) was developed against M marinum*, and an immunohistochemistry (IHC) test was developed to detect* M marinum *in fixed tissue. The monoclonal antibody appeared to be specific for* M marinum *but did not recognise all* M marinum *isolates. A reverse cross blot hybridisation polymerase chain reaction (PCR) test was developed for the detection and identification of* M marinum*,* M fortuitum *and* M chelonae*. Analysis of samples from Siamese fighting fish (*Betta splendens*) and snakehead (*Channa striata*) fish farms in Thailand revealed the presence of both* M fortuitum *and* M marinum *in fish and water samples. Siamese fighting fish farm workers were observed to have skin lesions, and analysis of skin biopsies by PCR revealed that* M fortuitum *together with unspeciated Mycobacterium appeared to be the main aetiological agents involved.*

Introduction

Mycobacteriosis, caused by aquatic *Mycobacterium* species, is a common disease in a wide variety of fish species world-wide. *Mycobacterium* belong to the family Mycobacteriaceae and the genus *Mycobacterium*, which consists of 54 species at present, although it seems likely that some still remain to be classified (Collins and others 1984). *M tuberculosis* complex, *M leprae* and some atypical mycobacteria species have been known to cause diseases in man (Wayne and Sramek 1992). In fish, three species of *Mycobacterium*, *M marinum*, *M fortuitum* and *M chelonae*, have often been

reported as causing disease (Nigrelli and Vogel 1963, Ross 1970, Giavenni and Finazzi 1980, Colorni 1992, Lansdell and others 1993, Puttinaowarat 1999). All three are also potential human pathogens (Poppe and Reichenbach-Klinke 1982, Paul and Gulick 1993, Lawler 1994, Guay 1996, Escalonilla and others 1998, Kullavanijaya 1999). This study was initiated by reports of mycobacteriosis in snakehead (*Channa striata*) and Siamese fighting fish (*Betta splendens*) in Thailand, and of granulomas on the hands and feet of Siamese fighting fish farm workers.

In Thailand, snakehead is an economically-important food species, while Siamese fighting fish is one of the main ornamental species cultured for export. Both species appear to suffer mortalities due to mycobacteriosis (Chinabut and others 1990, Pungkachonboon and others 1992). Classification of the different mycobacteria species is however difficult, and identification of the species causing infections in fish and fish farm workers in Thailand has not been accomplished.

Numerous mycobacteria species have been reported as pathogenic to fish, including *M piscium*, *M ranae*, *M marinum*, *M platypoecilus*, *M anabanti*, *M fortuitum*, *M salmoniphilum*, *M borstelense*, *M scrofulaceum*, *M gordonae* (Dulin 1979). Most of these species have been reclassified as *M marinum*, *M fortuitum* and *M chelonae*, but some remain unspeciated. Aquatic mycobacteria can be detected in tissue sections using Ziehl-Neelsen staining, and characterisation is usually based on growth rate, pigmentation, optimal growth temperature and biochemistry (Pungkachonboon and others 1992). Definitive identification of the type strains, *M marinum*, *M fortuitum* and *M chelonei*, is however, not possible using these conventional methods. Gómez and others (1993) and Adams and others (1995, 1996) introduced immunology-based techniques to characterise and detect mycobacteria in fish, but the results remained inconclusive with regard to species identification. Puttinaowarat (1995) subsequently re-examined some of these isolates using an enzyme linked immunosorbent assay (ELISA) and a polymerase chain reaction (PCR). Restriction enzyme analysis of the PCR products enabled identification of *M marinum*. Recently, Blackwell (1998) also reported the use of a monoclonal antibody (Mab) against *M marinum* to identify the pathogen by ELISA. This paper reports the development of methods for the detection and identification of *M marinum*, *M fortuitum* and *M chelonae*.

Materials and methods

Development of immunohistochemistry and ELISA methods

A Mab probe was prepared and then utilised in immunohistochemistry (IHC) and ELISA tests, as described by Puttinaowarat (1999). IHC was performed on fixed tissue sections from the spleen of fish, while ELISA was used with pure bacterial cultures.

Development of reverse cross blot PCR to detect mycobacteria

A PCR test was developed to detect the genus *Mycobacterium*, then a reverse cross blot step was added for the identification of mycobacteria to species level, as described by Puttinaowarat (1999). Fish, environmental samples and clinical biopsies were analysed by this method. DNA was extracted from the samples and reverse cross blot PCR performed using the method described by Puttinaowarat (1999).

Collection and analysis of field samples and clinical biopsies

Fish (kidney, spleen, liver and heart) and environmental samples (water, mosquito larvae) were collected from farms and analysed by PCR reverse cross blot hybridisation for the presence of *Mycobacterium* species. Samples were collected from snakehead and Siamese fighting fish farms. Farmers were interviewed to establish the incidence of *Mycobacterium* infection among farm staff. Biopsies were later taken by a clinician from some of the farmers reported to have lesions and these were analysed for the presence of *Mycobacterium* using PCR reverse cross blot hybridisation at the Royal Tropical Institute in Amsterdam (courtesy of Professor Kolk). Bacterial culture was attempted from biopsies at the Institute of Dermatology in Bangkok (courtesy of Dr Preeya Kullavanijaya).

Results

Detection of Mycobacterium marinum by IHC, ELISA and PCR

A monoclonal antibody (Mab 8F7) was produced against *M marinum* (S267). Characterisation of the Mab by ELISA using the various *Mycobacterium* species showed that the Mab was specific for *M marinum* (Table 1). An IHC test was developed using the Mab to detect *M marinum* in fixed tissue, as shown in Fig 1. A PCR test was developed to detect the genus *Mycobacterium*. As shown in Fig 2, a positive sample is recognised

TABLE 1: Cross-reaction of monoclonal antibody 8F7 with a variety of mycobacteria strains determined by an enzyme-liked immunosorbent assay.

Bacteria	Origin	Absorbance[a]	Reaction[b]
Mycobacterium avium	KIT	0·091	-
Mycobacterium kansasii	KIT	0·085	-
Mycobacterium tuberculosis	KIT	0·081	-
Mycobacterium gordonae	KIT	0·085	-
Mycobacterium vaccae	KIT	0·099	-
Mycobacterium intracellulare	KIT	0·127	-
Mycobacterium poriferae	NCIMB12538	0·138	-
Mycobacterium fortuitum	NCIMB1294	0·116	-
Mycobacterium chelonae	NCIMB1474	0·170	-
Mycobacterium marinum	NICBM1297	0·771	+
Mycobacterium marinum (S267)	Snakehead fish, Thailand	0·848	+

[a] The mean value of duplicate samples at 450 nm; the negative control was 0·077

[b] The cut-off point was three times of the average negative value

KIT= N.H. Swellengrebel Laboratory of Tropical Hygiene, Royal Tropical Institute, Amsterdam, The Netherlands.

NCIMB= National Collection of Industrial and Marine Bacteria, Scotland.

by the presence of a band on an agarose gel at 208 base pairs (bp). The products of the PCR were then analysed using a reverse cross blot to identify aquatic mycobacteria to species level, as shown in Fig 3. When identical samples were analysed by ELISA and reverse cross blot PCR, it became apparent that although Mab 8F7 was specific for *M marinum*, it did not recognise all *M marinum* isolates. All the isolates from Thailand were positive by ELISA, but isolates from Israel, and some from Germany and Greece were negative (Table 2).

Snakehead fish farms

The results of the PCR reverse cross blot hybridisation indicated that *Mycobacterium* were found either in the fish, the water, or in both, at all sites examined. Of the fifty-four snakehead fish sampled from the seven farm sites, 13% of the fish were positive for *M fortuitum*, 5·5% were
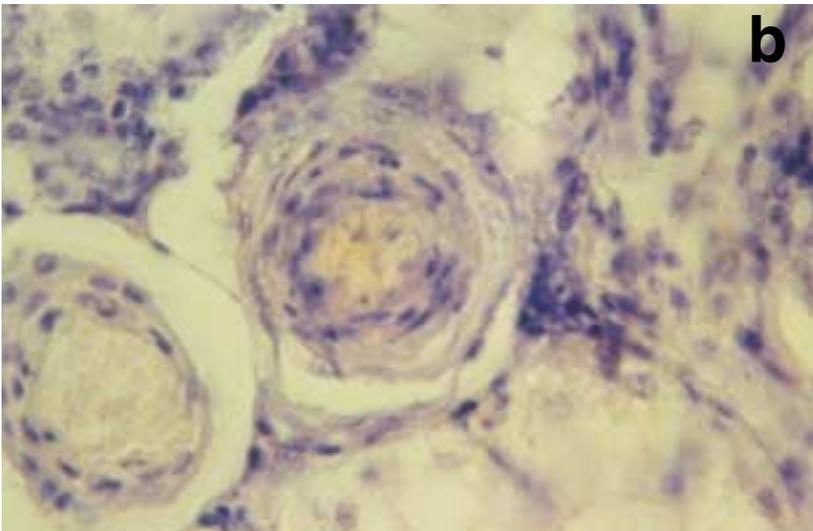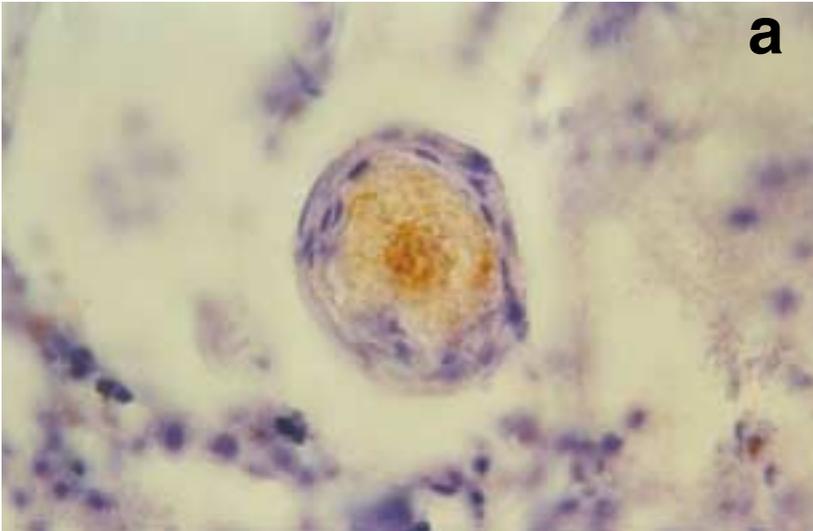
FIG 1: Spleen tissue of Siamese fighting fish (*Betta splendens*) infected with *Mycobacterium* species stained by immunohistochemistry using: (a) Mab 8F7 ×100; (b) normal mouse serum ×100
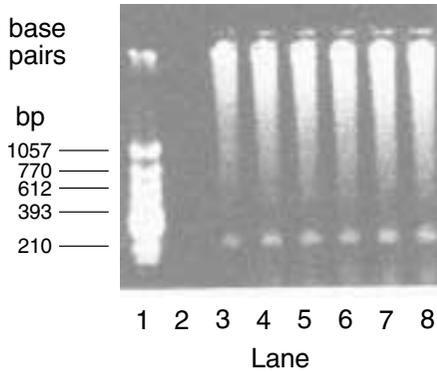
FIG 2: Agarose gel electrophoresis analysis of PCR product of mycobacteria strains. Lanes: (1) molecular weight markers [ØX/HincII], (2) tris-ethylenediaminetetraacetic acid [TE] buffer, (3) *M marinum* [NCIMB 1297], (4) M chelonae [KIT 4350], (5) *M chelonae* [KIT1474], (6) *M fortuitum* [NCIMB 1294], (7) *M fortuitum* [TB1], (8) *M poriferae* [NCIMB 12538]
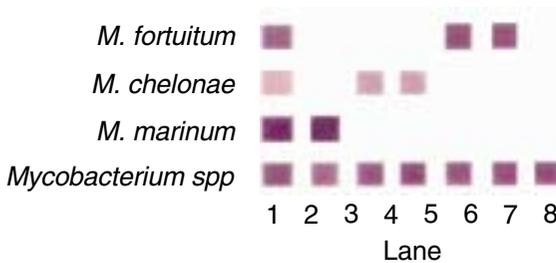
FIG 3: PCR-reverse cross blot hybridisation of mycobacteria strain. Lanes (1) DNA pool, (2) *M marinum* [NCIMB1297], (3) *M chelonae* [KIT 4350], (4) *M chelonae* [KIT11303], (5) *M fortuitum* [NCIMB1294], (6) *M fortuitum* [TB1], (7) *M poriferae* [NCIMB12538], (8) TE buffer

TABLE 2: Identification of *Mycobacterium* species isolated from different geographical regions by enzyme-linked immunosorbent assay (ELISA) and reverse cross blot polymerase chain reaction (PCR).

| Isolate	Fish	Origin	ELISA	PCR
E1	Sheepshead (*Puntazzo puntazzo*)	Israel	-	*M marinum*
E2, E11, E16	Sea-bass (*Dicentrarchus labrax*)	Israel	-	*M marinum*
E3	Sea-bass (*Dicentrarchus labrax*)	Greece	-	*Mycobacterium* sp.
E5	unknown	Germany	-	*M marinum*
E6	Red Drum (*Sciaenops ocellatus*)	Israel	-	*M marinum*
E7	Butterfly fish (*Chaetodon fasciatus*)	Israel	-	*M marinum*
E8	Sea-bass (*Dicentrarchus labrax*)	Greece	-	*M marinum*
E9	Rabbitfish (*Siganus rivulatus*)	Israel	-	*M marinum*
E10	unknown	Germany	+	*M marinum*
E12	Sea-bass (*Dicentrarchus labrax*)	Denmark	+	*M marinum*
E14, E15	Rabbitfish (*Siganus rivulatus*)	Israel	-	*M marinum*
S1, S2, S3, S4,S6, S9, S10, S15	Snakehead fish	Thailand	+	*M marinum*
S5, S7, S8, S11, S12, S13, S14, S18, S269	Snakehead fish	Thailand	-	*M fortuitum*
S267, S268	Snakehead fish	Thailand	+	*M marinum*
TB1, TB38, TB40,TB43, TB44, TB73	Siamese fighting fish	Thailand	-	*M fortuitum*
TB7, TB40, TB45, TB62	Siamese fighting fish	Thailand	+	*M marinum*

positive for *M marinum*, 5·5% were positive for both *M fortuitum* and *M marinum*, while 14·8% carried unspeciated *Mycobacterium* species. *M fortuitum* could be detected in the inlet water of four farms, while *M marinum* was detected in pond water at two sites and in the outlet water of one site. *M fortuitum*, was also detected together with *M marinum* at two of the sites. Of the eighteen farmers interviewed, none had any lesions on their skin or any previous history of skin lesions.

Siamese fighting fish farms

Mycobacterium species were detected either in the fish, in the water or in both. Of the 27 fish sampled from the nine farms, 29·6% were found to be positive for *M fortuitum*, 11·1% were positive for *M marinum* and 11·1% for unspeciated *Mycobacterium* species. *M fortuitum* was present in most of the water samples taken at the farms, while only one sample was positive for both *M fortuitum* and *M marinum*. Of the twenty-six farmers interviewed, 15% had nodule-like skin lesions on their fingers, hands, wrists, arms, ankles, feet and/or knees (Fig 4), while another 11·5% had previously experienced lesions, but had since been cured.

Additional environmental samples were collected from another five Siamese fighting fish farms around Bangkok. Both inlet water and pond water were sampled at these sites. Moina (mosquito larvae) were also sampled. Of the five pond water samples collected, one sample tested positive for *M marinum*, two were positive for *M fortuitum* and one contained both *M marinum* and *M fortuitum*, whereas one of the three inlet samples contained *M fortuitum* and another unspeciated *Mycobacterium*. Four of the moina collected from five farms contained *M fortuitum*, but no *M marinum* could be detected in any of moina samples.

