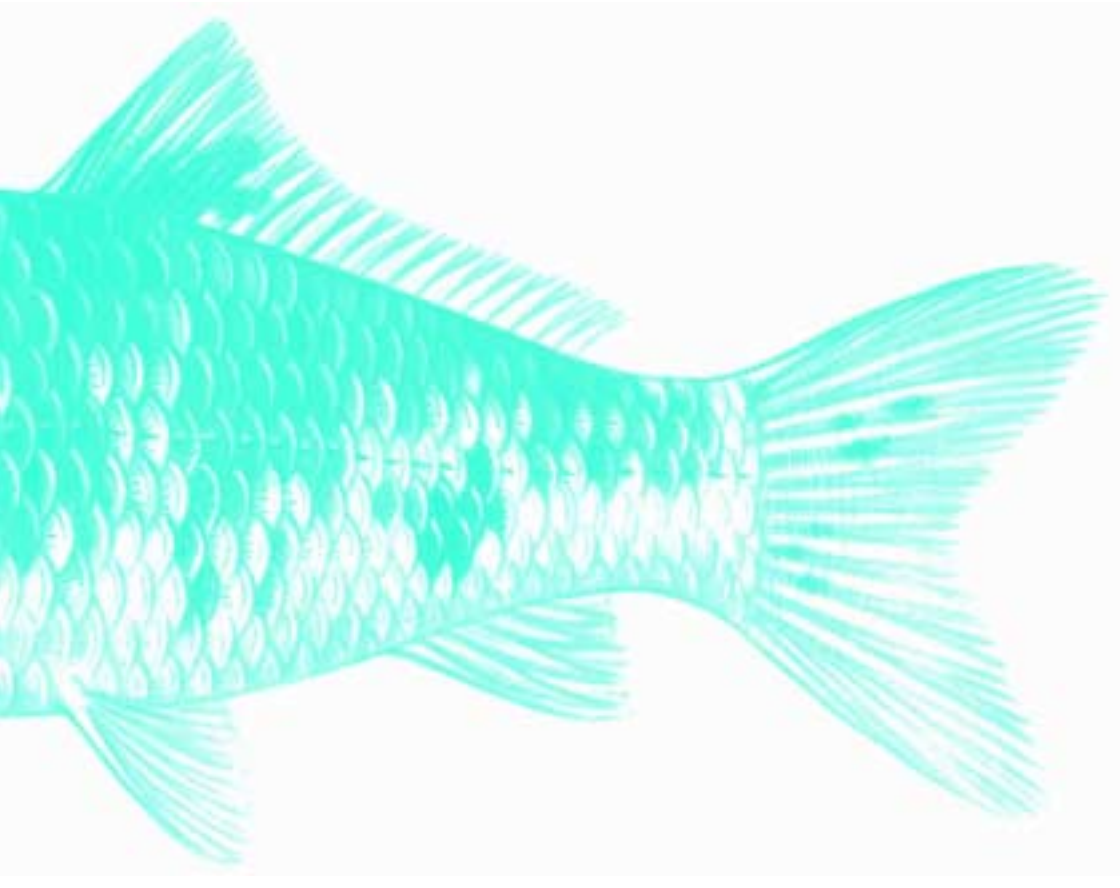
FISH VETERINARY JOURNAL

The Journal of the Fish Veterinary Society

Issue Number 6 • February 2002



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

The Society provides:

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

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 Honorary 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 J. McArdle, 119 Park Drive Ave, Castleknock, Dublin 15, Ireland. 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 a Word document on 3½" diskette (MS-DOS format) or by email to johnmcardle9@hotmail.com. 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, e.g. infectious pancreatic necrosis (IPN). All units of measurement should be given in the metric system and temperatures in degrees Celsius. 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, e.g. amoxycillin (Vetremox®, Vetrepharm). The full Latin name for each species should appear at least once when mentioned in the text.

Length of papers:

Papers should be concise. As a guide, the maximum length for scientific articles is 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 disease. *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, for example, 'Morrison et al 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 footnote (maximum of 100 words) for scientific articles.

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

President's Reflections

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

Since taking over the role of President of FVS, I have found the position very challenging and demanding. Despite excellent assistance and encouragement from Andrew Grant, our most recent past president, I quickly realised that the role of President of the FVS is not something you can easily take on without some knowledge of the issues involved. This is why we proposed the re-introduction of a vice president post on the committee to ensure some continuity from one committee to the next and to be able to drive forward on-going issues. This was agreed at the October 2000 AGM in Belfast and Edward Branson elected to the post. Edward brought with him a wealth of background knowledge and his input as Vice President has been invaluable. We have also set up a number of sub committees to help tackle the key issues facing the society and which we must address in a unified and constructive way.

We have made some progress in our relationships with the government fisheries authorities, such as with SERAD agreeing to issue results of diagnostic and statutory testing to vets upon the consent of their clients. Keith Treves-Brown has highlighted significant issues relating to the mixing of medicated feedstuffs on fish farms, and I'm grateful to him for driving this matter for the Society. I think the message is slowly getting through that the FVS and fish vets are an important part of the overall future fish health program within the UK & Ireland. I hope that ongoing discussions with various professional and statutory bodies will be useful in highlighting our difficulties and also how we as vets can and should be more closely involved in fish health. We also need to muster support in our efforts from the BVA and RCVS.

After much lobbying, the BVA has now agreed to recognise the FVS as a specialist branch. Full recognition required a change of BVA rules, which was ratified at the BVA AGM in October 2001. This means that FVS needs to send a representative to all BVA meetings and much more paperwork to trawl. Membership numbers are still an important issue and I would therefore ask you all to seek out further members to keep our numbers up and to expand our very dynamic and progressive society.

Chris Walster attended a meeting of RCVS in July 2000 of the animal production divisions of the BVA on behalf of the FVS. Many of the issues relevant to fish were aired including illegal medicine imports, availability of medicines, dispensing and cost of medicines, Special Treatment Authorisation etc. We need to address all these issues from a fish vet's perspective.

Andrew Grant continues as FVS representative on the Aquaculture Health Joint Working Group (AHJWG) of SERAD. A very successful self-financing IPN Seminar was held in Edinburgh in June 2000 and was attended by 45 delegates from across the salmon industry. A draft summary of the IPN seminar was presented to the AHJWG on September 1st by Andrew Grant and was well received. A copy of the final report was circulated to all participants and sponsors and is reproduced in this issue. As a consequence of our IPN seminar, the FVS was asked by the AHJWG to carry out a survey of members and appropriate groups in an effort to determine the real incidence and impact of IPN in salmon. **All the major vaccine companies generously sponsored this work and the results will be reported when available.** I have also been asked to join similar fish health working groups set up by MAFF now DEFRA and by the Department of the Marine in Ireland.

Ronnie Soutar organised a very practical and pertinent workshop on fish vaccination at the Institute of Aquaculture, Stirling in November 2000, where a broad range of topics related to fish vaccination were discussed. A short summary of the meeting will be included in the next issue of this Journal. In principle it was agreed that a code of practice for vaccination of fish should be drawn up by FVS. This meeting was followed up in September 2001 by an excellent practical workshop on vaccination reactions organised and hosted by Fish Vet Group in Inverness. The guest speaker was Aud Skrudland, a very experienced field veterinarian from Norway, who gave us the benefit of her observations on vaccination reactions and scoring. It is proposed that a FVS leaflet will be drafted to reflect the discussions and to help to standardise local reaction scoring worldwide. This meeting was kindly sponsored by AVL.

We now are involved in producing a very professional journal, providing representation on various working groups and committees as well as organising at least two scientific meetings per year. This all takes time, effort and huge

commitment especially on behalf of the committee members. If we want to continue to be proactive then we need volunteers to assist with lobbying, drafting guidelines, and driving particular issues. The committee work hard on your behalf but the workload does need to be shared otherwise no one will want to serve on future FVS committees. So if you are willing to serve on any of the following sub-committees or at least are prepared to be persuaded, please contact me. The proposed committees are:

- Review of EU Fish Health Regulations,
- Review of dispensing of veterinary medicines,
- Developing a code of practice for fish vaccination,
- Review of the Veterinary Surgeons Act,
- Medicated feeding stuffs regulations
- Liaison with professional and governmental bodies or
- just helping to organise one of our meetings.