Analysis of lesion biopsies

Biopsies taken from skin lesions were tested for the presence of mycobacteria by bacterial culture and PCR-reverse cross blot hybridisation. It was found that of the ten patients sampled, 50% were positive for *Mycobacterium* confirmed by bacterial culture. Using PCR-reverse cross blot hybridisation however, 40% of the farmers tested positive for *M fortuitum*, while 50% of the samples were positive for unspeciated *Mycobacterium* species. None of the samples tested positive for *M marinum*.

Discussion

The monoclonal antibody produced, Mab 8F7, appeared to be strain specific for *M marinum*. This probe worked well in IHC but has limitations as a diagnostic tool as not all *M marinum* isolates can be detected by it. With reference to epidemiology, the data obtained by ELISA and reverse cross blot PCR provided evidence that many of the *M marinum* isolates from

FIG 4: Lesions present on the skin of farmers of Siamese fighting fish: (a) hand lesion; (b) ankle lesion (Courtesy of Dr S. Chinabut).

Israel, and some from other locations such as Greece and Germany, are different in their antigenic make-up from the Thai isolates. The reverse cross blot PCR reported here detected *M marinum*, *M fortuitum* and *M chelonae*. The species-specific probes were able to detect 100fg DNA, equivalent to 20 mycobacterial cells (Puttinaowarat 1999).

Reverse cross blot PCR was utilised to screen fish and environmental samples from snakehead and Siamese fighting fish farms in Thailand for the presence of aquatic mycobacteria. Biopsies from lesions on the skin of Siamese fighting fish farm workers were also analysed by this method. *M fortuitum* appeared to be the most common species identified in fish and water samples (pond water, and inlet and outlet water) collected from the snakehead fish farms. The presence of *M fortuitum* in the water did not always correlate with its presence in fish. There was also a large proportion of unspeciated *Mycobacterium* present in fish. *M marinum* was frequently detected in both snakehead fish and in their environment, but unlike *M fortuitum*, when *M marinum* was detected in the environment it was also always detected within the fish.

Mycobacterium fortuitum has commonly been reported in the aquatic environment (Woods and Washington 1987). Stress and injury can make fish more susceptible to pathogens present in their surroundings (Nigrelli and Vogel 1963, Austin and Austin 1993, Smith 1996). This may explain why the *M fortuitum* is present in the fish's environment but sometimes not detectable in the fish and vice versa.

Mycobacterium fortuitum was also the main mycobacterial species detected at Siamese fighting fish farms, and was present in both the fish and the water in which the fish were maintained. *M marinum* was less frequently found. Some of the Siamese fighting fish farmers who were interviewed had lesions on their skin. Analysis of biopsies revealed that the main pathogen present in the lesions was *M fortuitum*, the main species identified in both the fish and the environment samples. This is contrary to the study by Kullavanijaya and others (1993) where *M marinum* was identified in skin lesions from Thai fish farmers.

Mycobacterium marinum has frequently been isolated from skin lesions of humans (Philpott and others 1963, Barrow and Hewitt 1971, Jolly and Seabury 1972, Kirk and Kaminski 1976, Huminer and others 1986, Gray

and others 1990, Lawler 1994), while the occurrence of *M fortuitum* in such lesions has been reported less frequently. *M fortuitum* was first isolated from a cold abscess in man in 1938 (da Costa Cruz 1938), and was detected in human skin lesions by Herndon and others (1972), Wolinsky (1979) and in Westmoreland and others (1990).

Only the Siamese fighting fish farmers appeared to have lesions on their skin, while none of the snakehead fish farmers interviewed were affected. This is probably due to differences in husbandry practised between the two fish species. Siamese fighting fish farming involves contact with fish and their environment every 3–5 days including changing the water from the bottles in which the fish are cultured, and gathering insect larvae. The insect larvae are grown in ponds, which contain fermented pig excrement to attract the insects. The incidence of skin lesions among Siamese fighting fish farmers has been found to be significantly higher in people responsible for collecting insect larvae (Nakhon Pathom Province Public Health Office 1997). Snakehead farmers are only in direct contact with the fish and the pond water during harvesting, when the farmer actually enters the water to catch his stock.

It was not possible to identify the unspeciated mycobacteria observed during the study, since only four probes were used here for the reverse cross blot hybridisation, specific for either *M marinum*, *M fortuitum*, *M chelonae* or genus *Mycobacterium*. Further analysis of the samples with the nine *Mycobacterium* probes as described by Kox and others (1995, 1997) may be useful for speciating other *Mycobacterium*.

The sampling method used here for fish was destructive and this is not acceptable with high value stock, such as brood stock. Analysis of blood or skin scrapings will therefore be assessed as an alternative in the future.

In conclusion, PCR-reverse cross blot hybridisation was shown to be a useful method to examine the prevalence *Mycobacterium* species in the aquatic environment. This technique will be utilised in future studies to monitor ornamental fish in exporting and importing countries to identify aquatic mycobacteria to species level. It may also prove useful for environmental monitoring in public aquaria.

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Fish surgery: an overview

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Health problems which can be treated exclusively by surgical means are relatively uncommon in fish. Consequently, only a limited number of procedures have been developed and reported in the literature. These are usually performed on individual cases and often restricted to pet fish with a high emotional and financial value, or large rare specimens kept in public aquaria. This article reviews several surgical procedures that have been performed in fish and highlights various aspects of surgical care which relate specifically to aquatic animals.

General considerations

In general, fish are considered good surgical patients, however in contrast to other animals, the nature of their aquatic life presents several limiting factors. In particular, the level of husbandry and management often plays a significant role in the success of any treatment. Ornamental fish disease is often complicated by the presence of concurrent disease or additional unidentified pathology. The diagnosis of internal disorders which might benefit from surgical intervention is difficult and often requires specialised imaging equipment. Anatomical differences between species can vary considerably: the intra-abdominal adhesions in carp and the orientation of organs in flat-fish are examples. The ingress of water into wounds in freshwater fish and the potential for secondary bacterial or fungal infection present additional obstacles.

Practical problems associated with the duration of surgery may require equipment to recirculate anaesthetic solutions. The cost for professional time, together with the guarded prognosis of many cases following surgery may further limit surgical opportunities in general practice. Despite all this, by adopting a logical surgical approach and taking these factors into account, it is possible to achieve good results.

Underwater surgery

The main advantage of this approach is that it caters for the aquatic needs of the patient. However, in addition to the obvious problems associated with wound contamination and osmotic tissue damage, the rippling surface caused by movement in the water and the presence of escaped blood usually obscures the field of view. Tying knots in loose floating suture material with needle holders can also prove difficult.

Out of water surgery

Most surgery on fish is performed out of the water, with the advantage that surgeons can operate using techniques similar to those used on terrestrial animals. However, only brief procedures lasting less than four minutes are possible without the use of an anaesthetic delivery system to provide a low maintenance dose of anaesthetic and fresh oxygenated water. Various techniques, using both recirculating and non-recirculating equipment, have been described in the literature (Brown 1987, Ross and Ross 1999, Lewbart and Harms 1999). Where submersible pumps are used, it is important to consider electrical safety for the operator. Due to the potential hazards of using 240 volt electrical supplies in the UK, it is advisable to use a residual current device (RCD) or low voltage (12 volt) pumps.

The lack of reflexes which can be used to assess the depth of anaesthesia in fish may necessitate the use of either an electrocardiograph or doppler pulse ultrasound probe, particularly during prolonged anaesthesia. In addition, it is essential to keep the external body surfaces moist by periodic irrigation of the skin to avoid dessication of the delicate tissues.

Anatomy of the skin

Some understanding of the structure of the skin is important in order to achieve surgical success. The cuticle is the outermost layer of the skin and allows the fish to move smoothly through water. It is about 1 μm thick and consists of mucus and cell debris. It also contains antibodies (IgM) and lysozymes which have antibacterial and antifungal properties. The epidermis consists mainly of fibrous malpighian cells and, together with the cuticle, forms a waterproof barrier. The thickness of the epidermis varies with species, age and site on the body. Mucus-secreting 'goblet cells' are

located in the epidermis, and in some species, 'club cells' secrete a potent alarm substance when the skin is damaged.

Unlike mammals, the epidermal cells at all levels are capable of mitotic division, although most occurs at the deepest level near the basement membrane. During wound healing there is a loss of the intercellular attachments and cells migrate rapidly to cover the defect and provide some waterproof integrity. This leads to a reduction in the thickness of the surrounding epidermis and produces a thin layer of epidermis at least one cell thick over the wound. This process occurs independent of water temperature but is inhibited by the presence of pathogens and necrotic tissue. Later, these malpighian cells undergo cell division to restore the epidermal layer. This is a slower process and is dependant on ambient temperature.

The dermis consists of two layers. The upper layer contains collagen and reticulin, which form a supportive network. The lower, deeper layer of the dermis is a more compact mass of collagen fibres which provides the main structural strength of the skin. The scales are flexible bony plates which develop in scale pockets in the upper loose connective tissue; they are not shed regularly but grow with the rest of the body. Some fish do not have scales and the epidermis is often thicker in these species. The dermis may also contain pigment cells called chromatophores. The hypodermis is the deepest layer of the skin and consists of loose fatty tissue connecting the skin to the underlying structures. Due to its design and good blood supply, bacterial diseases will spread rapidly along this layer.

Special equipment

Opportunities to perform surgery in a suitably-equipped operating theatre can be limited. In general practice, it is often restricted to using a makeshift operating surface on location, beside the tank or pond, using basic equipment and with brief surgical anaesthesia. However, a self-contained operating surface with drainage to allow the collection and recirculation of anaesthetic solutions, or drainage to waste, is useful. During prolonged anaesthesia using forced ventilation it is essential to avoid flooding the surgical site. This can be achieved by placing the fish's head through a slit rubber sheet, positioning it behind the operculae to deflect the anaesthetic solutions as illustrated in Stoskopf (1993).

Fish can be restrained using various mechanisms appropriate to the size of the fish and the site of surgical interest. Wet foam troughs and long foam wedges are commonly used supports.

Different materials have been used as surgical drapes, including waterproof and non-waterproof paper, and various plastic drapes including rectal gloves. Disadvantages of non-waterproof drapes are that they absorb water, tend to disintegrate easily and can damage the cuticle and epithelium when removed following surgery. Towel clips are not particularly useful in fish and drapes are usually held in position by moisture, vaseline, tissue glue or temporarily sutured to the wound margin.

The use of surgical gloves is a personal choice but failure to use them rarely causes additional damage to the delicate skin. In some cases, such as the debridement of external wounds, it is easier to use bare finger-tips to identify the rough surface of loose damaged scales since these are often transparent and difficult to see.

Various suture materials, both absorbable and non-absorbable, have been used in fish surgery, although the choice is often a personal one. Due to the small size of some fish, the use of monofilament material with atraumatic swaged-on needles minimises tissue damage and prevents bacterial ingress through the capillary effect of braided suture materials. Those commonly reported in the literature include polypropylene (Prolene®, Ethicon), polyglyconate (Maxon®, Cyanamid), polyglactin (Vicryl®, Johnson & Johnson), polydioxanone (PDSII®, Johnson & Johnson), chromic catgut and fine surgical steel. Absorbable sutures used in the skin do not usually require removal, although those used internally may not be absorbed and can often be found intact, years later. The knots of external sutures must be tied carefully and be very secure since they are more likely to loosen underwater: this can be avoided by applying a drop of tissue glue to the knot. Cyanoacrylate tissue glue can be used to seal small wounds and suture lines, and act as a protective patch on corneal ulcers. However, some fish can develop an allergic reaction to some adhesives.

Techniques

In general, surgical techniques employed in fish are often rather primitive but can prove very effective. In contrast to mammals, most fish are small,

and some tropical fish are very small. Consequently, familiarity and experience with microsurgery, together with a range of small scissors, forceps and needle-holders is helpful. Optical loupes with a magnification of ×2 to ×4 are generally adequate for most procedures.

Haemorrhage is rarely a problem and can be controlled by various routine methods. Bleeding from a capillary bed can be arrested by digital pressure and often ceases when the fish is returned to the water. Larger vessels can be crushed using artery forceps, or ligated using suture material or vascular clips. The topical application of adrenalin is also effective. Heat cautery is very effective as is electrocoagulation although the latter can be damaging to adjacent tissues in small fish.

The use of cryosurgery has been reported in the hobby press and demonstrated on video by Reynolds (1993) as a treatment for body ulcerations and external neoplasia. However, there has not been any critical appraisal or scientific study to show that this approach has any advantage over other surgical techniques.

Pre-operative preparation

To avoid complications with anaesthesia, such as regurgitation, it is advisable to starve the patient for 24 hours. Physiological stress can be reduced by thoroughly aerating the water and ensuring good water quality. Where possible, it is also helpful to treat any concurrent parasitic and bacterial infection prior to surgery.

Although it may appear desirable to prepare a sterile operating field, this is rarely possible. Fish skin is easily damaged by many skin disinfectants and alcohols, and wound healing may be delayed. Even dilute povidone-iodine solutions, commonly used on some species, are harmful to others. The protective cuticle contains various antibacterial components which limit bacterial wound infections. Therefore, skin preparation is usually limited to gentle swabbing to remove most of the mucus from the surgical site.

Depending on the degree of surgical invasion, it may be necessary to cut through the skin. This is rarely a problem in some scaleless species or varieties of carp, but in most fish, the scales at the site of incision should be removed carefully using forceps. However, this can be difficult in armoured species of catfish and sterlets which are covered in hard bony scales.

Fish do not have eyelids and cannot rapidly alter the size of their pupils. Consequently, they are sensitive to the glare of bright lights in the operating theatre and it is recommended that the eyes of unconscious fish are covered to avoid retinal damage.

Procedures

A variety of successful surgical procedures can be performed on fish. In practice, most operations are carried out on external surfaces, under brief anaesthesia or sedation. However, it is possible to carry out longer and more sophisticated procedures with the use of a recirculating anaesthetic system.

The surgical treatment of skin ulcers is commonly performed in pond fish such as koi (*Cyprinus carpio*) and goldfish (*Carassius auratus*). These ulcerations can can occur on all areas of the body, including the head, mouth, eye and fins. They may be the result of physical trauma from fighting, predation, ectoparasites and bad handling but also develop from chronic infection with *Aeromonas* species and other bacteria. Various methods of treatment have been described (Scott 1992***a***, Reynolds 1993) and much depends on personal preference. The author's approach is to anaesthetise the fish and remove it from the water. Debride the wound by removing necrotic tissue with dry cotton buds or gauze swabs and pluck out any damaged scales. Apply dilute povidone-iodine topically, dry the site and apply a waterproof preparation to the lesion: two commonly used products are Orahesive® and Orabase® (ConvaTec). Orahesive® is a fine powder containing equal quantities of gelatin, pectin and methylcellulose. Orabase® contains the same proportion of ingredients but is made into a paste with an equal amount of liquid paraffin. The debridement is performed only once, since epithelialisation will be impeded by further interference unless there is persistent tissue necrosis. An appropriate antibacterial is given by injection and repeated at suitable intervals or a suitable formulation is given as an in-feed preparation. The latter may be surface-coated or compounded into the food by the manufacturer.

Large traumatic skin wounds with a flap of loose tissue can be repaired by suturing the apposed edges of the wound following adequate tissue disin-fection. A similar method can be used to repair some lacerations on the fins.

Bacterial disease of the fins, often called 'fin rot' or 'tail rot', is common either as an extension of body ulceration or due to infection with *Flavobacterium columnare,* formerly *Cytophaga/Flexibacter columnaris*. To avoid the infection spreading up the 'rays' of the fins or tail, it is important to cut off the diseased portion with a healthy edge of tissue. The exposed healthy surface should then be treated like an ulcer as described above. In time, the fins will often regrow to their original shape and size.

Some parasites such as leeches (*Piscicola* species) and anchorworm (*Lernaea* species) require careful manual removal. *Dermocystidium koi* is an uncommon fungal infection in koi producing raised swellings, up to 1 cm on the body and fins, and may require surgical excision (Wildgoose 1995). Debridement of the underlying tissues may be required since the fungal hyphae often penetrate deep into the dermis. The sites of infection can then be treated like an ulcer as described above.

Various external tumours have been recorded in fish and these may grow to a significant size without causing clinical problems to fish in captivity. Several cases are reported in the literature (Reid and Backman 1988, Probasco and others 1994, Lewbart 1998, Wildgoose 1998) but many probably go unreported. Surgical intervention is a personal decision and the author uses the same criteria as used on other pets: tumours are removed if they interfere with normal activity, by causing difficulty eating, breathing or swimming, or if they are ulcerated, haemorrhaging or become infected. Total excision of tumours with a margin of normal tissue is rarely practical and wound closure is seldom possible due to the inelastic nature of the skin and presence of scales. Even pedunculated lesions often regrow due to remnants of neoplastic tissue. Excision as close to the body surface as possible may be the best that can be achieved by debulking procedures. Those masses which appear inoperable, can be investigated by taking a punch or wedge biopsy and treating the sampling site like an ulcer. Small sites can be dried thoroughly and covered with a drop of tissue adhesive. Some skin tumours have a viral aetiology and regrowth may occur at the same site or elsewhere on the body.

Puffer fish (Tetraodontidae family) and porcupinefishes (Diodontidae family) have fused teeth which form a strong 'beak' that is used to crunch up their natural diet of hard-shelled molluscs and crustaceans. However, in captivity, they are often fed on finely chopped meaty foods. Consequently,

overgrowth of this 'beak' through lack of dental exercise may occur and this may require trimming with scissors or fine saw (Rees Davies 2000). Great care should be taken when handling these species so as to avoid stress and thus causing them to 'inflate' and potentially increasing the risk of damage to the fish's skin and spines.

Oral and gastric foreign bodies have been reported in various species, including goldfish (Clark 1988) and red tailed catfish, *Phractocephalus hemioliopterus* (Sands 1995). In some cases, gravel or similar substrate materials are often regurgitated up to several days later. However, some foreign bodies will require manual or surgical removal and various cases have been reported (Scott 1992*b*, Lewbart 1998, Wildgoose 1999). In large fish, where endoscopic removal is not possible, foreign bodies can be removed manually *per os* under sedation or anaesthesia. The use of a short piece of rigid plastic pipe, large enough to fit into the patient's mouth will allow the operator to pass a lubricated arm through the pipe into the pharynx, oesophagus or stomach. The pipe will offer protection from the sharp teeth of some fish such as sharks. However, the gastric juices in some species can be very irritant to exposed human skin and protective gloves or lubricant is recommended. The successful removal of a papilloma from the posterior pharynx of a koi has been briefly described (Lewbart 1998) and the author has excised a granulomatous abscess from the pharynx of a marine porcupinefish, *Diodon holacanthus* (Wildgoose, unpublished data).

Superficial corneal ulcers in fish can be caused by trauma and infection by various organisms. Fluorescein stain can be used to reveal the extent of ulceration after the anaesthetised fish is removed from the water. Treatment of the underlying cause with the appropriate antimicrobial drugs and improving water quality is often successful. However, inadequate healing may require surgical intervention and cyanoacrylate tissue glue has been used as a temporary patch on minor corneal ulcers, following antibiotic treatment of the exposed surface (Whitaker 1993, Williams and Whitaker 1997). This patch can last for between five and ten days.

Severe ocular disease can result from trauma, uveitis or neoplasia. Penetration of the globe can follow deep corneal ulceration or trauma and is common in fancy varieties of goldfish which are specifically bred with over-sized eyes. Failure to respond to treatment, secondary infection and deterioration in the health of the fish may necessitate enucleation. The

globe sits freely within a large orbit and is held in place by an annular ring of cartilage and a thick scleral attachment to the skin. Under general anaesthesia, removal is relatively straightforward, requiring simple sharp dissection with a scalpel or scissors. Haemorrhage is often minimal but the optic vessels may require ligation or heat cauterisation. The socket should be packed with waterproof paste and systemic antibacterials administered. During healing, the orbit will become lined with epithelium and remain as an empty socket.

The implantation of microchip transponders in pet animals is now routine in general veterinary practice. These devices register a unique alpha-numerical code when activated by a suitable scanner, and this can be used to identify the individual against details which are kept on a national database. Recently, a number of valuable carp in stillwater fisheries have been microchipped in Cornwall as an anti-theft deterrent (H. Thresher, personal communication). Harmonisation of microchip standards in Europe has allowed a more widespread use of these implants but due to the limited range of detection of some scanners it is essential to implant the microchips at the same site in the same class of animal. In the case of fish, guidelines from the British Zoological Veterinary Association advises deep implantation in the midline, anterior to the dorsal fin. However, other sites used include the left side at the base of the dorsal fin in fish over 30 cm in length, and in the coelomic cavity if smaller.

Buoyancy problems are common in some species such as fancy varieties of goldfish. Although these are often mentioned in the hobby press they are rarely reported in the scientific literature. They are often of sudden onset, affecting individual fish, and can result in varying degrees of positive or negative buoyancy or producing abnormal posture. Many occur spontaneously with no obvious predisposing cause but some are secondary problems. Some fish may respond to conservative treatment such as short-term starvation and altering the water temperature. Those which fail to improve may be presented for veterinary treatment. Abnormal and excessive positive buoyancy in some fish can be due to dysfunction of the swimbladder, with subsequent skin damage resulting from dessication. Radiographs of these cases may reveal a displaced or over-inflated swimbladder. Careful aspiration of gas through the abdomen using a needle and syringe can occasionally resolve the problem, although this may need to be repeated periodically (Lewbart 1998, Wildgoose 1999). Most cases, however, recur due to untreatable complications. Negative buoyancy

disorders can also occur under similar circumstances and may produce skin lesions due to constant contact with the bottom of the tank or pond. These cases have a poor prognosis although one case, recently reported in the popular press, has survived for several months using a body sling attached to a polystyrene float (Anon, 1999).

Various diagnostic procedures are performed under sedation or general anaesthesia. Endoscopy of the oral cavity and upper gastro-intestinal tract is easily carried out although examination of the oesophagus in carp can be restricted by the presence of pharyngeal teeth. Paracentesis can be used to aspirate fluid from the coelomic cavity to assist the diagnosis of some internal disorders. However, it is seldom a successful method of treating ascites, even when performed repeatedly, since this is often due to various terminal diseases and neoplasia. Fine-needle aspirates from intra-abdominal masses can be performed, particularly if they can be palpated or felt by ballottement. Localisation of these may require supportive imaging procedures such as radiography and ultrasound. In some cases, magnetic resonance imaging (MRI) or computed tomography (CT) have also been used (Lewbart 1998, Lewbart and others 1998).

Some advanced procedures have been reported but often require specialist skills and equipment. Cataracts in fish have many different aetiologies, including nutritional imbalance, ultra-violet radiation, parasites, osmotic imbalance, genetic factors and toxic insults. Their surgical removal has been recorded and is illustrated photographically in Whitaker (1999). By comparison to the lens of other vertebrates, phacoemulsification has not proven effective due to the hardness of the cataracts in fish.

Invasive intra-coelomic procedures have also been performed for both diagnostic and surgical purposes. Laparoscopy, using an endoscope and insufflation of the abdomen with air, allows the operator to inspect, culture and biopsy most organs within the coelomic cavity through a small incision in the body wall. Exploratory surgery has been used to successfully remove an abdominal tumour in a koi (Lewbart and others 1998), correct a swimbladder abnormality in a cichlid, *Cichlasoma citrinellum* (Lewbart and others 1995) and excise an abdominal mass in a gourami, *Colisa labiosa* (Harms and others 1995). The surgical treatment of dystocia in a stingray, and the repair of a spinal fracture in a grouper using an external fixator are briefly described and illustrated in Lewbart (1998).

Most intra-coelomic surgery is performed through a ventral midline incision and its length is usually dependant on the nature of the procedure. Although it is important to avoid damage to the structures near the cloaca, it is usually necessary to cut through the pelvic bones. In immature fish, prior to fusion of the pelvic bones, it is possible to cut through the pelvic symphysis with a scalpel. However, in older fish this may require the use of an osteotome, but despite this, fish appear to have few post-operative complications following pelvic osteotomy. Where possible, the incision through the skin and abdominal muscle should be closed in two separate layers. To avoid excessive positive buoyancy, it is important to remove all free air within the coelomic cavity and this may require aspiration with a needle and syringe following wound closure.

Several advanced procedures have been developed for use in research and are reported in the literature. The implantation of an arterial cannula into the dorsal aorta is used to allow repetitive blood sampling, and intra-arterial drug administration in free-swimming fish (Smith and Bell 1967) is a well-known laboratory procedure. Pinealectomy (Goetz and others 1977) and gonadectomy in salmonids (Brown and Richards 1979) have been described in the literature as research tools to gather data on various endocrine and growth parameters. Surgical removal of the spleen, thymus and other lymphatic tissues have been performed during studies on the immune system in fish. Neurological function studies involving the removal of some brain tissue has also been described (Ott and Platt 1988). More recently, surgical enucleation and insertion of an ocular prosthesis has been performed on several stripped bass, *Morone* species (Nadelstein and others 1997).

Post-operative care

Hospitalisation is not always possible in the veterinary surgery and in the case of large fish, is not always practical. Unless large volumes of water from the tank or pond of origin can be transported to a hospital facility, a more rapid recovery is often achieved if the fish is returned to its original established environment. Unfortunately, post-operative follow-up of these cases can be limited.

The use of an isolation tank can be helpful, particularly if the patient is bullied by other fish. This enables the patient to be observed easily and

permits various environmental factors to be manipulated. In the case of coldwater pond fish, raising the temperature gradually to 20°C (68°F) can improve the rate of recovery. However, the water temperature should not exceed the preferred temperature range for the species since this may produce stress and delay recovery. Freshwater species can benefit from the addition of sodium chloride salt to the water. Initially, a dose of 1·5 gram/ litre (quarter ounce per imperial gallon) can be used, but this can be increased up to 6 gram/litre (one ounce per imperial gallon) over the period of a few days, if recovery is slow. Additional aids such as subdued lighting, suitable refuges or hides, and companion fish for shoaling species may also be of value. Despite all this, it is essential that the isolation tank is sufficiently large enough for the patient and that water quality is monitored regularly and maintained at a high level.

Although some surgical procedures in fish may not involve bacterial infections, it is rarely possible to ensure complete surgical sterility and consequently, systemic antibacterials should be used. These can be administered effectively by injection or in the food, or as a bath medication in the case of very small fish.

Where non-absorbable skin sutures have been used, their removal depends on the rate of wound healing. This is often related to the species of fish, its nutritional status and the water temperature. However, as a rough guide, sutures may be removed after four weeks in tropical fish, and up to eight weeks in coldwater species.

At present, the use of post-operative analgesia in fish has occasionally appeared in the literature. Lewbart and others (1998) used butorphanol at a dose of 0·1 mg/kg given subcutaneously to a koi following intra-coelomic surgery. In order to achieve accurate dosing of small fish, the proprietary product (Torbugesic®, Fort Dodge) must be diluted in water. While it is generally accepted that fish can feel pain (Kestin 1994, Sainsbury 1994), due to the unknown pharmacokinetic effects of analgesic drugs in fish, this aspect of surgical care requires further evaluation.

Conclusion

This article illustrates the range of surgical procedures that can be performed on ornamental fish, and highlights some of the problems relating

to their aquatic environment. It is hoped that this may encourage more veterinarians to perform surgery in these patients. Some cases will be unique events and readers are urged to write up their surgical cases and contribute to our knowledge of fish surgery. It is essential to follow up all cases, regardless of the difficulty of facing up to failure. This will quickly help to establish the limits of what can be achieved and often reward you with the surprise of success.

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Myxosporidiosis of fish and the bryozoan link with proliferative kidney disease (PKD) of salmonids

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Abstract

This paper provides a brief review of the Myxosporea as agents of fish disease and gives examples of their impact on wild and cultivated fish species. In particular, the effects of proliferative kidney disease (PKD) in salmonids and myxosporidiosis of cyprinids are described. Information on developments in the understanding of the life cycle requirements of these parasites is also provided, with emphasis on the recent breakthrough of the recognition of Bryozoa as hosts of Tetracapsula bryosalmonae*, the causative agent of PKD*.

Introduction

The Myxosporea (Phylum Myxozoa) are very common parasites of fish and include a few species that also infect amphibians, reptiles and invertebrates. They are multicellular organisms possessing a spore comprising one to seven capsulogenic cells that form the polar capsules, up to seven valvogenic cells enclosing the polar capsules and the infective sporoplasm, which consists of one to twelve cells. Spore morphology is extremely varied, particularly among the marine genera. Although some myxosporeans are serious pathogens, which have been associated with significant epizootics in wild populations and losses in aquaculture (Lom and Dyková 1992), most are relatively innocuous causing little harm to the host. Marine myxosporeans are also not usually associated with overt disease. Coelozoic species are known to induce pathological changes in their hosts (Feist and Bucke 1992), although these are usually minor. Amongst the histozoic species, perhaps the best-known marine myxosporean genus is *Kudoa*. Members of this genus, and especially *K thyrsites,* infect the musculature and upon death of the host the release of proteolytic enzymes, by a

mechanism as yet not exactly known, renders the flesh unmarketable (Lom and Dyková 1995). In mainland Europe, several myxosporean parasites have been identified as serious pathogens of cultured carp (Dyková and Lom 1988). Numerous scientific papers have been published on aspects of biology and pathogenicity of *Sphaerospora renicola* and it is well known that the extrasporogonic proliferative stages in the blood and swimbladder, and not the spores, induce the host response (Lom and others 1983, Molnár 1988). Similarly, the intracellular extrasporogonic stages of *Hoferellus cyprini* induce the dramatic cellular hypertrophy in the renal tubules of carp (*Cyprinus carpio*). The closely related parasite *H carassii* is responsible for kidney enlargement disease (KED) in goldfish (*Carassius auratus*) in which the principle pathology is primarily one of cystic papillomatous hyperplasia. Intracellular stages are also present and these result in hypertrophy of the infected cells.

Histozoic infections affecting the gills of freshwater fish can be lethal where the presence of numerous parasitic cysts impair respiratory function or facilitate the entry of secondary pathogens into the host on rupture of the cysts. One example is *Sphaerospora molnari*, which is regarded as a potential threat to cultivated carp (Dyková and Lom 1988). However, its occurrence appears to be sporadic. The factors influencing epizootics with *S molnari* and other myxosporeans are poorly known, but are probably related to the presence of oligochaete intermediate hosts. The need to understand these factors is particularly important since *Myxobolus cerebralis*, the agent of salmonid whirling disease, with a known oligochaete intermediate host, has recently gained prominence as the main factor implicated in catastrophic declines in wild rainbow trout populations in North America (Hedrick and others 1998).

Life cycles and proliferative kidney disease

Until the pioneering studies by Wolf and Markiw (1984) it was thought that myxosporeans were transmitted directly from fish to fish. Subsequently, it was established that an intermediate or alternate oligochaete host was an essential requirement in the development of many of these pathogens. Within the oligochaete they develop into the actinosporean stage (actinospore) which is the infective stage to fish and which usually has quite different morphology to the myxosporean stage (myxospore) within the fish host. Several studies have now demonstrated this link in a number of

different fish species (El-Matbouli and Hoffmann 1989, Kent and others 1993, Uspenskaya 1995, Andree and others 1997, Bartholomew and others 1997). It is now also clear that other invertebrate species can also act as intermediate or alternate hosts (Bartholomew and others 1997). However, few of the studies to date have successfully been repeated by other workers.