Two very successful meetings were held in Aberdeen and Belfast in spring and autumn and we hope to now set our annual meetings on set dates in March and October while still alternating the venues between North and South. The Spring meeting was scheduled for Cambridge on 21st March 2001, but due to the Foot and Mouth crisis it was cancelled and was re-organised for the 24th October 2001 in Mildenhall.

Sadly, Willie Wildgoose has resigned as publications secretary. I would like to thank him on behalf of all the membership for his dedication and professionalism in developing the Journal and for his wit and wisdom on the committee. We have had some hiccups in getting this issue published but I trust that it will flourish in the enthusiastic and capable hands of our new editor John McArdle.

Editor's Comments

John McArdle
119, Park Drive Avenue, Castleknock, Dublin 15

I have always believed that two of the great strengths of the FVS are the twice-yearly scientific meetings and the FVS Journal. The papers presented at the meetings are always of high quality, practical and interesting to veterinarians involved professionally with fish or those simply interested in them as a unique animal group. Papers from both veterinarians and non-veterinarians are welcome and the opening up of FVS associate membership to those from disciplines other than veterinary science was an enlightened move. The policy of publishing papers presented at our spring and autumn scientific meetings in the Fish Veterinary Journal is important and should be continued. It means that the information can be disseminated to a wider audience and enables the presenters of the papers to deal in more detail with the subject matter of their presentation. In addition, it enhances the profile of the FVS and leaves an accessible historical record of our deliberations.

The papers contained in this issue are chiefly made up of those papers presented at the Autumn Scientific meeting held in Belfast in October 2000. This meeting was memorable in many ways, not least because it was held in the home city of our then President, Marian McLoughlin. Held in Ireland for the first time, the venue of the prestigious Northern Ireland Dept. of Agriculture Veterinary Research Laboratory at Stormont was an excellent one. The attendance was high, the papers were relevant and interesting and the field trip and social programme were outstanding. Marian can claim all credit for its success. At Marian's suggestion, I have included a number of the images she captured on photograph which convey something of the enjoyment of the few days of the meeting and field trip.

In between issues of the Journal it is the intention to produce an FVS Newsletter and in this regard any short articles, opinions, news snippets or letters from members would be most welcome. If you have something to say or know of some interesting developments, put them on paper and send them to me.

Because my predecessor, Belinda Weigall, had already done much work on this issue my task was made easier and special thanks are due to her for her efforts. We wish her well on her return to Australia.

I would like to apologise to everyone for the delay in getting this issue out, despite my best efforts. I have to put this down to gross inexperience. I would like to mention specially Mike Williams of Akalat Publishing for his unfailing assistance without which this issue would not have appeared. Finally, I would also like to thank our sponsors and advertisers without whose continuing support and generosity it would not be possible to produce this Journal or hold our scientific meetings.

John McArdle

Fish Veterinary Society Autumn Scientific meeting
Belfast, October 2000

Photograph 1. Participants and speakers outside N.I. Veterinary Research laboratory.
Front row (l–r) Ed Branson, Tom Murphy, Julian Braidwood, Marian McLoughlin, John McHenery, Keith Treves-Brown, Norman Gault, Hugh Ferguson.
2nd row (l–r) Tom Turnbull, Fiona MacDonald, Willy Wildgoose, Dave Jackson, John McArdle, Bosco Cowley
3rd row (l–r). Myriam Algoet, Catriona Webster, Bob McCracken
Back (l–r). Dermot Sparrow, Andy Holliman, Jim Powell, Des Rice, Hamish Rodger, Brian Ross

Photograph 2. Field visit to Bushmills Whiskey Distillery, Co. Antrim.
From left: John McArdle, Brian Ross, Caitriona Webster, Chris Walster, Marian McLoughlin, Ed Branson, Andy Holliman.

Photograph 3. Field visit to Giants Causeway, Co. Antrim

Left–right: Mark Patterson, Andy Holliman, John McArdle, Brian Ross, Catriona Webster, Ed Branson, Chris Walster

Photograph 4. Andy Holliman explains the geology of the Giants Causeway to Ed Branson

(*Photographs courtesy of Marian McLoughlin*)

SCIENTIFIC PAPER

Fish Veterinary Journal (2002) (6) 1–21

Aerobic microflora of imported ornamental fish from Singapore and South America. Part 2: Antimicrobial Resistant Profiles of motile Aeromonads.

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Abstract

Motile aeromonads isolated from ornamental fish shipments from Singapore and South America were tested for their resistance to amoxicillin, apramycin, cephalothin, chloramphenicol, enrofloxacin, furazolidone, oxolinic acid, oxytetracycline, streptomycin, sulphamethoxazole-trimethoprim, and sulphamethoxazole. Although antibiotic multiresistant bacteria from both origins were discovered in similar proportions, bacteria responded differently to specific antibiotics, such as oxolinic acid, apramycin and enrofloxacin, and this response varied depending on the origin. Nevertheless, the frequency of resistance to antibiotics was more pronounced in the bacterial groups tested for Singapore when compared with South America. The possible source and implications of these results are discussed.

Introduction

One of the consequences of the increased use of antimicrobials in fish therapy has been the emergence of resistant bacterial fish pathogens, which in turn have limited the options of antimicrobials available for treating fish disease (Lewin 1992). Moreover, there is already widespread resistance to those available for fish therapy (Ansary et al 1992; Aoki 1992; Dixon 1994).

As in other aquaculture activity, antimicrobials have been used in the production of ornamental fish. In Britain, fish are not included in the Veterinary Surgeons Act (1966) so non-veterinarians can investigate, diagnose, treat fish, and even perform surgery (Wildgoose 1999). However, the prescription of antibiotics for use in fish remains the privilege of registered veterinarians. In the UK, only amoxicillin, potentiated sulphonamides, oxolinic acid, oxytetracycline and sarafloxacin are licensed for use to treat fish bacterial infections (Southgate 1999). However, only two of those (oxolinic acid and oxytetracycline) are licensed for use in ornamental fish (Wildgoose 1999).

In Singapore a large number of antibiotics commonly employed in human and veterinary medicine have been used for the treatment of diseases in the ornamental fish industry (Chan, personal communication), although it is not clear if those antibiotics have been used off-license or for experimental purposes. According to Ferraz (1995), in South America the use of a wide range of antimicrobials is still not a common part of the husbandry while the fish are held in facilities before they are packed and dispatched. In South America ornamental fish are mostly shipped to the USA where they are trans-shipped to other parts of the world. In the USA only tetracycline, sulfamerazine and a potentiated sulphonamide are licensed for use (Dixon et al 1990).

Singapore and South America are the two main suppliers of ornamental tropical fish to the UK (Cheong 1996; Davenport 1996). However, despite the supposed risks that ornamental fish represent as vector of potentially harmful bacteria to other fish and man (Anonymous 1997), research on this risk is poor and the scarce information is scattered in the scientific literature.

This paper is the second report of a larger study on ornamental tropical fish imported into Scotland and discusses the findings of antibiograms performed on motile aeromonads isolated from ornamental tropical fish shipments from Singapore and South America during 1996–1998 (del Río-Rodríguez and Turnbull 1999).

Materials and methods

Antibiotic resistance study

The motile *Aeromonas* spp. isolates tested in this work were obtained from the shipping water, fish slurry and from internal organs of fish (Table 1, see del Río-Rodríguez and Turnbull 1999). The isolates were maintained on TSA

slopes in the laboratory at room temperature (18°C). Before the tests, all 73 isolates were checked for purity by re-isolation on TSA agar and incubated at 22°C. After overnight growth, some colonies were removed with a sterile loop and suspended in 0.85% (w/v) saline solution. The bacterial suspensions were diluted to the density of a MacFarland number 0.5 barium sulphate turbidity standard. Colony counts were carried out on randomly chosen diluted suspensions (n=30) that produced an average of 1 x 10^8 CFU/ml (CFU = colony forming units).