Proliferative kidney disease (PKD) and the causative agent, *Tetracapsula bryosalmonae* (Canning and others 1999), formerly known as 'PKX', have been the subject of intensive research investigations for many years (Hedrick 1993). Although it has long been known that *T bryosalmonae* was an unusual myxosporean (Fig 1) because it did not appear to form spores with fully formed valves, it has only recently been established using molecular data that the *T bryosalmonae* organism is only distantly related to other myxosporeans (Kent and others 1998). A breakthrough in understanding regarding *T bryosalmonae* has resulted from a pivotal discovery by Dr Okamura at the University of Reading, of free-floating parasitic sacs in the freshwater bryozoan *Cristatella mucedo* in the UK. These sacs were recognised as an unusual myxosporean and in 1996 a paper describing a new genus and species (*Tetracapsula bryozoides*) was published (Canning and others 1996). After publication, Feist (1997) and Kent and others (1998) pointed out the similarity of small electron-dense structures: 'haplosporosomes' in 'PKX' and the sporoplasmosomes of *T bryozoides*. However, molecular evidence showed that 'PKX' and *T bryozoides* were not conspecific. Subsequently, similar myxosporean (*Tetracapsula* species) sacs were discovered in the North American bryozoans *Plumatella rugosa* and *Pectinatella magnifica*. DNA sequence data showed that some of these isolates were in fact *T bryosalmonae* (Anderson and others 1999). Most recently it has been confirmed for the first time in the UK and Europe that *T bryosalmonae* infects different species of bryozoans, *Plumatella* species and *Fredericella sultana* (Longshaw and others 1999) (Fig 2). On the basis of morphological differences and the DNA sequence data, Canning and others (1999) were finally able to name the 'PKX' parasite as *T bryosalmonae*.

The discovery has opened up significant possibilities for the elucidation of the transmission requirements of *Tetracapsula* to farmed and wild fish. During the summer of 1999 transmission experiments conducted at CEFAS Weymouth successfully induced PKD in naïve rainbow trout exposed to bryozoans known to be infected with *T bryosalmonae*. Briefly, the
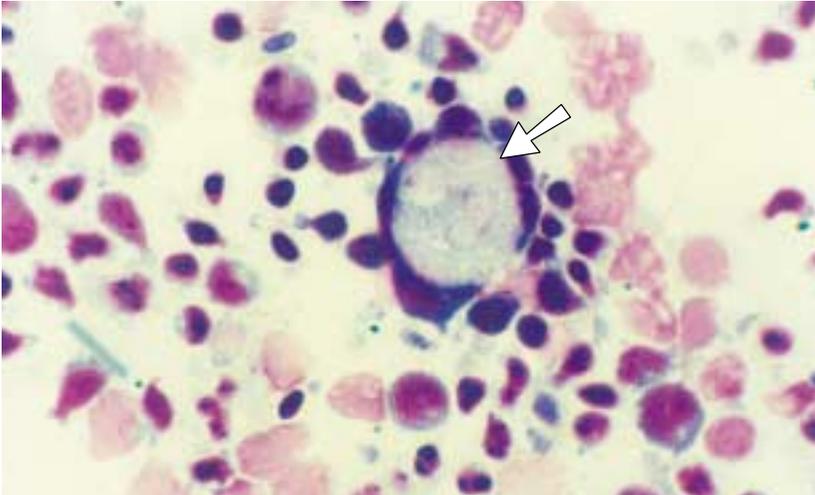
FIG 1: Renal impression smear of kidney from rainbow trout clinically infected with PKD. *Tetracapsula bryosalmonae* cell (arrowed) is clearly visible, surrounded by host phagocytes and lymphocytes. (May-Grünwald Giemsa) ×400

FIG 2: Portion of a colony of *Fredericella sultana* showing a single zooid with the characteristic lophophore (horseshoe-shaped crown of tentacles surrounding the mouth) clearly visible. Fresh preparation ×30
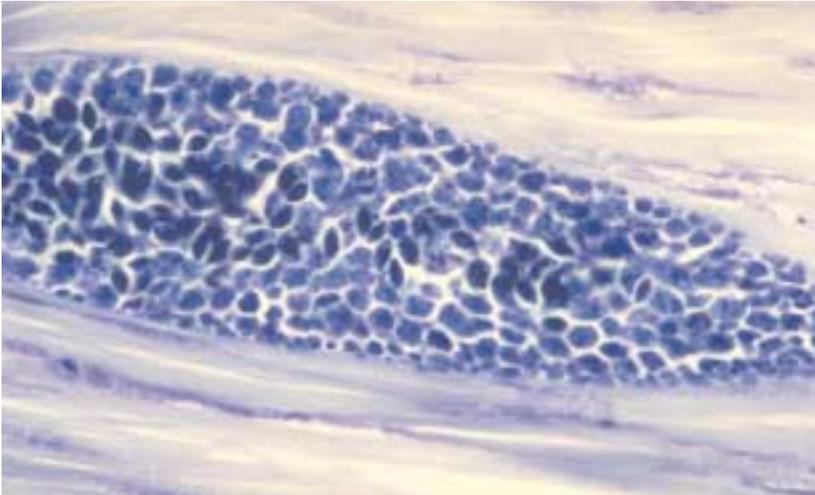
FIG 3: Cyst of *Myxobolus* species within the skeletal muscle of a chub fry. Note the numerous sporogonic stages with mature spores present towards the centre of the cyst. (Giemsa) ×200
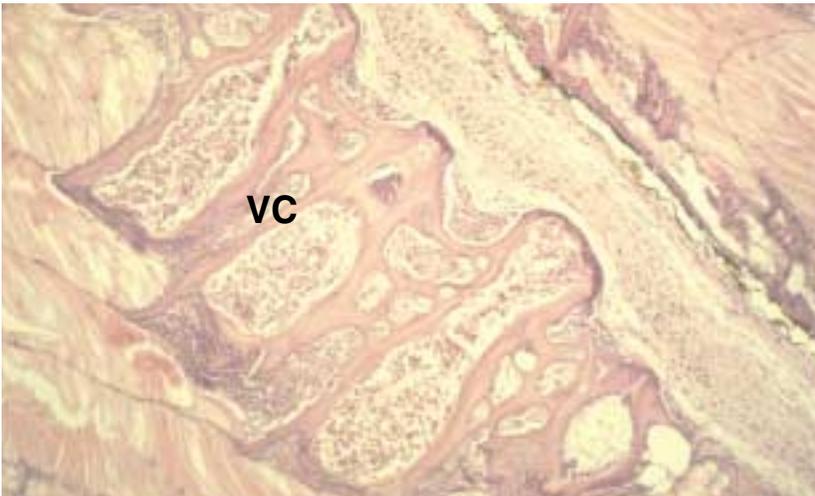

FIG 4: Section of vertebral column (vc) from a chub fry showing severe pathological change associated with cysts of a *Myxobolus* species. Note the pressure exerted on the spinal cord by the cysts. (H&E) ×90

experiments involved challenge of fish by short-term bath exposure to disrupted colonies of infected bryozoans; longer-term exposure of fish to intact infected bryozoan colonies, and injection of isolated *Tetracapsula* sacs and spores into the peritoneal cavity. Fish from the two bath exposure groups became infected and fish showed clinical signs of PKD. However, the group injected directly with the parasite did not become infected. The full scientific paper describing this work is in preparation. Further studies are in progress to investigate in detail the behaviour of the parasite once released from the bryozoan host and in particular the mechanisms of infection and early pathogenesis.

In summary, the breakthrough in identification of the true nature and probable origin of the infectious stage of PKD to fish has stimulated research efforts in this area and provided many new promising leads. These will certainly give rise to more discoveries which, it is hoped will facilitate the eventual control of PKD.

Myxosporidiosis of cyprinids

Epizootics in wild freshwater fish have been the subject of ongoing research at the CEFAS Weymouth Laboratory for a number of years and have focussed on the impact of myxosporidiosis caused by *Myxobolus* species on juvenile cyprinids and on the possible effects on recruitment of infected fish to adult populations.

To date, over three thousand cyprinid fry, mainly roach (*Rutilus rutilus*), chub (*Leuciscus cephalus*), dace (*Leuciscus leuciscus*) and minnow (*Phoxinus phoxinus*) collected from rivers in Yorkshire and Humberside have been examined histologically for myxozoan infections. The main parasite of concern is the muscle myxozoan *Myxobolus cyprini* sensu lato. The parasites develop within the muscle fibres and, whilst contained within a cyst (Fig 3), cause minimal damage. Most infections are innocuous, although there is some evidence of pressure atrophy on the surrounding tissues caused by increasing size of the cyst. When cysts rupture as a result of parasite maturation, the spores are released into the surrounding tissue. They are antigenically active at this stage and elicit a vigorous host response. The pathological changes that ensue can be debilitating to the fish host and in severe cases it is unlikely that affected fish will survive and be recruited into the population. However, levels of infection can fluctuate

dramatically from year to year and between different rivers sampled in the same year. Chub are most severely affected by the parasite, followed by minnow, roach and finally dace, which are least impacted.

Additionally, in recent years we have monitored for the presence of *Myxobolus ellipsoides*. This is a parasite of the vertebral column that causes spinal compression (Fig 4) and fusion of the vertebrae. It is a parasite specifically of chub, and is very occasionally found in roach and bream (*Abramis brama*). It is likely that the resultant pathology reduces the fishes' ability to capture food items and avoid predators. In Yorkshire, it appears to be restricted in its distribution to three rivers where up to 90% of chub fry can be infected. Whilst we have no direct evidence of an impact on recruitment of affected fish, it is interesting to note that few 'stumpy' adult chub are caught. It appears likely that infected chub fry are removed from the population prior to reaching adulthood.

The work of investigating factors that determine why certain populations and certain fish species become more parasitised than others is ongoing. Whilst it is appears that myxozoans can have an involvement in determining the population structure, there are many other factors including other parasites and diseases, habitat degradation and changes in food availability which also have an effect on the fish. Progress in understanding the complex interactions between hosts, parasites and environmental factors will only be made by further studies using a multi-disciplinary approach, with additional input from fisheries biologists and ecologists.

Conclusions

The Myxozoa are a diverse group of parasites which seem to continually surprise those who study them, either as agents of disease or for their biological interest. Understanding of some aspects of their complexity in developmental cycles within the fish host, and then the recognition of their requirement for alternative hosts (oligochaete and polychaete worms) has surprised many experts in the field. In the case of whirling disease, this important information provided a management solution based on the avoidance of the infective stage. Now the discovery of bryozoans as yet another host has increased the number of animal phyla that they utilise for survival. It remains to be shown whether Bryozoa are the 'true' hosts for *Tetracapsula* and whether infection of the fish hosts and PKD are

'accidental', a hypothesis which had often been suggested in the past. Further discoveries will undoubtedly emerge and some will challenge current ideas of myxosporean evolution and life cycle strategies, perhaps as more host Phyla are recognised. In particular, the marine environment contains by far the majority of known bryozoans and there are surely many undiscovered links with marine fish Myxosporea.

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An overview of carp diseases in the UK

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'Hops and turkeys, carps and beer,
Came into England all in a year'.
Dame Juliana Berners, (Attrib.) 'The Treatyse of Fysshynge with an Angle'
in the Boke of St. Albans (London, Wynyn de Worde, 1496)

Abstract

The hypothesis in this paper is that the high numbers of carp deaths that have occurred for the past 40 years are related to a number of interacting variables. These include the massive numbers of carp and other fish that are transferred into and around the country; poor environmental conditions; bad fishery management; increased angling pressure, and nutritional problems associated with dietary deficiencies. Added to those factors, the high value of larger sized carp has led to illegal imports of fish, bringing with them their parasites and other disease organisms. The paper discusses the fact that the changing coarse angling scenario that has taken place in recent times has been a major factor in exacerbating the well known 'spring mortality syndrome of carp', a condition that has been known in European carp farming communities for many years.

Introduction: historical background

The common carp (*Cyprinus carpio* L.) is the most recognisable representative of the cyprinids. Its origins are in the tropical and subtropical waters of Asia. However, man, notably religious orders including monks, gradually introduced the fish into the cooler, temperate waters of mainland Europe. The first mention of carp in the UK was in one of the first books to appear in print (above). Carp initially were raised in 'stew' ponds but as the demand for fresh fish increased, farmers in Europe, including the former Union of Soviet Socialist Republics, cultivated carp in massive earth ponds.

Carp were selectively bred resulting in chunky 'mirror' and 'leather' varieties compared to the leaner 'wild' strains. The Japanese went even further with the art of strain selection and over the centuries perfected coloured varieties going under the names of 'koi', 'hi-goi' *etc*. Furthermore, the huge demand for ornamental varieties of carp has created a specialised market. All varieties are *C carpio*.

Carp are now spread around the world as a food and sporting fish, and in North America and Australasia are regarded as environmental pests. In the UK, over the past 20–30 years, the carp has become the most popular fish for sport fishing.

The carp and its diseases has interested historians, naturalists, philosophers and writers throughout history. For example:

• Conrad Gessner (1558) in his '*Historiae Animalium Liber IV, qui est de Piscium et Aquatium Animantium Natura*' described the disease 'carp pox'

• Izaak Walton (1593–1683) regarded as the father of angling, wrote in 'The Complete Angler' (Walton and Cotton 1895) about 'carp with decays, sick and lean with frogs attached to their heads'. The 'decays' were states of debilitation associated with over-wintering carp; and male frogs attach themselves to the heads of carp when there are not enough female frogs available to satisfy their urge to mate

• Frank Buckland (1826–1880), eminent naturalist, first inspector of salmon fisheries and member of Government Fishery Commissions, described the fungus disease caused by *Saprolegnia* species as 'pond carp with white moss growing upon their heads' (Buckland 1883).

Carp are extensively exploited as food animals and it is not surprising that their diseases and parasites have been well documented in the scientific literature for the past 100 years. In the earlier investigations it was recognised that most health problems occurred in the springtime following severe winters. Older literature also revealed considerable understanding of the pathogenesis and control methods for many of the health problems. For example, because of persistent pseudomonad and aeromonad infections in carp, research into methods for vaccination were made in the 1930s (Schäperclaus 1938). Those early studies showed much promise and clearly

demonstrated that fish were able to produce antibodies against the pathogens. It was also understood that in 'over-wintered' carp, immunity was reduced in the spring in European carp farms (Snieszko 1972).

Although carp have been resident in this country for more than 500 years, they were never abundant. Their scarcity was because of intermittent spawning success, little demand as a food item and unpopularity as a sporting fish. The reason why there is a paucity of information about the health of carp in the UK before the second world war is because the fish were not exploited for food as the population had access to a good supply of sea-fish. However, since then the carp population has multiplied to the extent that today they are regarded as one of the country's most common freshwater fish. It must be remembered that in the UK, the old 'native' populations of carp are most likely to be naïve to diseases exotic to this country. The introduced carp are from mixed populations originating from several countries which have varieties of pathogens and parasites presenting those fish with different degrees of disease resistance.

Carp diseases up to 1975

The sequence of events associated with an increase of carp diseases could be summarised as follows:

- increasing popularity of coarse fishing,
- a demand for more fisheries, and
- pecuniary interests of entrepreneurs.

The reason for the rise in popularity of carp as an angler's fish possibly owes much to one single event: on 12 September 1952, a British rod-caught record carp, weighing 44 lbs (approximately 20 kg) was captured in Redmire Pool, Gloucestershire, by a well known angler/writer, Richard Walker. From that September day, carp fishing in the UK caught the imagination of the angler, first of all as a cult sport; and as more carp were introduced into waters, it changed the face of angling. Carp fishing was given lots of publicity by the press; London Zoo which exhibited Walker's record carp, and his own excellent articles on carp fishing in angling magazines and books. Photographs of anglers holding big fish are good for media sales, but probably not good for the carp.

This changing angling scene led to the creation of new fisheries, even the replacement of trout fisheries, to meet the demand. However, on some fisheries, little thought went into the management and many were over-stocked, over-fished and quickly degenerated into 'mud holes'. The anglers' interest is for big fish which are often caught, handled and returned several times, unlike trout fisheries which are run on a 'put and take' system.