TABLE 1: Number of isolates tested for antimicrobial resistance

Isolates	Singapore				South America			
	W	FS	F	Subtotal	W	FS	F	Subtotal
Motile Aeromonas spp.	3	11	44	58	3	3	9	15
Pseudomonas spp.	4	14	4	22	10	10	5	25
Alcaligenes faecalis	9	12	6	27	5	6	1	12

W = water, FS= fish slurry, F= fish internal organs

The isolates were tested by the Kirby-Bauer disc diffusion method (Lorian 1986; Frerichs and Millar, 1993) for sensitivity to 11 antibiotics on Mueller-Hinton agar plates (Table 2). The antibiotics selected included antimicrobials which are currently licensed for the aquaculture industry in the UK.

The Mueller-Hinton agar plates were inoculated with 200 µl of bacterial suspension and spread to produce a lawn of growth. Three or four antibiotics were placed on each Petri dish (4,4,3) per isolate immediately after inoculation and the plates incubated at 22°C and each test was duplicated. Inhibition zones were read after 24 and 48 hours of incubation. The zones were measured to the nearest millimetre using callipers (Camlab, Cambridge UK).

The description of isolates cultured as susceptible, intermediately susceptible and resistant followed other published reports. However, in this study bacteria falling into the categories of susceptible or intermediately susceptible (Table 3) were both considered to be susceptible. This approach was adopted since at the moment of performing the tests, no official standards of susceptibility (disc diffusion method) existed for fish pathogens.

TABLE 2: Antibiotic sensitivity discs and their contents

Antibiotic	Symbol	Content in mg
Amoxicillin*	AML	10
Apramycin	APR	15
Cephalothin	KF	30
Chloramphenicol	C	10
Enrofloxacin	ENRO	5
Furazolidone	FR	50
Oxolinic acid*	OA	2
Oxytetracycline*	OT	30
Streptomycin	S	10
Sulphamethoxazole-Trimethoprim*	SXT	25 (23.75–1.25)
Sulphamethoxazole	RL	25

*Antibiotics currently licensed for veterinary use for the Aquaculture Industry in Britain.

TABLE 3: Bacterial inhibition zone diameter (mm) and level of antibiotic sensitivity*

	Resistant	Intermediate Susceptible	Susceptible	Source
Amoxicillin	≤13	14–20	≥21	Acar and Goldstein 1986
Apramycin	≤12	13–14	≥15	Plumb and others 1995
Cephalothin	≤13	14–17	≥18	Plumb and others 1995
Chloramphenicol	≤12	13–17	≥18	Lorian 1986
Enrofloxacin	≤16	17–20	≥20	Plumb and others 1995
Furazolidone	≤16	–	≥17	Inglis and others 1991
Oxolinic acid	≤16	–	≥17	Inglis and others 1991
Oxytetracycline	≤13	14–20	≥21	Plumb and others 1995
Streptomycin	≤11	12–14	≥15	Acar and Goldstein 1986
Sulpha -Trimethoprim	≤14	15–19	≥20	Plumb and others 1995
Sulphamethoxazole	≤12	13–16	≥17	Acar and Goldstein 1986

*All discs supplied by Unipath, Ltd. UK.

Statistical analysis

The percentages of resistant isolates were square root arcsin transformed, tested for normality by the Anderson-Darling test and compared by Student *t* test for two independent samples (Zar 1996). The groups were compared by origin (i.e. Singapore with South America). The level of significance chosen was $P<0.05$. Data analysis was performed using Minitab ver 12.0 (Minitab Inc. ®, USA).

FIG 1: Antibiotic Resistance of Motile Aeromonads

RESULTS

Percentage of resistant isolates (Singapore and South America)
Inhibition zones on motile *Aeromonas* spp. inoculated plates were readily measurable after 24 hours. A higher proportion of *Aeromonas* spp. isolates from Singapore were multi-resistant compared with South America (Figure 1). At least 60% of isolates from both origins were resistant to either AML, KF, RL, OA and OT. There was a difference in the percentage of resistant isolates for OA and SXT since only 20% of *Aeromonas* spp. from South America were resistant to OA while up to 88% of isolates from Singapore were resistant to this antibiotic. Of South American *Aeromonas* spp. 26% were resistant to SXT while for Singapore more than 50% were resistant (Figure 1).

Multiple antibiotic resistance (Singapore and South America)
All of the isolates from both origins were resistant to at least one antibiotic. Two isolates from Singapore shown to be resistant to all 11 antibiotics tested, although most of the isolates were resistant to either 3 or 10 antibiotics. Few were resistant to ENRO (Table 5). The highest proportion of South American isolates seemed to be commonly resistant to 2 antibiotics while only two isolates were resistant to 10 of the 11 antibiotics tested. No resistance to ENRO was detected for the South American isolates.

TABLE 4: Multiple antibiotic resistance of *Aeromonas* spp. isolates

	Origin and number of isolates	
Number of antibiotics to which resistant	Singapore	South America
None	0	0
1	1	0
2	1	6
3	3	1
4	4	1
5	10	1
6	10	3
7	7	1
8	9	0
9	8	0
10	3	2
11	2	0
Total	58	15

TABLE 5: Patterns of antibiotic resistance of *Aeromonas* spp. Singapore

Patterns*											Num. of isolates.
OT	OA	AML	KF	RL	SXT	S	APR	FR	C	ENRO	2
OT	OA	AML	KF	RL	SXT	S		FR	C	ENRO	2
OT	OA	AML	KF	RL	SXT		APR	FR	C	ENRO	1
OT	OA	AML	KF	RL	SXT	S		FR	C		6
OT	OA	AML	KF	RL	SXT	S	APR		C		1
	OA	AML	KF	RL	SXT	S	APR	FR	C		1
OT	OA	AML	KF	RL	SXT	S			C		6
OT	OA	AML	KF	RL		S		FR	C		1
OT	OA	AML	KF	RL		S	APR	FR			1
OT	OA	AML	KF	RL	SXT	S					3
OT	OA	AML	KF	RL		S			C		2
OT	OA			RL	SXT	S		FR	C		1
OT	OA	AML	KF	RL	SXT					ENRO	1
OT	OA	AML	KF	RL	SXT						3
OT	OA	AML	KF	RL					C		1
	OA	AML	KF	RL	SXT	S					1
OT	OA		KF	RL				FR	C		1
OT	OA	AML		RL				FR	C		1
OT	OA	AML		RL	SXT				C		2
OT	OA	AML		RL		S			C		1
		AML	KF	RL		S					2
OT	OA	AML	KF	RL							3
OT	OA	AML	KF	RL							1
	OA	AML	KF	RL	SXT						1
OT	OA	AML		RL				FR			1
OT	OA			RL		S	APR		C		1
OT		AML	KF	RL		S					1
OT		AML	KF		SXT	S					1
OT	OA	AML	KF								2
OT		AML	KF	RL							1
	OA	AML	KF	RL							1
	OA	AML	KF								1
	OA		KF	RL							1
		AML	KF	RL							1
		AML		RL							1
OT											1
									TOTAL		58

*OT=oxytetracycline, OA= oxolinic acid, AML= amoxicillin, KF= cephalothin, RL= sulphamethoxazole, SXT= potentiated sulphonamide, S= streptomycin, APR= apramycin, FR= furazolidone, C= chloramphenicol, ENRO= enrofloxacin

Patterns of antibiotic resistance (Singapore)
Twenty-four isolates of *Aeromonas* spp. from Singapore shared a resistance pattern to OT, OA, AML, KF, RL and SXT (Table 6). Only 4 isolates shared susceptibility to both sulphonamides (RL and SXT). However, 38 isolates were susceptible to both APR and FR.

Although APR and S are closely related antibiotics, not all APR susceptible isolates (52) were also susceptible to S (24).

The higher frequency of resistance for exact patterns of Singapore aeromonads corresponded to the patterns OT, OA, AML, KF, RL, SXT, S, FR, C (6 strains) and OT, OA, AML, KF, RL, SXT, S, C (6 strains) (Table 6). This seems to indicate that antibiotic resistance patterns in aeromonads from Singapore are more consistent.