Simultaneous to the demand for carp as a sport fish, the interest in keeping koi and other varieties of coldwater fish was gaining popularity among hobbyists. These fish were imported originally from Japan and later from several other countries including Israel. After importation, the fish are distributed by dealers to retail outlets. Some dealers and retailers held and supplied carp as well as ornamental fish, a practice that still continues. Furthermore, some trout farmers set aside raceways that had been used for trout to hold coldwater ornamental fish as a sales item. Of course, most ornamental fish ended up in garden ponds, but there were some that found their way into fishing ponds and rivers.

The mid-1960s was a bad time for fish diseases in the UK, with ulcerative dermal necrosis (UDN) occurring in salmonids, 'roach ulcer disease', 'perch disease' and subsequently, carp health problems. The latter appeared to affect two separate groups of carp. Namely:

1. Mortalities occurred in the spring and early summer. The affected carp, which were sometimes up to 10 kg in weight, had been imported into the country within the previous year. They gathered in small groups at the edge of ponds, were lethargic and reluctant to swim away when disturbed. Externally, the carp had skin ulcerations which were circular with a red centre and a white periphery in the mirror and leather varieties of carp. Internal examination usually revealed 5–10 mls of clear fluid in the coelomic cavity. Virology examinations were negative and bacteriological tests only revealed mixed growth of common aquatic bacteria from the skin lesions. Histological examinations revealed varying degrees of inflammation at the site of ulceration, but little else (Mawdesley-Thomas and Bucke 1973). A tentative diagnosis of carp erythrodermatitis (CE) was considered (Bucke, McCarthy and Hill 1975). According to Fijan (1972), CE represented the chronic form of the 'spring viraemia of carp' (SVC) complex, however, as stated, no viruses was isolated from these disease outbreaks (Bucke and Hill 1977)

2. Mortalities in natural stocks of 'wild' carp (0·5 to 5 kg size) occurred in waters which had been recently stocked with imports of foreign carp. There had been no previous history of health problems in these waters, and the imported fish remained healthy. The moribund fish appeared listless, with their heads pointing downwards in the shallow areas of the lakes. Death followed after several days.

   Post mortem examination did not reveal any obvious external lesions. Internally, there were numerous fibrous adhesions binding together the visceral organs with attachments to the abdominal walls. The intestines were empty and inflamed distally near the vent. No viruses or other significant pathogens were detected. Histological examinations confirmed inflammation of the intestine and other minor changes.

In both the above cases, mortalities appeared in the spring. The actual cause of the mortalities was not identified but clinical and pathological signs suggested an infectious, possibly viral, aetiology.

The first official isolation of *Rhabdovirus carpio*, the causative agent of SVC was made in 1977. This was from a population of 1200 common carp (8–10 cm in length) used for toxicity trials and kept in an aquarium system. The carp were subjected to stress from temperature change and prophylactic treatments for ectoparasites. Subsequently, the fish showed abnormal behaviour, stopped eating, and started dying. Histological results revealed marked degrees of necrosis in all organs, and in the intestine in particular. Virus isolation was successful. These fish had been obtained from a fish dealer and originated from English stock (Bucke and Finlay 1979).

Around that time, three more SVC virus isolations were made from other small populations of common and koi carp. In all cases, the fish were destroyed and the holding premises disinfected. To all intents and purposes, the disease was contained and eradicated.

It should be noted that it was perfectly legal to import wild non-salmonids into the UK until 1980, but thereafter imports of fish and fish eggs (excluding ornamental fish) required health certification. It was suggested that the introduction of health certification for importation of wild fish was significant in controlling carp virus diseases. However, in 1988 there were many reports of dead and dying carp throughout the country. Investigations

resulted in the isolation of SVC virus from 38 of approximately 140 affected sites. The strain of virus proved to be virulent. The outbreak was serious as losses of carp on affected sites were reported to be between 10% and 100%, averaging between 40% and 50%. The affected sites involved open-water fisheries as well as garden centres, fish farms and garden ponds (MAFF 1989).

Most of the disease outbreaks were associated with fish transfers and stress, possibly arising from sudden temperature changes following a cold winter. Whatever the reason, it was a fact that the demand for carp did not diminish although there was an increase in the value of the fish. Despite the introduction of health certification for foreign imports of carp, illegal imports of carp were known to occur and it was possible that a virulent form of SVC virus could have been introduced into the country by that means.
Another possible entry route of pathogens was with the importation of ornamental fish, since imports of coldwater ornamental fish were not, at that time, subject to health certification. Customs officers on occasion intercepted mixed consignments of ornamental fish containing large specimens of common carp. In those cases, destruction of imported fish and successful prosecutions were obtained.

There is still a strong possibility for the import of diseases through the illegal trade in several species of imported fish. There is much evidence to show that ornamental varieties of *C carpio* are susceptible to exactly the same infections as common carp, including the well-known viruses, bacteria and parasites. Imports of ornamental fish may have been treated with anti-microbials before export. This action can induce drug-resistant strains of bacteria. Several metazoan parasites have been imported with exotic fish over the past few years. For example, the cestodes, *Atractolytocestus huronensis, Khawia sinensis* and *Bothriocephalus acheilognathi* are reported to have entered the UK with imports of cyprinid fish species (Environment Agency 1999).

Despite the risk of introducing exotic diseases, fishery owners continue to transfer and introduce fish into their waters. These transfers are legal but subject to a Section 30 Order (*Salmon and Freshwater Fisheries Act 1975*). This order requires that fish intended to be transferred must be accompanied by written permission from the Environment Agency before a transfer can take place. In most cases, a health check is required on a sample of the fish

that is due for transfer before the fish can be moved. Currently, the demand for coarse fish transfers is on the increase, creating a colossal amount of work for the Environment Agency's National Fisheries Laboratory. Because of the work load, inspections are limited to visual examination for parasites and clinical signs of disease. This exercise could allow unscrupulous dealers to be selective about the fish they provide for inspection. There is no requirement for health inspections on fish transferred between closed waters. In those instances, transfers are subject to a 'Buyer Beware' guide for stocking fish. The introduction of rapid diagnostic tests for the more important diseases and parasites would alleviate the screening situation.

Emerging pathogens

In addition to SVC virus, it has been documented that at least nine Rhabdoviruses have been isolated from carp and other cyprinids. Most of these viruses have been incidental findings and not all have been associated with fish mortality. A recent report on research into these virus types has shown that all these viruses have slight genetic variations from *Rhabdovirus carpio*. In fact one recent isolation which was associated with mortalities in cyprinids in the UK had some similarities to pike fry rhabdovirus (PFRV). This new rhabdovirus has been proposed for reclassification (Way and others 1999).

Mass mortalities have occurred in koi and common carp in Israel (Ariav 1999, Walster 1999). A similar disease has been reported in koi in the USA, Germany, Belgium and the UK. Exports of koi from Israel may be one explanation for the outbreaks of disease, except that in Germany affected koi were said to be from stock imported from several countries. Gill changes were the main pathological feature described in all cases. A herpesvirus was isolated from affected koi (Bretzinger and others 1999, Neukirch and others 1999, Hedrick and others 2000).

The current situation is that most carp mortalities investigated have rather inconclusive diagnoses. To date, there have not been reports of herpesvirus isolation from carp in the UK. In 1999, some 450 cases involving carp and other cyprinid mortalities were investigated by the Environment Agency in collaboration with CEFAS, Weymouth. Results of diagnoses have revealed that less than a dozen cases have been positive for SVC virus. For others, the causes of disease were suggested to be the result of over-stocking,

bacterial or parasitic infections, angling pressure, bad husbandry or poor water quality.

The problem of carp mortalities has led to a government-funded study of the problem. At the time of writing, this study has just been completed and the report is due to be published in January, 2000. Press releases indicated the cause of mortalities followed the pattern of an infectious disease and in most cases were associated with the introductions of new fish. Although detailed investigations were made in the study, it was clear that progress would be inhibited because of under-funding.

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This paper is based on a presentation given at the winter meeting of the Fish Veterinary Society in Weymouth on 2 December 1999 and was submitted for publication on 10 December 1999.

Disease management and control in carp fisheries in the United Kingdom

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Angling is one of the top leisure activities in the United Kingdom, with an estimated 1·2 million people who currently hold an Environment Agency rod licence, a legal requirement for all coarse fishing. 'Coarse fish' is the term used to describe all those species of fish found in the British Isles with the exception of the salmonids which are referred to as 'game fish'. Coarse fisheries are stocked with cyprinids, European perch (*Perca fluviatilis*) and pike (*Esox luscius*), and a limited number of sites stocked with exotic species such as Wels catfish (*Silurus glanis*) or zander (*Stizostedion lucioperca*). By far the most popular coarse fish species with the majority of anglers in England is the carp (*Cyprinus carpio*), not just because some individuals can attain weights of up to 18 kg but also because it can prove wily and difficult to catch. The carp is not native to England: it is believed to have naturally inhabited the Danube basin and western Asia but was spread to southern Europe by the Romans and was certainly on the royal menu of King Richard II in the 14th Century (Giles 1994). Carp thrive best in regions warmer than England and therefore this species is on the extremity of its geographic range in this country. As a result, its growth rate tends to be slower than in the warmer climates of continental Europe. Spawning and recruitment success of carp fry in natural waters in England would seem to decrease progressively towards the north of the country (B. Brewster, personal observation).

The popularity of carp probably verges on the obsessive, which has led to an artificially inflated value for these fish and created a market for theft, illegal movement and unauthorised importation from continental Europe. The latter may be either on a casual basis, such as the angler who lands a decent sized, attractive fish and wishes to stock it into a home stillwater, or as a result of criminal activity. In the case of organised crime, vast numbers of carp have been illegally imported from Europe and it is now difficult to be certain of the origin of many carp which have been stocked in the UK. Illegal importation of carp has had a significant impact on coarse fisheries

in this country. As part of Zone 1 status, Britain is deemed to be free of notifiable diseases such as spring viraemia of carp (SVC) which is widespread in Europe. Illegally imported carp originate from countries throughout Europe and are introduced into English waters with a total disregard to the potential spread of any disease or exotic parasite. Such illegal introduction has resulted in infected or carrier fish causing outbreaks of disease in coarse fisheries, with mortalities of between 85% and 90% of the carp. In many instances, the managers of infected fisheries will contact The Centre for Environment, Fisheries and Aquaculture Science (CEFAS) to investigate the mortalities, either directly or through the Environment Agency, veterinarian or fish consultant. Where SVC is identified as the cause of mortalities, the site becomes a 'designated order area', restricting the movement of fish into or from the site, although angling may continue. Following an outbreak of a notifiable disease, the fishery is visited by fisheries inspectors to sample the fish on an annual basis. The designated order area is only lifted after the site has tested negative for SVC in three successive years. A small minority of fishery owners or managers have no desire to involve CEFAS, fearing the repercussions of handling carp from a dubious source more than the effects of mortality in their fish stock.

Historically, there have been springtime mortalities of carp for many years but with the increased popularity of this fish for angling the incidence of these outbreaks would appear to have increased. The mortalities have been termed 'spring mortality of carp syndrome' and often follow the introduction of carp into a stillwater. Although no infectious agent has been formally identified, there have been suggestions that the mortalities are due to a virus, with sentinel fish developing similar symptoms to infected fish when introduced to a coarse fishery during an outbreak (P. Bolton and I. Wellby, personal communication). Mortalities of carp resulting from an outbreak of spring mortality of carp syndrome, are between 85% and 90%.

Some fishery managers use iodophor disinfectants for dipping boots and equipment prior to entering and on leaving any fishery, which theoretically could reduce the transmission of infectious disease. In practice, many of the dips are not routinely maintained and often the concentration of disinfectant is inadequate.

Until 1995, it was mandatory that prior to any movement of fish, a sample of the population for re-stocking was subjected to a health examination.

This was part of the requirement to obtain a movement consent from the Environment Agency. From 1996 onwards, the Environment Agency has operated a 'buyer beware' policy for stocking stillwaters, advising fishery managers to buy fish which have been subjected to a health examination. Health examinations of fish for stocking rivers and canals remain compulsory. The health examination of the fish requires an overall assessment of the health of the fish and identification of the parasite fauna associated with the fish. This assessment is totally dependent on the experience of the person carrying out the examination but no viral screening is included, unless specifically requested. The value of health reports on a sample of fish is questionable, because of the possibility of fraudulent submission of fish from a different source to those supposedly covered by the report. The health report may be valid for between six and twelve months, during which time much can happen to the health of a population of fish.

In many instances, the parasites are akin to a passport and the appearance of exotic species can confirm the origins of a batch of fish. Exotic parasites and those which cause List 2 diseases, including the gill maggots (*Ergasilus* species, *Neoergasilus japonicus*, *Paraergasilus longidigitus*) and the segmented tapeworm *Bothriocephalus acheilognathi* are reasons for the imposition of movement restrictions by the Environment Agency. Where these parasites have been identified as infecting fish on a coarse fishery, the fishery managers may seek information on the feasibility of treatment and eradication. Unfortunately, the only species of gill maggot likely to respond to treatment is *Neoergasilus japonicus*, which lives on the body surface, usually around the base of the fins. On contact with any treatment, most of the ergasilids which infect the gills move to the proximal part of the primary gill lamellae, where they are protected from the effects of medication. It is equally difficult to irrigate the nostrils of fish infected with *P longidigitus*.

Infection with *B acheilognathi* tends to be confined to young carp of 0 to 3 year class, that is those fish which are in their first to third years of age and which feed heavily on the intermediate copepod host. Older carp prefer to feed on larger organisms and plants, so are less likely to become infected. It would also seem that the tapeworm (*B acheilognathi*) has difficulty in attaching to the intestine of carp in excess of 1·5 kg body-weight (D. Pool, personal communication). A single copepod may be infected with a large number of procercoids. In most instances of infection with *B acheilognathi*, the intestine of the young carp is completely occluded by the tapeworms,

leading to nutritional disorders as well as changes in blood biochemistry (Williams and Jones 1994). The spread of this tapeworm cannot be effectively controlled in any natural environment, but carp in re-circulating systems can be treated using anthelmintics which may be incorporated into the feed.

Some exotic parasites have now become so widespread in the UK that they have been down-graded by the Environment Agency from List 2 to List 3. Examples of these include the lytocestid, *Khawia sinensis* and the blood fluke, *Sanguinicola inermis*. List 3 diseases are only subject to movement restrictions where a clinical infection is identified.

Khawia sinensis is a nonsegmented tapeworm, which lays eggs that are shed with the faeces, with oligochaetes acting as an intermediate host. In most instances, the numbers of worms infecting carp is low: often only one per fish examined, and at this rate of infection the parasite does not appear to produce any significant pathology. Williams and Jones (1994) indicate that a number of anthelmintics are effective in reducing the numbers of this parasite. Praziquantel is not effective in eradicating *K sinensis*, although the worms appear to become detached from the anterior section of the intestine, which is their preferred attachment area (B. Brewster, unpublished data).

The blood fluke (*Sanguinicola inermis*) was commonly found in carp fisheries during the early 1990s. The blood fluke is very degenerate; the body is devoid of suckers or hooks and adults are usually found in the *bulbus arteriosus* but these can be difficult to detect unless present in large numbers. It is more common to identify the presence of this parasite from the characteristic, triangular eggs, in which the miracidium is clearly visible. The blood flukes shed a single egg at a time into the blood circulation. These eggs often become lodged in the gill tissue but it is also common to find them in the kidneys, either with live miracidia, or as characteristic granulomata, indicating a long-standing infection. The miracidia actively escape from the egg and surrounding gill tissue. In heavy infections this may cause severe damage to the blood vessels and epithelium compromising respiration and osmoregulation (Kirk and Lewis 1994). The intermediate hosts of the blood fluke are lymnaeid snails.

The numbers of fisheries stocked with carp infected with blood fluke seem to have declined over the last few years (B. Brewster, personal observation).

Often the introduction of a novel or exotic parasite allows it to flourish initially before establishing a stable level of infection within the host populations. Large carp will certainly eat lymnaeid snails and it is possible that the stocking patterns of fisheries have created a situation where the fish themselves may have eradicated the intermediate host.

Water quality is a commonly encountered cause of disease and stress in stillwaters. Many land owners have capitalised on the popularity of angling by digging a lake, filling it with water and adding fish. Plants efficiently remove the ammonia waste produced by the fish on an established water but a new lake often has insufficient quantities of submergent or marginal growth to utilise the nitrogenous waste. As a result, it is common to encounter water quality problems typically associated with the ornamental fish industry and 'new pond syndrome', where fish stocks are added in the absence of any established filtration system. The recommended stocking ratio for a stillwater is 300 kg of fish per hectare of water (Templeton 1995). This stocking rate is appropriate for growing-on stocks but entails continually thinning out numbers of growing carp or other species, to stay within this limit (A.C. Wheeler, personal communication). Very few fishery managers would be willing to reduce their fish population on a regular basis to maintain this level. On many sites, the stocking level is greater and waters holding in excess of 1000 kg per hectare are not unknown. The consequence of stocking this heavily is poor water quality, often with unacceptable concentrations of ammonia or low dissolved oxygen. These fish stocks often suffer common, stress-related diseases such as white spot (ichthyophthiriosis) or fin rot (columnaris infection). The effects of poor water quality are often compounded by the application of herbicides to control the growth of submergent aquatic plant or nuisance algae such as blanket weed, predominantly *Cladophora* species. In recent years, it has also become fashionable to use barley straw to control nuisance algae but standard practice is to place entire bales at strategic points around the lake, usually at the margins where they will not obstruct any of the anglers' pegs. The whole bales are not conducive to the production of hydrogen peroxide, which is the algae-inhibiting factor, but they cause the straw to decompose and mineralise, giving rise to further ammonia pollution or the release of other toxic products.

Many carp suffer injuries which are related to both the damage caused by catching the fish and through poor handling. Numerous fisheries have

banned the use of barbed hooks on the site but despite this, the removal of the hook from a fish is often undertaken in a brutal manner, especially where it becomes entangled in the bones and associated cartilage of the mouth. Large carp, in excess of 5 kg, are usually weighed and often photographed before being returned to the water. It is common to find these fish with lesions or large areas of lateral replacement scales where they have been dropped or badly handled.

In recent years, large numbers of cormorant (*Phalacrocorax carbo*) have moved inland owing to the depletion through over-fishing of juvenile fish stocks in the estuaries. Particularly during the winter months, these inland populations of cormorant have taken to feeding on many of the heavily stocked fisheries. The quantities of fish eaten is a hotly-debated issue between the anglers and the Royal Society for the Protection of Birds. There is no doubt that cormorant are responsible for some loss of fish stocks and it is possible to find large numbers of fish with beak marks on the body but increasingly these birds are becoming the scapegoat for poor management and the resulting outbreaks of disease.

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Novel methods to reduce disease in aquaculture

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Over recent years there has been an enormous advance in our understanding of bacterial fish diseases and in preventative methods such as vaccination. Despite these advances the rainbow trout and salmon industry in the UK still suffers from a small number of troublesome bacterial diseases such as furunculosis (*Aeromonas salmonicida*), enteric red mouth (ERM) (*Yersinia ruckeri*) and rainbow trout fry syndrome (RTFS) (*Flavobacterium psychrophilum*). Once bacterial diseases occur it is often necessary to turn to antibacterial agents to treat them. At present it is true to say that the use of antibiotics remains essential to the industry. However, as many in the industry are all too aware such use these days is not always trouble-free. This can be for a number of reasons, including bacterial resistance, drug residues and an increasing concern among retailers and their customers, about the use of antibiotics in food animals.

For all these reasons alternative methods for disease control and prevention in aquaculture are required. Potentially there are a number of different approaches that could be taken and might prove beneficial in preventing or controlling diseases.

Non-invasive stress measurement

At the Centre for Environment, Fisheries and Aquaculture Sciences (CEFAS) Weymouth Laboratory a series of studies investigating the links between stress and disease susceptibility is currently being carried out. Nearly every basic text on fish diseases highlights the link between stress and disease. In fact the relationship between stress, the immune system and disease susceptibility is a complex one and is by no means fully understood. There are many situations in commercial farming such as transport, crowding, handling and poor water quality that can produce acute and chronic stress.

An invariable response of stressed fish to all forms of environmental stress is an activation of the hypothalamic-pituitary-interrenal axis with a resultant elevation in the blood plasma of the steroid hormone, cortisol. It has previously been shown that plasma cortisol levels are elevated in rainbow trout subjected to all forms of environmental stress and that chronic cortisol elevation is directly responsible for many of the damaging effects on survival (disease resistance), growth and reproduction (Pickering 1993). However, a major problem with carrying out stress-related experiments is that sampling (approach, capture, immobilisation and bleeding) is an acute stress and causes elevated cortisol levels. Although the first one or two fish in a tank may be sampled before their cortisol levels become significantly elevated, the remaining fish will have been affected and need to be left for at least 24 hours before cortisol levels return to normal (Pickering and Pottinger 1989).

At CEFAS Weymouth and Lowestoft laboratories a joint project is underway to develop a novel non-intrusive and non-invasive technique removing the need for the inherently stressful procedures of capture and blood sampling. The technique essentially involves measuring levels of cortisol or its metabolites in outflow tank water rather than in the fish themselves. It can be seen from Fig 1 that when fish are stressed significant levels of cortisol are released into the water when measured using the new technique. It is hoped that this non-invasive cortisol assay will also be used later in the year as an objective measure of fish welfare to examine the effect of stocking density in a project to be carried out in conjunction with the Institute of Aquaculture, Stirling and the British Trout Association.

Immunostimulants

Currently, there is considerable renewed interest in aquaculture in the use of immunostimulants and other feed additives to enhance the non-specific immune response to a range of pathogens, and the specific immune system where the response is weak or developing such as in fry or larval fish. A number of compounds have been proposed as immunostimulants, including inflammatory agents such as silica and carbon particles, bacterial cell walls, glucans, levamisole, lipopolysaccharides, sodium dodecyl sulphate and lectins. For some immunostimulants the mode of action is understood but for the majority the exact mechanism remains a source of speculation.
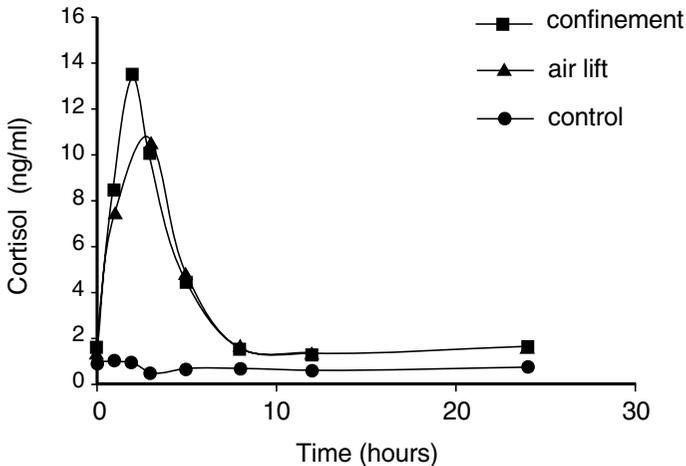
FIG 1: Levels of cortisol released into the water after netting fish out of the tank (air lift) and after increasing stocking density through reducing the area of the tank with screens (confinement).

Although immunostimulants seem to offer considerable potential for disease prevention they have largely been met with scepticism by the industry. This is due to a number of reasons. First, early immunostimulant candidates were relatively crude and ineffective. Secondly, dosing regimes were not clearly understood. It is now clear that too low a dose may be ineffective and that excessively high doses may in fact suppress defence mechanisms. Recommendations for more sophisticated dosing regimes and newer immunostimulant agents are likely to prove far more effective in the future.

Prebiotics

A third potential approach to reducing disease susceptibility of farmed fish is the use of prebiotics. This is the subject of a research project that will be started at CEFAS in the spring. Prebiotics are defined as "nutrients added to feed to selectively stimulate already present and established populations of bacteria" and typically are non-digestible oligosaccharides, lactulose, lactitol and inulin, which occur naturally in foods or have been derived by enzyme-catalysed processes from various carbohydrates (Crittenden 1999).

They pass in to the distal intestinal tract where some bacteria can use them as an energy source by fermentation to short-chain fatty acids (SCFAs) like acetate, propionate, butyrate; lactate, and the gases carbon dioxide, methane, hydrogen (Cummings & Macfarlane 1997). Prebiotics can stimulate growth and/or metabolic activity of beneficial bacteria and suppress potentially deleterious ones, thus modifying the composition of the intestinal microflora. Two studies on Arctic charr (*Salvelinus alpinus*) carried out by Ringo (1993) and Ringo and others (1998) indicate that diets supplemented with polyunsaturated fatty acids (linoleic acid, linolenic acid, and high unsaturated fatty acid [HUFA] mix) can potentially influence the lactic acid bacteria populations in the intestine and stomach. In addition, an increased growth stimulation of up to 20% was achieved with modified diets over fish maintained on non-supplemented feeds. Only a few prebiotic studies have previously been carried out on fish and although the approach does have potential, a great deal of work needs to be completed before that potential can be realised. For example, bacterial populations in the fish intestine need to be more fully characterised, an understanding of the mode of action of prebiotic candidates has to be established, and efficacy trials need to be carried out under carefully controlled laboratory conditions.

Genetic improvement

Of all the approaches that can be taken to increase disease resistance, the area of genetic improvement holds the most promise for significant gains in fish health in the medium and longer term. Genetic improvement comprises a number of approaches spanning natural selection to selective breeding through to genetic engineering. Selective breeding programmes have been going on within the aquaculture industry for some time. Probably, the best known are the salmon selective breeding programmes conducted in Norway and Scotland. However, there are a large number of other such programmes centred on many diverse species such as tilapia, catfish, oysters, shrimp, carp, rainbow trout, sea-bass and bream. These programmes are largely aimed at improving growth rates and overall performance under commercial aquaculture conditions rather than at increasing natural resistance to disease. However, in the near future it is likely that world-wide an increasing number of selective breeding programmes aimed at improving disease resistance will be carried out. The efficiency of these programmes is likely to be increased using genetic marker based approaches. Genetic markers such as variable number tandem repeats (VNTR) and polymorphisms

(microsatellites) can be used as a means of identifying both individuals and family groups that are reared together. They can also be used to identify marker loci which are linked to nuclear loci that control traits or characteristics that may be important commercially (quantitative trait loci or QTL). These markers can then be used to accelerate the progress of selective breeding programmes, such as marker assisted selection (MAS). At the CEFAS Weymouth Laboratory a long-term research project on genetics and disease resistance is scheduled to start in April 2000. This project uses rainbow trout (*Oncorhynchus mykiss*) and susceptibility to viral haemorrhagic septicaemia (VHS) as a model. It is widely accepted that different species and strains of fish vary in their susceptibility to VHS. For example, salmon and rainbow trout differ significantly in their susceptibility to the disease. Indeed, even within a species, such as rainbow trout, different strains vary enormously in their natural ability to resist the virus. The reasons for these differences in disease susceptibility are likely to be both numerous and complex. However, it is certain that the most significant component lies within the inherited genotype of the fish. The ultimate aim of the project is to develop a genetic marker for use in selective breeding programmes to accelerate improvements in health among farmed fish. Such a programme would also lead to a dramatic increase in the protection of UK rainbow trout stocks in the case of inadvertent VHS introduction.

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Koi carp mortality syndrome: an update

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In recent months there have been a number of scientific papers published on the subject of koi carp mortality. This article will present an update on this syndrome.

During 1999, there were further outbreaks at new sites in Israel, South Africa, continental Europe and the United States. The worst affected area of the USA was southern California. Although there were several suspected outbreaks within the UK, all were eventually ascribed to other causes. This highlights the difficulty in differentiating between this syndrome and other conditions. A diagnosis should be made only after exclusion of all other possible causes.

Across the world, there was a decrease in the number of reported outbreaks. Only Israel reported outbreaks at previously affected sites in 1999, although in 1998 there had been recurrences at previously affected sites in the UK.

Aetiology

It is widely accepted that a virus causes the syndrome. In Israel, virus-like particles have been seen on electron microscopy of samples from affected fish. These particles were considered to be herpes-like (Ariav and others 1999). In both Germany (Bretzinger and others 1999) and the USA (Hedrick and others 1999) a herpesvirus has been isolated. Following transmission studies, this virus has produced an identical syndrome and has been called koi herpesvirus (KHV).

Although this virus would appear to be the causative agent, it has not been confirmed. It can be isolated from some affected fish in small quantities but not from others. It is possible that not all outbreaks can be linked to the same virus. Indeed there is speculation that some of the outbreaks in the USA were caused by a different virus (M. Haimi, personal communication).

Immunity

It is apparent that fish which have been previously exposed to the causative agent whilst being held at water temperatures between 18°C and 26°C, exhibit immunity to the agent upon re-exposure. In Israel, they have produced fish immune to the causative agent by exposing the fish to the agent and manipulating water temperatures. These fish have been called 'naturally immune fish' and it has been found that this decreases mortalities from the normal level of 80% or higher, to as low as 5%. In addition, the fish appear resistant to re-infection when further challenged (Ariav and others 1999).

It has also been possible to identify specific strains of *Cyprinus carpio* resistant to the disease process (M. Haimi, personal communication).

Treatment

No pharmaceutical preparation has been found to alter the course of the disease. The addition of salt to the water may be of benefit in reducing secondary disease. Clinical signs only occur in infected fish following a rapid change in water temperature within the range of 18°C and 26°C. By manipulating the water temperature, the outcome may be altered. It is best to raise the temperature to above 26°C, maintaining it until mortalities stop, and then decrease the temperature to below 18°C.

Control

Since most cases occur after the introduction of new fish, it is obvious that the disease is spread by direct contact. No other method has been shown to disseminate the disease. There are anecdotal reports that it can be spread by contaminated water or by ectoparasites such as *Argulus* species.

To prevent introduction of the disease, it is recommended at present, that all new fish are quarantined for between two to three weeks, together with sentinel fish, at water temperatures between 18°C and 26°C. As already mentioned, where large stocks of fish are at risk, exposure to the agent and manipulation of water temperatures could be considered. However, this may only slightly decrease the mortality level.

Carrier fish

Since this syndrome is probably due to a herpesvirus, there is a distinct possibility that there may be carrier fish. This has been shown to be the case with cyprinid herpesvirus 1 (CHV) infection, the causative agent of 'carp pox'. This may explain cases that have occurred in ponds where no new fish had been introduced for several years. The factors that trigger the disease process is unknown. In 1999, tens of thousands of koi were exported from Israel to Europe after exposure to infected fish and after testing to ensure they could not pass the infection to naïve fish (M. Haimi, personal communication). There have been no reports of outbreaks due to these fish.

Further research

Although it would appear that koi herpesvirus is the cause of this syndrome it has yet to be confirmed. The author is aware of another herpesvirus that has been isolated from carp at Mie University, Japan: this appears to be antigenically different from koi herpesvirus. As yet, the significance of this is unclear. Several research groups are investigating the possibility of producing a PCR test for the rapid diagnosis of this syndrome. To date, insufficient viral DNA has been recovered from affected fish to allow research on this to progress speedily. It would also be of benefit to investigate various aspects of carrier fish.

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Slice® : good news for salmon, bad news for sea lice

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Schering-Plough Animal Health has an established range of medicinal products for the therapeutic treatment of food and companion animals and has begun to develop a portfolio of products which address the needs of the aquaculture industry world-wide. The first of these to gain marketing authorisation in Europe was the antibiotic florfenicol marketed in the UK as Florocol® for the treatment of fish diseases including furunculosis. As part of the development programme liaison with the aquaculture industry identified the need for a medicine for salmon which could effectively treat the parasite which was at the time, and still arguably is, the single largest welfare problem for farmed salmonids, the sea louse. In order to reduce the input of medicines into the environment, and better target the fish, it was decided to develop an in-feed formulation. The information presented in this paper will soon be published in the scientific literature by those who undertook the work.