Patterns of antibiotic resistance (South America)
With respect to the South American isolates, 6 displayed a resistance pattern to AML, OT, KF and RL which was a similar to the pattern obtained for the Singapore isolates (Table 6). However, South American *Aeromonas* spp. were mostly susceptible to both OA and ENRO (12 isolates).

Seven isolates resistant to RL were also resistant to SXT, but alike to Singapore isolates, S susceptibility in 12 isolates was accompanied by sus-

TABLE 6: Patterns of antibiotic resistance of *Aeromonas* spp. South America

Patterns*										Number of isolates.
OT	OA	AML	KF	RL	SXT	S	APR	FR	C	2
OT		AML	KF	RL	SXT	S			C	1
OT	OA	AML	KF	RL	SXT					1
OT		AML	KF	RL	SXT					1
OT		AML	KF	RL	SXT					1
OT		AML	KF	RL						1
OT		AML		RL						1
OT		AML								3
OT				RL						1
		AML	KF	RL	SXT					1
		AML		RL						1
		AML	KF							1
								TOTAL		15

*OT=oxytetracycline, OA= oxolinic acid, AML= amoxicillin, KF= cephalothin, RL= sulphamethoxazole, SXT= potentiated sulphonamide, S= streptomycin, APR= apramycin, FR= furazolidone, C= chloramphenicol

ceptibility to APR. Also 12 isolates were susceptible to both FR and C. For South American aeromonads, the highest frequency corresponded to the pattern formed by OT, AML (3 strains) (Table 7).

TABLE 7: The percentages of *Aeromonas* spp. resistant isolates from ornamental tropical fish from Southeast Asia and Singapore

Antibiotic Groups	Geographic Source*		
	Southeast Asia[a]	Singapore[b]	Singapore[c]
ß-lactams			
Amoxycillin	-	-	88.1
Ampicillin	91	100	-
Penicillin	69	-	-
Macrolides			
Apramycin	-	-	8.5
Erythromycin	-	64	-
Neomycin	-	26	-
Streptomycin	35	-	54.2
Nitrofurans			
Furazolidone	-	-	32.2
Nitrofuradantoin	-	51	-
Unspecified nitrofuran	13.6	-	-
Quinolones			
Enrofloxacin	-	-	11.9
Naladixic Acid	None	30	-
Oxolinic Acid	-	27	88.1
Sarafloxacin	-	8.5	-
Tetracyclines			
Oxytetracycline	-	-	76.3
Tetracycline	43	96	-
Sulphas, Diaminopyrimidines and Potentiated Sulphonamides			
Sulpha	84	-	-
Triple Sulpha	80	-	-
Sulphamethoxazole	-	-	88.1
Trimethoprim	-	44	-
Ormetoprim-sulphadimethoxine	-	67	-
Sulphamethoxazole-Trimethoprim	-	60	57.6
Cephalosporins and Choramphenicol			
Cephalothin	-	-	83.1
Chloramphenicol	19	-	52.5

*The sign minus (-) denotes not tested in a particular work.
[a]Shotts and Gratzek 1984
[b]Dixon et al. 1990
[c]The present study

Statistical comparison of antibiotic resistance
The square-root arcsin transformed percentages of antibiotic resistance data for *Aeromonas* spp. from both origins passed normality, therefore the *t* (independent) Student test was used. Results of the probability values (P=0.16) suggested no significant difference, i.e. the proportion of aeromonad isolates resistant to multiple antibiotics from Singapore were not different from the proportion of aeromonad isolates resistant to multiple antibiotics from South America.

Discussion

General findings
It was found during this study that most of the bacterial isolates were resistant to various antibacterials tested. It would appear that some isolates from Singapore and South America shared similar resistance patterns. However, although many of the bacterial isolates from Singapore and South America were found to be resistant to several antibacterials, they differed in their susceptibility to individual antibacterials.

The statistical test comparing the transformed percentages of resistance of the isolates from South America with Singapore detected no significant difference. The statistical test in this case suggested that similar proportions of motile aeromonads, from Singapore and South America are resistant to multiple antimicrobials. These proportions would only differ if particular antibiotics could be tested separately, for example, as with the case of oxolinic acid for motile aeromonads from both sources.

The results of this study would suggest that enrofloxacin, apramycin and furazolidone would be the best choice for treating fish infected with motile aeromonads from both Singapore and South America. Oxolinic acid should also be a good option for fish from South America and potentiated sulphonamide may have some effect on bacteria from both sources. Due to the wide spectrum of activity, apramycin and enrofloxacin have been already suggested as the best antimicrobials to treat fish bacterial infections (Plumb et al 1995). However, at the time of writing these antibiotics were not currently licensed for fish chemotherapy in the UK.

Four of the antibiotics included in the test are currently licensed for use in the aquaculture industry in the UK. During the planning stages of this study,

it was intended to include sarafloxacin in the tests, which has been recently licensed for aquaculture. Unfortunately sarafloxacin was not available during this study. Therefore, it was decided to include enrofloxacin instead – because of its similarity to sarafloxacin. However, enrofloxacin has received considerable attention from fish pathologists and veterinarians due to the role of second generation fluorquinolones in the treatment of infections in man. The other 10 antibacterials tested are being used routinely during antibiograms of bacterial fish pathogens under study by the Diagnostic Services of the Institute of Aquaculture, University of Stirling.

Antibacterial resistance (Singapore)

The results obtained in this study suggested that imports of ornamental fish to be treated for motile aeromonad infections, should respond favourably to treatment with enrofloxacin and apramycin. Some success in controlling the infection could be achieved if furazolidone was employed. However, none of these antibiotics are licensed for treatment of ornamental fish in the UK.

Although the *Aeromonas* spp. group remained un-speciated in this work, it is possible that several species were represented. To discover if a species of aeromonad were more common within the group would require further work. However, it was interesting to note that 83% of the strains tested here showed resistance to cephalothin. Cephalothin has been used in the identification of motile aeromonads (Janda and Motyl 1985). Cephalothin susceptibility has been significantly associated with *A. sobria* (Janda and Motyl 1985). Therefore, it is reasonable to assume that since 83% of motile aeromonads tested in this study were resistant to cephalothin most of the aeromonads isolated in this work were *Aeromonas* species other than *A. sobria*.

The reasons for multiple resistance in the motile aeromonads isolated from Singapore in this study may have originated from the way ornamental fish are raised in that country. Ornamental fish are extensively produced in fish farms or large aquaria systems where diseases are common and antimicrobials are routinely used for prophylaxis and treatment, including in the shipment water (Meier & Schmitt 1992). Therefore it is reasonable to assume that the antibacterial resistance displayed by motile aeromonads from Singapore may be a direct result of previous exposure to antimicrobials, since resistance to several antibiotics was shared by most of the isolates tested.

Evidence of multiple resistance may be found on previous work carried out on motile aeromonads isolated from imported ornamental fish from Southeast Asia. The emergence of antibiotic multiresistant motile aeromonads in the ornamental fish industry in Southeast Asia was detected as early as the mid 1970's. Shotts et al (1976) found antibiotic multiresistant isolates of the '*A. hydrophila*-complex' in water and ornamental tropical fish imported from Southeast Asia. '*A. hydrophila*-complex' was a term used in the past by researchers and diagnosticians to include all motile aeromonads (Thune et al 1993). A high percentage of the *A. hydrophila* isolated by Shotts and others (1976) were resistant to ampicillin, an unnamed tetracycline, sulphamethoxazole, a sulphamethoxazole-based drug (named as 'sulpha drug') and streptomycin. Of the isolates, 25.5% recovered from diseased fish and 14.1% from the aquarium water carried plasmids imparting conjugal transmissible antibiotic resistance to tetracycline and/or antibiotics based on sulphates.