Following screening studies, the second generation semi-synthetic avermectin 4``-epimethylamino-4``-deoxyavermectin B1 benzoate, emamectin benzoate, was selected for further development. This molecule, like the other avermectins, acts by binding to specific cell membrane sites, increasing membrane permeability to chloride ions, being particularly active on glutamate-gated chloride channels on nerve cells (Vassilatis and others 1997). Whilst it had been demonstrated that the related compound ivermectin was effective in controlling lice it had not been approved for use in salmon and had been reported to be toxic to fish, with fatalities when administered at four times the therapeutic dose (Palmer and others 1987).

Dose determination and efficacy

As a result of dose determination trials, an emamectin benzoate dose of 50 µg/kg body-weight per day for seven days was selected (Stone and others 1999). When fish were dosed for seven days at nominal doses of 100 µg/kg

and 250 μg/kg there were no signs of toxicity. At the highest dose tested, 500 μg/kg nominal and 356 μg/kg determined, there were signs of toxicity but no mortalities arising from a seven day treatment.

A formulation of emamectin benzoate as a 0·2% pre-mix for application to feed has been developed, Slice®, and following the determination of the dose a number of trials were undertaken, initially in Scotland but also in Norway, Chile and Canada. In the Scottish field trials it was found that following treatment, the efficacy relative to the control fish was initially 25–63% on day 7, rising to 74–93% by day 14, and 90–99% by day 21. The efficacy values were determined from the total lice counts on the fish and it was subsequently discovered that the early efficacy values may have been underestimates as killed lice, including copepodites and chalimus stages, did not always detach but failed to develop. In the first commercial trial it was found that there was 89% efficacy at day 35, and the control fish had to be treated with hydrogen peroxide at day 21. Another indication of the efficacy of emamectin was in the reduction of visible damage by lice in the early field trials with 0–2·5% of treated fish showing damage compared with 20–25% of control fish. In addition to the efficacy against *Lepeophtheirus salmonis* it was also shown in the field trials that emamectin was active against *Caligus elongatus*. It was subsequently shown in Chile that emamectin had greater than 90% efficacy against *Caligus flexispina* and *C teres* on rainbow trout (*Oncorhynchus mykiss*) at day 42 after the seven day treatment. From the extensive range of studies which have been undertaken it can be concluded that emamectin is effective under a wide range of environmental conditions, namely at water temperatures of 5–15°C and salinities of 23–35 ppt.

In a second commercial trial in Scotland 186,000 Atlantic salmon (*Salmo salar*) were treated (108 tonnes biomass), whilst a similar number of fish in neighbouring cages on the same pontoon served as untreated controls, and a potential source of re-infestation. The concentrations of emamectin arising in the environment and potential impacts were monitored in the same study. Following the seven day treatment the efficacy rose to 97·8% on day 42, and was maintained at more than 95% at day 64 despite continual exposure of treated fish to lice from the untreated controls. After this, the efficacy declined although gravid females, present on untreated fish throughout the study, were not detected on the treated fish until day 77. From the data generated in the study it can be estimated that the seven day treatment with

emamectin had reduced the sea lice infestation potential by 80% for up to 70 days following treatment by eliminating the source of the eggs and copepodites, namely the gravid females. The welfare benefit that the treatment presents is shown by the fact that whilst 50% of the untreated fish exhibited dermal lesions 70 days after the treatment, less than 10% of the treated fish showed any signs of lice damage. These observations show that emamectin treatment has the potential to not only benefit treated fish but also to dramatically reduce sea lice populations in treated areas, thus reducing the potential for the establishment of infestation. The periodic and transient nature of infestation with *C elongatus* makes it more difficult to quantify efficacy against this species under farm conditions but 100% efficacy was demonstrated at days 13 and 27 in the same trial.

Duration of efficacy

An interesting property of emamectin benzoate demonstrated early in the development programme was that it undergoes hepatic recirculation, namely excretion into the intestine via the liver followed by reabsorption and recirculation. This property accounts for the prolonged degree of protection seen in field trials yet with only slow release, following metabolism, at low concentrations into the environment.

The duration of efficacy in treated salmon has also been investigated under laboratory conditions. Treated and control fish were placed together in tanks and exposed to 76–200 copepodites per fish, added at weekly intervals to the water in which they were swimming from day 27 to 83 from the start of the treatment. Each group of fish was exposed to only one challenge and subsequently held for 8–15 days to monitor development of the parasite on the fish, with mean numbers of lice on untreated fish of 47 to 151 following challenge. The efficacy reached a maximum of 97%, based on total numbers of all life stages, at day 34 and was maintained with better than 90% efficacy at day 58 before declining to 74% on fish challenged on day 69. Examination of the life stages on the challenged fish revealed that whilst the copepodites could settle on the treated fish their development was prevented for at least 41 days with chalimus numbers remaining low up to day 81 on fish challenged on day 69. Even on fish challenged on day 83 efficacy of 35% was found, with reduced numbers of recruited chalimus maturing to motile pre-adults and adults by 15 and 20 days post-challenge. It was observed by the researchers that, when lice did eventually start to develop

through to pre-adults on treated fish, survival of female lice was lower than that of male lice when compared to the populations on control fish.

The efficacy data gathered for Slice® indicates that it can offer a major step forward in reducing the risk and damage caused by lice. The extended protection that Slice® offers further benefits the fish and the farm, as frequency of treatments can be reduced and fish do not have to be crowded as required for bath treatments.

Consumer and environmental safety

An extensive database has been generated on the toxicology of emamectin benzoate and the safety of Slice® has been assessed by the UK and European Union authorities, as well as those in the countries where it is already in use, namely the Faroe Islands, Norway, Canada and Chile. Emamectin has been demonstrated to be neither carcinogenic, mutagenic nor teratogenic and it has no effect on development or reproduction at levels below those which are directly maternotoxic. Based on this information, a Maximum Residue Limit (MRL) of 100 μg.kg^{-1} of emamectin in edible tissue, muscle and skin in natural proportions of salmon has been set within the European Union together with an Acceptable Daily Intake (ADI) of 1 μg.kg^{-1} body-weight per day. This MRL is above the highest concentration determined in salmon muscle and skin following treatment and has enabled a zero withdrawal period to be set by the UK authorities.

As with all medicines which are administered to animals in the aquatic environment, there are justified concerns over the potential for environmental impact. A comprehensive data set has been developed which encompasses studies required by the regulators and those which Schering-Plough and its consultants considered necessary. This has enabled an environmental risk assessment to be undertaken to establish the fate of emamectin in the environment and safety to animals which might be exposed in the water column, or in sediments, or those which might eat medicated feed settling in the vicinity of treated salmon. That the use of Slice® poses no un-acceptable risks is supported by studies carried out in the field where no environmental impact was detected over 12 months following the use of Slice® to treat salmon under commercial conditions.

Regulatory status

The marketing authorisation for Slice® has now been granted in the United Kingdom and the product is being submitted for wider European registration under the Mutual Recognition Procedure. Schering-Plough is committed to ensuring that Slice® is administered in strict compliance with the law in the countries where it is used. In the UK and Ireland, Slice® will only be supplied to veterinary surgeons, and a condition of supply will be that the veterinary surgeon must provide details of the farm and stock to be treated to enable its use to be monitored. In the UK, farms will require a discharge consent before the use of any sea lice treatment can be permitted. Past experience has shown that most of the objections to the use of medicines are likely to be on environmental grounds. Whilst scientific data can be presented to show that these would be unfounded for Slice®, they are likely to pose problems to farmers in obtaining discharge consents.

The combination of killing all parasitic stages of sea lice from a low dose given in feed and the duration of efficacy should in itself ensure that the welfare of fish is improved and the frequency of sea lice treatment is reduced. However, these same properties of the product allow the potential for strategic treatment of whole loch systems with prevention of subsequent reinfestation, either from untreated cages or from migrating wild fish. If this approach gains acceptance this could lead to a dramatic reduction in the frequency of sea lice treatment with benefits for all, but especially for the fish.

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John McHenery graduated from the University of Glasgow with a BSc and PhD in microbiology in 1976 and 1980 respectively. Following a research fellowship at UMBS Millport he joined the Marine Laboratory in Aberdeen where he first became involved in research on sea lice treatments. Leaving Aberdeen in 1991 he worked for British Petroleum before working as an environmental consultant specialising in pharmaceuticals. John has been involved in the research and development of Slice® since 1994 and joined Schering-Plough as the aquatics technical manager in 2000.

Jeremy Johnson graduated from the Liverpool University Veterinary School in 1986. He spent five years in mixed animal practice before joining Pitman-Moore in 1991. He obtained a diploma in marketing and another in accounting and finance, and became brand manager. He stayed with the company through the period when they changed their name to Mallinckrodt and were then acquired by Schering-Plough. He is currently the director at the Poultry and Aquatics Business Unit within the company.

This paper is based on a presentation given by Jeremy Johnson at the winter meeting of the Fish Veterinary Society in Weymouth on 2 December 1999 and was submitted for publication on 21 March 2000.

SCIENTIFIC MEETINGS

The Fish Veterinary Society are grateful to the sponsors of the last scientific meeting held at the CEFAS Laboratory in Weymouth on 2 December 1999.

Schering-Plough Animal Health, Breakspear Road South, Harefield, Uxbridge, Middlesex *for sponsoring the evening meal*.

The Society also wish to acknowledge the sponsors of previous meetings:

29 April 1999	at Ross Breeders, near Edinburgh **Ross Breeders Ltd**, Newbridge and **Intervet Norbio**, Bergen, Norway
12 November 1998	at Veterinary Laboratories Agency, Penrith **Veterinary Laboratories Agency**, Penrith and **Grampian Pharmaceuticals Ltd**, Leyland
23 April 1998	at the Queen's Moat House Hotel, Edinburgh **Alpharma**, Skøyen, Norway and **Vetrepharm Ltd**, Fordingbridge
28 November 1997	at Royal College of Veterinary Surgeons, London **Vetrepharm Ltd**, Fordingbridge
25 April 1997	at Ewos Technology Centre, Livingston **Ewos**, Livingston and **Novartis Animal Health UK Ltd**, Cambridge
27 September 1996	at the BVA Congress, Chester **British Veterinary Association**, London

IFM Fish Disease Discussion Group
Wednesday 15 September 1999, Sparsholt College, Hampshire

The second meeting of the Fish Disease Discussion Group, a specialist group formed under the auspices of the Institute of Fisheries Management (IFM), was held at Sparsholt College during the 30th IFM Annual Study Course. A report of the first meeting was published in the fourth issue of the *Fish Veterinary Journal* (pp 69–70). Twenty fish health colleagues attended the meeting including several members from the Environment Agency, the Centre for Environment, Fisheries and Aquaculture Sciences (CEFAS), college workers and fish health consultants. Chris Walster and myself represented the Fish Veterinary Society.

The meeting, a forum for discussing current fish disease issues, opened with an account of the 'koi carp mortality syndrome' by Chris Walster. This problem has caused severe mortalities, up to 100% in koi ponds and dealers premises, during summer months. It usually presents as an acute mortality with severe gill necrosis in many cases. A résumé of the events, as reported in the third issue of the *Fish Veterinary Journal* (pp 54–58), was given, followed by an update on the investigations that have been carried out in Europe, Israel and the United States. Despite speculation that this disease is caused by a virus, laboratory results have been inconclusive. A herpesvirus was isolated in America, but not from gill tissue. Many carp farms in Israel are currently affected and movement restrictions have been enforced by the Israeli government.

In the UK, mortalities in the summer months of 1999 were considerably lower than the previous year, and some of this is thought to be due to changes in the style of exhibition at koi shows around the country. Prior to 1998, koi were shown using the 'English style', where only fish from one owner are held in the same show vat. However, since it was considered difficult to judge fish of the same variety in several different vats, it was decided to use the 'Japanese style' of show in 1998. Here, fish of the same variety but from different owners are held in the same vat. Significant health problems followed as a result of this method and the shows then reverted back to the 'English style' in 1999. At present, the situation is being monitored by the relevant authorities, but since the causative agent

has not yet been identified, no further action can be taken to control the problem by trade restrictions. It was decided that the group should continue to be aware of the syndrome and monitor future developments.

A brief account was given by Phil Bolton from the Environment Agency, on progress made into the investigation of 'Spring carp mortality syndrome'. This is a recognised syndrome that has produced large-scale mortalities of carp in freshwater fisheries in recent years. In 1995 and 1996, it was estimated that losses in the UK amounted to £100,000 and £80,000, respectively. Despite detailed investigation, no primary cause was identified. Consequently, a research project began in 1998 involving the National Fisheries Laboratory at Brampton and CEFAS, Weymouth. The aims are to investigate carp mortalities and provide advice on various management techniques to prevent further mortalities. Since a full presentation was due to be given at the Conference on the following day, his account was abbreviated. All aspects of affected fisheries had been examined, including management, nutrition and water quality. Although all the fish showing similar clinical signs there were no consistent histopathological findings. Although a primary agent was not isolated, the disease could be transmitted by moving affected fish into previously unaffected waters with subsequent mortalities from this syndrome. As a result, it was thought that an infectious agent was responsible and further investigations are in progress.

Bernice Brewster, a private fish health consultant, brought up the issue of common otters (*Lutra lutra*) reappearing in the British countryside. Although these animals were being welcomed and encouraged in some parts, their preferred diet consists of European eels (*Anguilla anguilla*). Concern was raised about the increasing incidence of the nematode parasite, *Anguillicola crassus*, and the longterm effect that this may have on the number of eels in British rivers. Prior to 1990, the parasite was only seen occasionally, but in the last decade it has gradually become more prevalent, to a point where it is now found in most samples. Eels are the final host for this parasite which has an indirect life-cycle involving copepods and occasionally other intermediate hosts. Extensive damage is caused to the swim bladder, with fibrotic changes and collapse of the organ resulting in severely impaired swimming ability. In addition, affected eels are often in poor health and easily caught. Adult eels migrate to the Sargasso Sea to reproduce. After spawning, they die. The juveniles, glass eels, then return to the freshwater rivers after two years, where they grow to adults over the

following 10 to 15 years. Due to the period at sea and the length of the life-cycle, it may be several years before the full impact of this disease becomes apparent. It is not yet known how widespread this parasite has become, and more effort should be made to collect this data. At present, live glass eels which are caught in river estuaries are sold for between £200 to £300 per kilo. Although this trade is not yet controlled, it was thought that trade restrictions could help limit the spread of the disease. Equally, the use of a vaccine against the larval stages could, in theory, prove effective if used on eels as they enter the rivers. At present, research into the development of artificial spawning techniques and controlled reproduction is being carried out in France and Japan, and application of these methods should be investigated in the UK.

David Hawkins, the secretary for the group, and Ash Girdler voiced concern about fish welfare in sport fishing. Due to the intensive management of clubs and fisheries, there were growing concerns that this would become a significant issue in the future and that steps should be taken now, to improve the situation. This related mainly to hook damage but also includes severe scale loss, nutritional problems, emaciation and chronic mortalities. The problem primarily involves pleasure and competition lakes where the expectation is to catch fish, especially when matches are held. Fish are often held in keep-nets for up to six hours, and a 'good' catch is 100 pounds of fish. It was hoped that, following the successful introduction of hooking mats and topical antiseptics which are now widely used in the hobby, a similar approach could be used to promote the benefits of good fish welfare.