Shotts et al (1976) suggested that the presence of plasmids among motile aeromonads was an indication of increased use of antibiotic therapy on a prophylactic basis rather than for treatment of diseases. These authors also suggested that antibiotics such as ampicillin, antibiotics based on sulphates, tetracycline and streptomycin were of little use, while other 'not commonly used' drugs such as nitrofurans, chloramphenicol and nalidixic acid appeared to be more effective *in vivo*. It was interesting to note that the suggestions of Shotts et al (1976) fit the patterns of resistance/susceptibility found in this study. The motile aeromonads isolated during this study were resistant to amoxicillin, which is a compound related to ampicillin; to sulphamethoxazole which is a sulphate based drug; to oxytetracycline, which is a close compound to tetracycline, and to streptomycin. This pattern of resistance is similar to that found in the aeromonads isolated from Southeast Asia in 1976 by Shotts and co-workers. Aeromonads isolated in 1976 were also susceptible to a nitrofuran type drug (not described); a high proportion of aeromonads in this study were susceptible to furazolidone, which is a nitrofurandantoin derivative. Almost 50% of the isolates of the present study were also susceptible to chloramphenicol, again similarly to the motile aeromonads isolated in 1976.

Shotts et al (1976) also pointed out that the aeromonads isolated on that occasion were susceptible to nalidixic acid, which is a first-generation quinolone compound from which oxolinic acid is a derivative. In this study, a high percentage of aeromonads tested showed resistance to oxolinic acid,

which might suggest that resistant aeromonads to first-generation quinolones have emerged in the ornamental fish trade at some stage.

Studies on motile aeromonads isolated from ornamental fish by Shotts and Gratzek (1984) and Dixon et al (1990) may support the last suggestion. Shotts and Gratzek (1984) tested the antibacterial resistance of 215 isolates of motile aeromonads to 10 different compounds. The isolates were recovered from ornamental tropical fish shipments from Singapore, Hong Kong, Thailand and Taiwan. These researchers found all tested aeromonads susceptible to nalidixic acid. However, Dixon and others (1990) tested 70 motile aeromonad isolates recovered from freshwater tropical fish from Singapore, and found that 30% were resistant to nalidixic acid and 27% were resistant to oxolinic acid. Comparing the results of the present study with the two studies described above (Table 7), it seems that the prevalence of resistant aeromonads to first generation quinolones has increased. However, in order to provide further evidence to support this suggestion, more work would be needed on aeromonad isolates from ornamental fish from Southeast Asia.

Conversely, from the reports of *Aeromonas* spp. resistance of Shotts and others (1976), Shotts and Gratzek (1984), Dixon et al (1990) and the current study, it would appear that the resistance of these bacteria to certain antibiotic groups has not changed significantly. Such is the case for penicillins, tetracyclines and sulphonamides for which antibiotic resistance seem to range from medium to high percentages. What is more, the data of Table 7 suggests that aeromonads might be naturally resistant to Beta-lactam antibiotics and Sulphas.

With the exception of furazolidone, for which resistance is scarcely known (Singleton and Sainsbury 1994) oxolinic acid and enrofloxacin, the rest of the antibiotics tested in this work can be rendered ineffective by plasmid mediated resistance (Katzung 1992). It is largely known that *Aeromonas* spp. carrying plasmids are common among ornamental tropical fish from Southeast Asia. Although plasmid detection was not carried out for the isolates in this study, it is very likely that plasmids are playing a role in the antibiotic profile of these *Aeromonas* spp.

The emergence and establishment of resistant bacteria among ornamental fish from Singapore is also similar to the findings in food fish from Southeast Asia. The study of antibiotic multiresistant *Aeromonas* spp. and its plasmids isolated from food fish production sites in Southeast Asia has been encour-

aged since it represents a major economic problem for the fish producers (Saitanu et al 1994). There is some concern regarding the spread of drug resistance among *Aeromonas* spp. that have emerged as human pathogens causing diarrhoeal diseases (Singh and Sanyal 1997).

Ansary et al (1992) defined the antibiotic resistance profile and frequency of plasmid carriage of 34 strains of *A. hydrophila* isolated from several species of cultured food fish in Malaysia. They found a high frequency of resistance to ampicillin, carbenicillin and sulphonamide and low level of resistance to chloramphenicol, trimethoprim and gentamicin. Plasmids were isolated from 14.7% of the *A. hydrophila* isolates. However, conjugation did not occur when these bacteria were mated with a strain of E. coli as recipient, indicating that they were non-conjugative plasmids, in other words, transmission of resistance was not feasible amongst those *A. hydrophila* isolates.

Saitanu et al (1994) tested the antibiotic susceptibility and detection of plasmids conferring resistance to antibiotics in 68 strains of *A. hydrophila* isolated from wider sources comprising of cultured fish, pond water, humans and turtles in Thailand. Nineteen antibiotics were included in the test and 100% of the strains were resistant to ampicillin and 79% were resistant to either cephalozine, chloramphenicol, streptomycin, tetracycline, sulphamonomethoxine, trimethoprim or furazolidone. All strains were highly susceptible to the 10 quinolone antibiotics tested (Saitanu et al 1994).

These two recent studies in Asia support the hypothesis that the patterns of resistance, detected in this present study for isolates of *Aeromonas* spp. are widespread among motile aeromonads from cultured aquatic animals in Southeast Asia (Inglis et al 1997). These multiresistant capabilities and homogeneity may in turn account for their survival and prevalence in fish systems where antibiotics are applied (Sugita et al 1989).

A low percentage of Singaporean aeromonads displayed resistance to enrofloxacin in this study. A low prevalence of bacterial resistance from ornamental fish has been reported against the new 4-fluoroquinolones. Dixon et al (1990) reported that 8.5% of motile aeromonad isolates were resistant to sarafloxacin. In this study 11.9% of *Aeromonas* spp. from Singapore were resistant to enrofloxacin. Sarafloxacin and enrofloxacin are very similar compounds, however, there is no plasmid-mediated resistance known for the 4-fluoroquinolones and the existing resistant bacteria are mostly mutants

obtained in laboratory conditions (Hooper and Wolfson 1993). Concern has arisen among British veterinarians since the prevalence of ciprofloxacin resistant strains of *Salmonella typhimurium* (human and animal pathogen) has increased, and there is a high correlation of emerging resistance with the licensing of enrofloxacin (Threlfall et al 1998). Therefore, British veterinarians are reluctant to recommend the use of fluorquinolones for fish therapy and Threlfall et al (1998) suggest that the restrictions of these drugs applied in USA should be implemented in UK.

Antibacterial resistance (South America)

With the exception of some antibacterials, the antibiotic resistance profile of *Aeromonas* spp. isolated from South America is slightly different to the isolates from Singapore. The South American strains isolated in this work showed a consistent pattern of resistance to oxytetracycline and amoxicillin, but varying degrees of resistance to the remaining antibiotics tested. In general the percentages of resistance detected was lower than their counterparts from Singapore. However, although the percentages of resistant isolates were not as high as the percentages for Singapore, still, the proportions were similar. From South America, no resistant strains to enrofloxacin were detected.

Shotts and Gratzek (1984) detected a high percentage of South American *Aeromonas* spp. (89%) resistant to only one (ampicillin) of the ten antibiotics tested. Percentages of resistant strains to the other antibiotics in that study were generally low. However, the percentages of South American aeromonads resistant to multiple antibiotics observed in this study does not agree with the findings of Shotts and Gratzek (1984). Antibiotic multiresistant aeromonads appear to have become common in South American imports of ornamental fish.

Nevertheless, the presence of antibiotic multiresistant bacteria in fish from South America cannot be attributed to the same factors as for Singapore due to fundamental differences in the way that ornamental fish are entered in the trade. Ornamental fish from South America are captured in the wild and maintained in their own natural waters during transportation. South American exporters hold these fish for 3 to 5 days (Ferraz 1995), and they are then shipped to the USA where they spend a maximum of 5 days. It can only be speculated whether the period of time that these fish spend in transit is enough to allow multiple antibiotic resistance aeromonads that would thrive in the exporters facilities to invade these fish and overcome the original microflora. Alternatively, it is possible that these bacteria are naturally

resistant to several antibiotics, or an uncontrolled use of antibiotics has commenced among the South American ornamental fish exporters, explaining the profiles observed here. However, aeromonads from South America carrying plasmids conferring resistance to antibiotics were not commonly found during the 70s and 80s; only 1.3% isolates of aeromonads from South America contained these plasmids (Shotts et al 1976; Shotts and Gratzek 1984).

An interesting clue to the probable origin of these bacteria might be the percentage of aeromonads resistant to oxolinic acid and enrofloxacin, both quinolones. The percentage for oxolinic acid was low and resistant isolates to enrofloxacin were not detected. This suggested that aeromonads carried in fish and water from South America were more antibiotic naïve than their counterparts from Singapore. One possible reason for the lack of resistance to these antibiotics is that they might not be used in South America or during transit to USA with the consequent low prevalence of aeromonads quinolone-resistant mutants. Alternatively, these bacteria might have originated from open, natural environments (i.e. the Amazon region) and their exposure to these types of antibiotics may have been negligible.

Resistance to oxytetracycline can be due to its extensive use as prophylactic treatment in ornamental fish during transportation by the intermediary to the exporter in the South American Amazon. Fish are dipped into a highly concentrated bath of oxytetracycline during transportation (Ferraz 1995). However, a more comprehensive appraisal of the current antibacterial resistance of these aeromonads isolates would be difficult to obtain, since there is a lack of information regarding the antibiotic resistance profiles of motile *Aeromonas* spp. related to fish in South America.

The tropical ornamental fish trade in UK as a risk

Legislation in the UK with respect to aquarium fish has tried to balance the protection of native stock, the aquaculture industry and introduction of fish diseases against the survival of the ornamental fish industry as a whole. However, legislation in this country, at least for ornamental tropical fish, has not largely relied on scientific knowledge or assessment of the risk involved in the importation of ornamental tropical fish. In spite of the size of the industry in the UK, few researchers have dedicated their efforts towards the diseases of ornamental fish or ornamental fish as vectors of potential food fish and/or human pathogens (Rodger et al 1997; Wildgoose 1999).

Besides motile aeromonads, other prevalent groups of bacteria were found to be multi-resistant to various antibiotics, such as pseudomonads and *Alcaligenes faecalis* (data not included in this report). There is concern among some researchers regarding the transference of these multiresistant bacteria from tropical aquarium fish to cold-water species, or the transference of plasmids conferring antibiotic resistance from tropical aquarium fish bacteria to bacteria that cause disease in cold water species. Meier and others (1992) have suggested that this transference could occur if tropical and cold-water ornamental fish are kept in the same facilities. Cold water species in turn would transmit these plasmids to widespread cold water fish pathogens (e. g. *A. salmonicida*). These risks can be assessed with simple experiments, but they can also be reduced with strict management and operation when both cold water and tropical fish are kept in the same site.

The threat posed by multiresistant bacteria harboured in ornamental tropical fish to native fauna, aquaculture and humans is arguable. So far, the literature concerned shows little involvement of these bacteria as a source of human disease. However, it is obvious that the level of resistance displayed by these bacteria poses a problem for the industry when therapy is required to solve disease problems caused by opportunists.

At present, the ornamental fish industry and aquaculture are running out of chemotherapy options due to the emergence of resistant strains and the strict control on the licensing of antimicrobials for use in fish therapy. Some authors have expressed their concern with respect to the emergence of antibiotic resistant bacteria in aquaculture activities (Aoki 1992, Smith et al 1994). There seems to be no clear way to stop the emergence of resistant bacteria as long as antibiotics are used. However, in order to stop the emergence of these bacteria, researchers have suggested ways of dealing with the problem. One suggestion is to have constant monitoring and national surveillance programmes (Inglis et al 1991), or the use of potentiated antibiotics to deal with plasmid-mediated resistance (e.g. amoxicillin and clavulanate) (Wildgoose 1999). Another suggestion is the use of probiotic bacteria in aquaculture to balance bacterial populations (harmful and harmless bacteria) (Gildberg et al 1997), and the shifting of the ecological balance from resistant to susceptible bacteria by sensible chemotherapy programmes (Levy 1997). So far, the possibilities described above have not been fully explored in the ornamental industry.

Nevertheless, the ornamental fish industry could have more strict control of disease processes or outbreaks compared with other aquatic farming sys-

tems. Aquaria are enclosed environments, which due to their characteristics are easier to control and are not subjected to environmental changes as with open-aquaculture production sites. The ornamental fish industry could take advantage of this unique characteristic, which represents a good opportunity to diminish the risk of bacterial disease outbreaks by sensible programmes of antibiotic therapy. This would be achievable if communication between fish health specialists and ornamental fish trade is enhanced and more scientific research on chemotherapy or alternative treatments are addressed to this particular group of fish.

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Approaches to the eradication of infectious disease from farm animal livestock.

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Abstract

The successful eradication of an infectious disease requires the implementation of a programme based on a sound scientific approach, coupled with appropriate legislative powers. In most instances, where a programme is initiated, it is to be expected that both of these are achievable. Thus there is normally no merit in attempting to eradicate a disease if the infection cannot be diagnosed in an animal at an early stage, and if the pathogenesis and epidemiology are not clearly understood. Furthermore, the necessary legislative powers need to be implemented in advance of an eradication programme.

Equally important, and more difficult to achieve, is the necessity to involve all relevant players within the industry in the programme. Experience indicates that lack of ownership by all relevant industry players results either in failure to eradicate or in an unacceptable delay in achieving eradication. In any proposed eradication programme ownership by all relevant players within Government and the wider industry is of paramount importance and must be the aim of all involved.

ARTICLE

Experience indicates that infectious diseases are eradicated for one of the following reasons:

- ➢ Public health – e.g. BSE, Rabies;
- ➢ Killer disease – e.g. Rinderpest, African Swine Fever;
- ➢ Economic impact – e.g. Foot & Mouth disease (FMD)
- ➢ Differential diagnosis from the above three – e.g. Swine Vesicular disease.

In justifying an eradication programme it is also important that it should be cost effective to the industry/Government or be, at least, cost beneficial where there are public health implications.

However, there is no merit in eradicating a disease if its re-introduction to the region or country cannot be prevented. Northern Ireland was free of many diseases such as Infectious Laryngotracheitis (ILT) and Porcine Reproductive and Respiratory Syndrome (PRRS). However, following our becoming a member of the European Community it became necessary to abide by the Community rules. These rules include free trade in animals and their products except in animals infected with diseases for which there is Community legislation. Since legislation does not exist for the above diseases, Northern Ireland lost its ability to ban the import of live animals even where these diseases were known to be present in the herds/flocks of origin. It thus has to be recognised that such diseases will gain entry to Northern Ireland, as Government does not have the authority to impose import conditions.

In formulating an eradication programme certain prerequisites are highly desirable:

- A knowledge of the incidence of disease and infection;
- A clear understanding of the pathogenesis & epidemiology of the disease;
- Availability of effective diagnostic tests for detection of infection;
- A knowledge of the means of transmission;
- A knowledge of all susceptible hosts;
- An ability to restrict the movements of suspected, infected animals;
- Sufficient legislative powers to conduct the programme;
- An ability to prevent the re-introduction of the disease through importation of infected animals;
- Trained personnel to implement the programme; and
- Ownership of the programme by all relevant players;

To state the obvious, the more we know about a disease the more likely are we to be successful in its eradication.

Incidence of infection

Eradication is more attainable if we are aware of the level of infection in the country and of the herds/flocks in which it exists. An understanding of these issues is essential in the formulation of a cost effective/benefit analysis.

Pathogenesis
Knowledge of the pathogenesis of a disease can eliminate unnecessary work. An example of this is Aujeszky's disease where it has been established that pigs excrete the virus but, whilst many other species are susceptible to infection and disease they are dead end hosts and do not transmit the virus. An understanding of the pathogenesis of a disease often facilitates the development of effective diagnostic tests through recognition of the most effective mechanisms – humoral antibody, cell mediated response or antigen detection.

Epidemiology
It is important that we have knowledge of aspects such as the range of natural hosts, reservoir hosts, intermediate hosts, any carrier status and the agent's ability to survive outside the animal. The carrier status is a significant factor in the eradication of Aujeszky's disease but of no significance in the eradication of Newcastle disease.