The background factors that attract anglers to particular sites and the changes in the profile of the hobbyist were discussed. This is now a major leisure industry which almost guarantees a catch, and one which provides the customer with a range of additional comforts such as ease of access to sheltered sites, catering and toilet facilities. The image of the angler sitting on the river-bank with the occasional catch now represents only a small sector of the hobby. Carp fishing has increased dramatically over the last 10 years with the introduction of large specimen fish, some weighing up to 40 pounds. The financial value of these fish is substantial and 20 pound carp often cost £1,000 or more. The size of the individual fish caught appears to be more important that the total weight of all the fish caught throughout the day. The hobby is continuing to change and the future may include the introduction of other exotic fish species.

At present, the IFM are drawing up a 'Code of Conduct' to promote health issues and educate the owners of clubs and fisheries about the benefits. These include a reduction of stress and disease in their stock with a better survival following their release by anglers, and consequently a longer lifespan. Although stocking densities are often regarded as a quantitative measure for indirectly assessing fish welfare, it was considered that this varies significantly with the level of management and would not be a useful guide in fisheries. Identifying other factors that could be used to assess welfare was desirable. It was decided that a draft of the Code would be circulated to group members on the email list for constructive comment.

The meeting closed with an update and discussion of future developments for the email list service which links all group members by an email bulletin board. Readers who are interested in joining the group should contact the
Secretary: David Hawkins,
               Environment Agency,
               National Fisheries Laboratory,
               Bromholme Lane,
               Brampton,
               Huntingdon,
               Cambridgeshire PE18 8NE

Report by
William H. Wildgoose

The UFAW Handbook on the Care and Management of Laboratory Animals (seventh edition). Volume 2: Amphibious and aquatic vertebrates and advanced invertebrates.
Edited by Trevor Poole (1999)
208 pages, hardback, £49.50
Blackwell Science Ltd., Oxford. ISBN 0 632 05132 9

Fish and aquatic invertebrates are important animals that are used in the laboratory for a vast number of purposes which include physiology and genetic studies, and ecotoxicology tests. The choice of species is often determined by the purpose of the study or various protocols. Laboratory fish must be maintained in good health in order to obtain meaningful results that are not affected by stress or other husbandry-related factors. Consequently, they must be kept in optimal conditions and receive a high standard of care. The aim of this book is therefore to convey good husbandry practice rather than disease diagnosis and treatment. The Universities Federation for Animal Welfare (UFAW) is an internationally recognised scientific and educational animal welfare charity. Founded in 1926, it was established with the aim of reducing the pain and suffering inflicted by man on animals. The charity funds animal welfare research, publishes extensively and provides expert advice and information on the subject.

Volume one of this two volume set has 864 pages devoted to terrestrial vertebrates and is in three parts: (1) the laboratory animal, a section about background issues relating to husbandry and breeding; (2) animal units, their design and transportation; (3) chapters on the various species kept in the laboratory. In addition to birds and reptiles, the latter covers rodents, lagomorphs, carnivores, ungulates, non-human primates and a few Australian mammals.

Volume two is shorter and also in three parts: (1) the captive environment; (2) vertebrates; (3) invertebrates. The nine chapters have be written by seven well-known international authors. The book opens with a foreword on the history and origin of the UFAW laboratory animal handbook which was first published in 1947. It has become the standard text for those concerned with the husbandry and welfare of laboratory animals but it is also widely read by those in zoos and public aquaria because it contains a wealth of information on the care of animals in captivity. The last edition was published 12 years ago and has now been extensively rewritten to include

important recent information for an international readership. Mention is made of the Three Rs — fundamental principles for making laboratory experiments more acceptable. These are *replacement* of conscious living animals by insentient material such as tissue cultures; *reduction* in animals used to obtain results; and *refinement* of experimental procedures.

In the brief introduction, the editor explains that the need for two volumes is to cater for the demands of different readerships. Despite its detailed content the book discourages complacency and acknowledges that scientific information on the behavioural needs of most species is lacking. The laws relating to laboratory animals varies considerably between countries and is constantly being updated. As a result and unlike earlier editions, it was decided to exclude a chapter on legislation but emphasises that it is the responsibility of the reader to ensure compliance with their own national requirements. The editor concludes with an important paragraph which clearly expresses the UFAW philosophy: by publishing this handbook, the charity may be criticised for facilitating the keeping of animals for experiments. However, it have always taken the view that as long as governments consider it necessary to use live animals for testing the safety and efficacy of products, UFAW has a duty to promote their welfare.

The multi-author chapter on life support systems for aquatic research centres gives an accurate summary of setting up and maintaining a suitable facility. This includes general background information, illustrated with clear diagrams of filter units and several useful tables about water chemistry.

The third chapter gives an introduction to fish, highlighting their anatomy, feeding requirements, reproduction and other aspects relating to the captivity of commonly kept groups of fish. Although there are about 25,000 species offering a wide choice for research purposes, many have very specific needs in captivity and only a few species are used in the laboratory.

The following chapters follow a standard outline describing the biology, capture, handling, transportation, management in captivity, feeding, rearing, laboratory procedures, anaesthesia, euthanasia, diseases and treatment.

The lengthy chapter on freshwater fish provides an excellent overview of all the important aspects of maintaining freshwater species. In the section on anaesthesia, carbon dioxide from Alka Seltzer® tablets is suggested as a method of last resort but I unsure why these would be kept in a laboratory instead of a recognised fish anaesthetic. If it is because they are easily available over the counter in pharmacies, then clove oil (active ingredient

eugenol) would be a safer and equally obtainable non-prescription alternative. There are good practical notes on the collection of various body fluids and a simple method for heparinising blood sampling equipment is described. There are several pages on diseases, diagnosis and common disorders but they include only brief notes on a few pathogens. The chapter concludes with a section on a few commonly used therapeutic agents but includes only two antibacterial agents, namely nifurpirinol and oxytetracycline. It does however, emphasise that veterinarians should always be consulted in this area, and highlights the growing concern about the inappropriate use of some medicines.

The fifth chapter covers similar aspects of marine fish and since most of these are caught from the wild, it provides extensive notes on safe capture methods. The important issues of minimising handling stress and providing correct environmental conditions are also discussed at length together with correct management and various laboratory techniques. The common parasites and diseases are briefly summarised, as is the reproductive data.

The chapter on amphibians has been shortened to less than half the size of its predecessor. This has resulted in a more general, but nonetheless valuable chapter. It highlights important information on their collection and correct handling, and lists some of the uses of amphibians in the laboratory. Recognising individual animals within a group is important and this may require some form of identity marking. Several different methods are listed: where different skin patterns exist, photocopying the animal's body is possible and less destructive than toe-clipping (digit amputation). Although there are several useful references for further reading, the previous edition contained more useful notes on some common species.

The following chapter on aquatic reptiles is short, reflecting the fact that these animals are not widely used in the laboratory. Although it advises that euthanasia is performed by intravenous injection of an anaesthetic overdose or using inhalation agents in a sealed chamber, it also suggests freezing the animal. While this may be necessary because of the constraints of a specific protocol it is no longer considered humane or acceptable in some circles.

The third part of the book is entirely new and devoted to the higher aquatic invertebrates. It spans 57 pages and discusses cephalopods and decapod crustaceans in two chapters. Studies have demonstrated that cephalopods have a remarkable capability for sensory discrimination and true learning, confirming that their welfare should be given greater consideration. *Octopus*

vulgaris is the first invertebrate to be covered by the *Animal (Scientific Procedures) Act 1986* but other species may be included in the future. Many features of the nervous system in the octopus are similar to those in vertebrates, making them useful for studies on nerve function. Detailed knowledge of the requirements of a captive environment is still limited to a few cephalopods but this book provides much valuable information. Small but important points are highlighted: octopuses are particularly prone to escape and their the holding facility must be very secure. Some cephalopods particular health hazards by having painful bites and salivary toxins. Their relatively short life span of 1–2 years and the fact that both sexes die after reproduction, limits their time in captivity. There are some notes about bacterial and parasitic disease, and the problems of cannibalism when cephalopods are held collectively. Anaesthesia is still at an early stage and has traditionally been carried out using magnesium chloride, and more recently, ethanol by immersion.

The final chapter on decapod crustaceans provides information on shrimps, lobsters and crabs which is useful to the hobbyist as well as laboratory staff. There are extensive notes about their complicated biology and many line drawings of most animals in this group. The exact requirements for keeping decapods in captivity are not fully understood but factors affecting water quality and their special requirements are described. There are useful pages about a range of diseases that affect crustaceans and this is followed by some interesting comments on the treatment of these animals.

Most chapters present concise notes on their subject but contain extensive references which are found in the 16 pages at the back of the book. Unfortunately, the brief index is more like an expanded contents section relating to subsections within the chapters, and is of limited use.

Although this book may be of limited value to veterinarians in practice, it is immensely useful to those unfamiliar with the complex environmental needs of aquatic species. The overall style is clear and concise but it is also extensively referenced and directs the reader to more detailed publications. Despite a few minor points, it is impossible to fault this compact and carefully written book: it is full of valuable and practical information which will be of great value to laboratory staff and anyone involved in keeping aquatic animals in captivity.

William H. Wildgoose

On Mary Brancker's elevation to the rank of Honorary Life Member of the Fish Veterinary Society

Mary Brancker qualified from the Royal Veterinary College in 1937, one of the first women to do so. Over the following years she was a tireless practitioner of the veterinary art, working continuously in general practice, but also finding time to become the first and only woman president of the British Veterinary Association (BVA) in 1967/68. Mary's great enthusiasm, organisational ability and determination to get things done have left many trailing in her wake. For these efforts she has been rewarded with, amongst other things, a Fellowship of the Royal College of Veterinary Surgeons, and an OBE and, latterly, a CBE by the State.

What is of particular interest to the Fish Veterinary Society is her pioneering role in the veterinary care of fish. Coinciding with her term as BVA president, culture of marine fish suddenly became a reality in the UK. Realising the potential for fish farming, she used her position to promote veterinary interests in this new field. One of the important achievements of this phase of her life was to be instrumental in setting up the Institute of Aquaculture at the University of Stirling, a fact that was remembered by the Institute during their 25th anniversary, at which Mary was awarded an honorary doctorate.

During these formative years of fish culture in the UK, Mary made a visit to the Sea Fish Industry Authority's research facilities at Ardtoe in Scotland, a visit which began a working relationship which continues to this day. It was at Ardtoe that she first became acquainted with her beloved halibut. To hear the wonders of the halibut extolled by Mary, is to hear a true enthusiast.

While most of her contemporaries were denying the existence of fish as a serious area of study for the veterinary profession, Mary showed that it was possible for vets to apply their skills effectively in the treatment of these animals; and also demonstrated to fish owners/farmers that vets could make a valuable contribution to the health and welfare of the fish. She has always been willing to help people to get to grips with this subject and, once introduced to the fascination of fish, one succumbs easily to her infectious enthusiasm.

Mary was one of the founding members of the Fish Veterinary Society, so it is fitting that the Society recently voted unanimously to offer Mary an Honorary Life Membership of the Society as a way of thanking her for her part in opening up the field of fish health to the veterinary profession. And I'm sure that the fish would agree!

Edward Branson

New Web Site

The Fish Veterinary Society has a new web site which can be found at:

www.fishvetsociety.org.uk

Not only does it contain information about the Society itself, but there are regular updates on relevant forthcoming events and scientific meetings. It will soon be possible to download the membership application form and check the contents of previous editions of the *Fish Veterinary Journal*. It is hoped that the site can be developed further and incorporate a 'chat room' or an on-line discussion forum. We are open to any suggestions about the content and format — this is your chance to become more directly involved with the public face of the Fish Veterinary Society.

Associate membership for non-veterinarians

The Fish Veterinary Society (FVS) was formed in July 1990 to provide veterinarians working with fish the opportunity to benefit from the experience of others through regular meetings and latterly through the pages of the *Fish Veterinary Journal*. The formation of the FVS was recognition that there are scientific, legal and ethical issues of particular relevance and importance to the profession which were not being addressed elsewhere. For this reason, membership to date has been confined to veterinarians since it was not felt that other fish health professionals would be particularly interested in joining the Society. However, in the 10 years since its inception the FVS has gone from strength to strength and has credibility in the wider fish health community.

For this reason, and as result of interest expressed by non-veterinarians, a proposal to amend the constitution and to extend membership was put to those who attended the annual general meeting (AGM) in Weymouth in December 1999. After much debate, it was agreed without serious dissent that a new category of member, Associate, be created for those who might be interested in the Society's activities. Associate Members will enjoy all the benefits of Full Members but without the right to sit on the committee or vote at any of the Society's meetings or attend the AGM. This is not an attempt to disenfranchise new members but is intended to ensure that the Society retains its unique identity and particular objectives identified at its formation. Prospective members should be proposed and seconded by current Full Members.

We hope that new members, both within and outwith the profession, will be encouraged to apply to the Treasurer and allow the FVS to fulfil its purpose to advance the care and welfare of fish. Further details of the Society can be found at the new web-site at **www.fishvetsociety.org.uk**

Andrew Grant
President 1997-1999

BACK ISSUES

Copies of earlier issues, except number 1, are still available. These can be purchased using the order form overleaf, with preferential rates for packs of ten copies. The main contents of the last two issues are listed below.

Issue No. 3 (February 1999)
Successful removal of a gastric foreign body from a red tailed catfish, *Phractocephalus hemioliopterus*. *W.H. Wildgoose*
Koi health care in the UK: a veterinary overview. *W.H. Wildgoose*
Preliminary investigations into the bacteriology of skin lesions of Atlantic salmon reared in seawater in Scotland. *L.A. Laidler, A.N. Grant & S. Wadsworth*
'Vibrio viscosus', the agent of winter sores. *E. Benediktsdóttir*
Parasites, resistance and control strategies. *M.A. Taylor*
Sea lice, medicines and a national strategy for control. *G.H. Rae*
Health care in a large public aquarium. *D.R. Gibson*
Sarafin®, a novel quinolone for bacterial disease. *P.J. Southgate*
Clinical observations of severe mortalities in koi, *Cyprinus carpio* with gill disease. *C.I.Walster*
Description of a myxosporean disease in cultured turbot (*Scophthalmus maximus*).
E.J. Branson & A. Riaza
Infectious salmon anaemia in the United Kingdom. *T. Turnbull*
Halamid® = Biosecurity. *D.J.C. Campbell & D.G. Parsons*
Avian influenza ecology: a brief review. *D.J. Alexander*
Bronopol: an alternative to malachite green? *G.D. Cawley*
Book & video reviews:
 Fish Stress and Health in Aquaculture (Iwama, Pickering, Sumpter & Schreck 1997)
 Handbook of Trout and Salmon Diseases, 3rd ed (Roberts & Shepherd 1997)
 Koi Health and Disease (Johnson 1998)
RCVS diploma in fish health and production

Issue No. 4 (September 1999)
Aerobic microflora of imported tropical ornamental fish from Singapore and South America.
Part 1: Characterization. *R.E. Del Rio-Rodriguez & J.F. Turnbull*
Acid-fast bacteria found in granulomatous lesions in a koi (*Cyprinus carpio*). W.H. Wildgoose
Use of Supaverm® for the treatment of monogenean infestation in koi carp (*Cyprinus carpio*).
C.J. Marshall
Availability of medicines for fish. *K.M. Treves-Brown*
Infectious salmon anaemia in the UK: an update. *T. Turnbull*
The role of biosecurity in disease prevention: a poultry primary breeding company perspective.
R.J.W. Currie
Salmon Health Group. *A.N. Grant*
Institute of Fisheries Management: fish disease discussion group
Book reviews:
 Anaesthetic and Sedative Techniques for Aquatic Animals, 2nd edition (Ross & Ross)
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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 and those with an appropriate interest/ degree as set out in the Constitution of the Society (available on request from Treasurer).

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.

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*The sum of £50 is enclosed for full enrolment into the Fish Veterinary Society and membership for the current year. Future payments will be made by Standing Order each year in January (mandates available from Treasurer)

*I am a veterinary undergraduate and wish to become an associate member of the Fish Veterinary Society and I am due to graduate in _____ (year)

delete as appropriate

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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.

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Other Membership:

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