Diagnosis of infection
Laboratory diagnosis of disease is frequently possible but many diseases exist for which there is no effective diagnostic test that will detect the infection at an early stage. Examples of the latter include Johne's disease and Caseous Lymphadenitis. An eradication programme would have been implemented in many countries for Johne's disease if an effective diagnostic test for infection had been available. In contrast, the eradication of Aujeszky's disease has already been achieved in several countries, largely because there are effective tests that can identify the infected pig at a very early stage of infection. The existence of virulent and avirulent strains of an infectious agent is a complicating factor in the diagnosis of infection. This complication is very apparent in avian influenza where virulent and avirulent strains exist and where avirulent strains have the ability to mutate to virulence.

Transmission
Knowledge of the means of transmission allows controls to be introduced to eliminate or reduce the potential for transmission. The Northern Ireland experience indicates that Newcastle disease virus can be spread by wind and the ability of the FMD virus to spread by wind is well documented. It is important to have knowledge of the direct and indirect methods of transmission of an infectious agent if success is to be the outcome. Animal feed is an effective means of transmitting Salmonellae to animals and prevention of this means of transmission must be an integral part of any Salmonellae erad-

ication programme. Vaccines have been incriminated in the spread of infectious diseases and strict protocols now exist to prevent such an occurrence.

The integrated poultry industry has demonstrated the obvious advantages in having an "all in/all out" policy in relation to animal premises. Such a policy has been a major factor in the eradication of *Salmonella enteritidis* from the poultry industry and in maintaining that freedom. The world-wide pig industry has, belatedly, adopted the same policy with considerable benefits in the control and eradication of pig diseases.

Movement control

Infection moves with infected animals! It must be accepted that the primary means of spread of infectious agents is through the movement of infected animals to non-infected animals in other herds/flocks. Consequently, most eradication programmes must include forward and backward tracing and testing and contiguous testing as essential elements of the programme. The speed of such tracings and subsequent testing is a very important element in an eradication programme. Computerisation of all cattle identities and movements in Northern Ireland in 1988 was a significant tool in the Northern Ireland Veterinary Service's ability to control or eradicate diseases such as bovine Brucellosis and Tuberculosis. Forward and backward tracings took 8 weeks by the manual system; computerisation achieved a more accurate trace overnight. Whilst this is important in diseases such as bovine Tuberculosis it becomes almost essential with FMD where the agent can spread rapidly by wind.

Legislative powers

Having a detailed knowledge of the scientific aspects of a disease forms a sound basis for considering an eradication programme. If a programme is to be progressed it is essential to ensure that effective legislation is implemented in advance of the implementation of the programme. The powers that emanate from such legislation normally include the ability to test susceptible animals, slaughter infected animals, restrict relevant herds/flocks, cleanse and disinfect infected premises and prohibit the importation of potentially infected animals. Such powers are essential if success is to be achieved. The legislation also normally lays down the means by which eradication will be achieved. Vaccination may be an element in the initial stages of eradication especially where a deletion vaccine exists. This is the case in Aujeszky's disease where a deletion vaccine exists and where it is possible to differentiate between the infected animal and the vaccinated animal.

Prevention of the re-introduction of the disease
Legislative powers must also ensure that imported, susceptible animals are free of the infection. Furthermore, there should be a facility to monitor all imported animals to ensure freedom from infection. Consideration must also be given to the role of the importation of animal products, vehicles and equipment that have the potential to harbour the infectious agent.

Trained personnel
A further element in achieving success is in ensuring that all personnel involved, at both field and laboratory levels are adequately trained to carry out all necessary work. An effective programme can only be successful if the personnel are effective in their duties. Credibility is readily lost if such knowledge and ability are not clearly evident to all involved. Additionally, it is important that the industry is regularly updated on progress and that it has a clear understanding of its role in the eradication programme. Eradication involves the full and effective participation and co-operation of all relevant players. Education of the industry, where necessary, must also be a component of a programme.

Ownership by all relevant players
Finally and importantly, programmes have failed in the past or have been unnecessarily protracted because of a lack of ownership by all relevant players in the industry. All relevant players need to be involved in the formulation and implementation of an eradication programme. It is not sufficient for such players to be involved only in the implementation aspects. This "ownership' by all relevant players is normally the most difficult element to achieve and consequently becomes the most important element because of the difficulties in achieving it. Success will not be achieved if the industry does not accept the need for eradication as lack of co-operation by the industry is the death-knell of any eradication programme! Those involved in an eradication programme (including veterinarians) are generally very competent in the science of disease eradication but may not be so competent in ensuring ownership by all relevant players.

"Ownership" is rarely guaranteed by asking relevant players to sign up to a programme devised by Government officials. All interested parties should be involved from the outset if such ownership is to be achieved. Experience indicates that this aspect is very difficult to achieve especially as some of our livestock industries are in the hands of a disparate group of producers and where meaningful representation of all such players is difficult to achieve.

Many diseases have been eradicated successfully from countries and FMD eradication from the European Community is a recent example of such success.(*) Successful eradication of a disease is achievable if the above points are an integral part of the eradication programme. The following will also aid the success of such a programme:

- ➢ Rapid removal of infected animals;
- ➢ Effective cleansing and disinfection;
- ➢ Forward & backward tracing and testing; and
- ➢ Effective monitoring for continuing freedom

It is incumbent on the leaders within a livestock sector to identify those diseases that should be eradicated. The opportunity of a lifetime occurs only during the lifetime of the opportunity. All leaders must grasp this opportunity and responsibility so that the health and welfare of our livestock can be maintained and improved.

Dr McCracken qualified from Edinburgh in 1966 and spent a short time in mixed practice before joining the Veterinary Research Laboratories of the Department of Agriculture for Northern Ireland as a pathologist. He was involved in diagnostic, research and regulatory pathology and also in lecturing to undergraduate students at the Queens University of Belfast. Dr McCracken's special interests include neuropathology and Aujeszky's disease; indeed his Ph.D. thesis covered "The neural pathway of Aujeszky's disease virus in calves"

His duties as a pathologist encompassed all farm livestock, companion animals and farmed salmon and trout. He served as Head of the Pathology Department and Deputy Director of the VRL before being appointed as Deputy CVO in 1990. Dr McCracken was appointed Chief Veterinary Officer in 1998 and is now responsible for animal health, animal welfare and veterinary public health in Northern Ireland.

* Please note that Dr McCracken's paper was presented at the October 2000 meeting of the FVS in Belfast and was accepted for publication prior to the current outbreak of FMD in the United Kingdom.

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Integrated lice management on Irish Salmon Farms

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Abstract

On the basis of information gathered in surveys of lice infestation on salmon farms in 1991 and 1992 the Department of Marine in Ireland put in place a new initiative in salmon farm management. This initiative, termed Single Bay Management, has been progressively introduced over the past six years and has resulted in significant and sustained improvements in lice control on farmed fish. Two case studies are presented where the mechanics of integrated lice control strategies and the barriers to its successful implementation are explored. Crucial elements are identified as separation of generations, annual fallowing of sites, strategic application of chemotherapeutants, good fish health management and close co-operation between farms. The process of integrating the elements of Single Bay Management into a co-ordinated local aquaculture management system is outlined together with the implications for sea lice control.

Introduction

Sea lice (*Copepoda: Caligidae*) have long been recognised as the most commercially limiting parasite in salmonid aquaculture in northern Europe (Costello, 1993). They weaken and kill salmon, reduce their commercial value and may be implicated in the transmission of microbial pathogens within farms. In northern Atlantic waters infestations are dominated by two species, *Lepeophtheirus salmonis* (Kroyer) and *Caligus elongatus* (Nordmann). In southern Chile native caligids have become a problem for farmed salmonids reared in netpen systems. Carvajal et al (1998) identified *Caligus flexispina* as the dominant caligid species found on salmonids. *C. flexispina* is causing significant economic losses due to slower growth of fish and the direct costs of treatments. In Ireland monitoring of sea lice infestations on farmed salmonids was initiated in 1991 (Jackson & Minchin, 1993), with a view to collecting baseline information on the true level of infestations and improving the techniques available for the control of lice

FIG 1: Spring mean ovigerous female *L. salmonis* on one sea winter salmon, 1991–2000

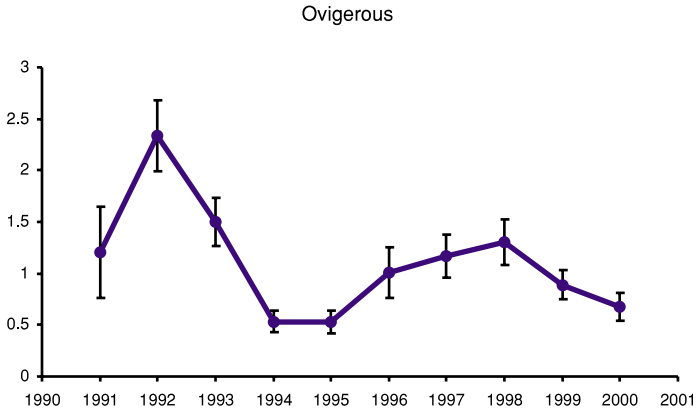
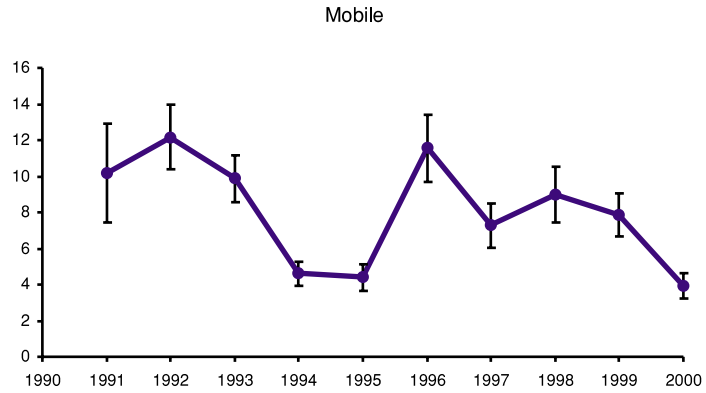


FIG 2: Spring mean total mobile *L. salmonis* one sea winter salmon, 1991–2000



infestations. As a result of these studies a programme of Single Bay Management (SBM) was developed and progressively put in place from 1993 onwards. At certain sites, lice infestation levels had been reduced to the practical minima by 1995 (Jackson et al, 1997). The maintenance of good control of lice infestation, as the industry becomes more efficient and develops to meet more stringent commercial demands, requires the integration of lice management into an overall coordinated management system. The first steps towards this goal have been taken and progress to date is described, with the aid of two case studies.

FIG 3: Spring mean ovigerous female *L. salmonis* on one sea winter salmon at Killary and Kilkieran farms, 1991–2000

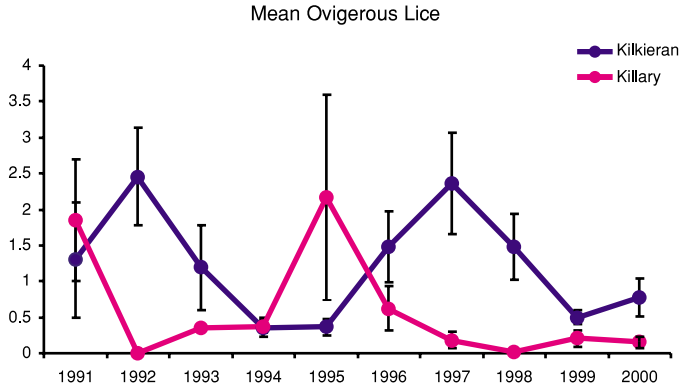
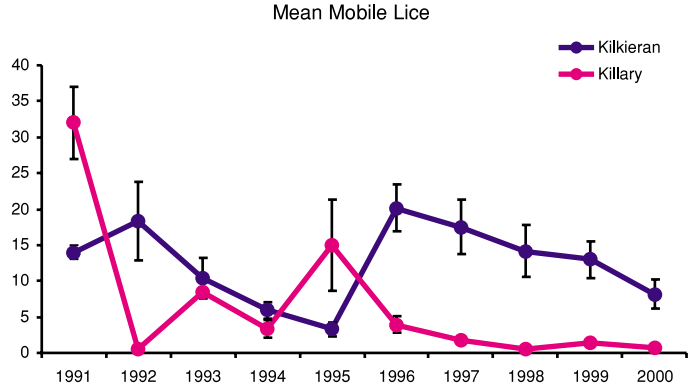


FIG 4: Spring mean total mobile *L. salmonis* on one sea winter salmon, at Killary and Kilkieran farms, seven year trend, 1991–2000



Materials and Methods

Estimations of lice infestation levels were obtained from all licensed fish farms by regular sampling of the production fish on site. Sampling was carried out bi-monthly for the months March to May inclusive and monthly for the remainder of the year, with the exception of the December - January period when only one sample was taken. Generally two cages of fish were sam-

pled for each population of fish on site; a standard cage, which was sampled at each inspection, and another cage selected at random at each inspection. Different year classes of fish were considered as different populations, as were different species (i.e. *Salmo salar* L., *Oncorhynchus mykiss* (Walbaum)). A sample of approximately thirty fish was taken from each cage. The fish were anaesthetised and all mobile lice were removed and preserved in alcohol after Jackson & Minchin (1993). All lice remaining in the anaesthetic after the sample was processed were retained and included in the sample. Lice counts presented in this paper are based on arithmetic means.

Results and Discussion

National trends in lice infestation

On the introduction of Single Bay Management in 1993-1994 there was a strong downward trend in infestation levels of the salmon louse, *L. salmonis*, as demonstrated by the spring means for ovigerous females (Figure 1). Over the period from 1991 to 2000 there has been a downward trend in lice infestation parameters nationally. For mobile lice (Figure 2), which includes immature stages, the trend is less obvious in the month of May as the control strategy is targeted at minimising ovigerous females during this period. Infestation parameters in individual bays and on individual sites have varied considerably and, in general, in those bays where the implementation and synchronisation of the new management practices has been more straightforward, lower infestation levels have been attained. This is illustrated by the lice infestation parameters in two case studies, Killary Harbour and Kilkieran Bay (Figures 3 and 4). The level of control achieved in Killary Harbour is greater than that obtained in Kilkieran Bay, where the greater complexities of implementing the new management practices have delayed their full implementation. The degree of complexity ranges from well defined bays, with a single cultured species and all sites in the bay under the one management, to complex embayments where multiple species are cultured, including finfish and shellfish, under a diversity of commercially distinct management regimes. The diversity of solutions required to successfully address this heterogeneity is demonstrated by these two case studies.

Case Study 1

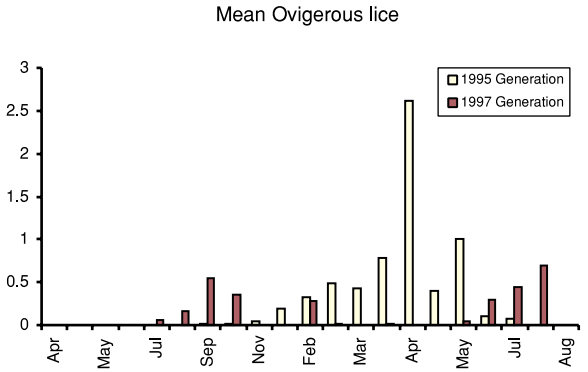
Killary Harbour (Figure 5) is a fjord some 14 kilometres long. There are two salmon farming sites licensed, both under the one management and two salmon and sea trout fisheries enter the sea within the fjord. There is a sig-

nificant run of spring salmon into the fjord. A Single Bay Management plan has been in place since 1994. In 1997 the farm entered into a legal agreement with a neighbouring salmon and sea trout fishery on lice control. A key element in the agreement was an undertaking by the farm to maintain the mean number of ovigerous *L. salmonis* to a level as close as possible to zero but not greater than 0.3 per fish during the "critical period" of the spring smolt migrations. This level of control has been achieved over the three seasons from 1997 to 1999 as reported by the Department of Marine and Natural Resources monitoring programme.

The steps taken in order to achieve this level of control included; complete physical separation of generations, annual fallowing of all sites, early harvest of growers and a strict regime of targeted lice treatments. There were a number of obstacles to the achievement and maintenance of this level of control. The physical separation of the generations required the granting of a new license for a fallowing site outside the mouth of the fjord. This was to accommodate smolts during their first summer in the sea and to facilitate fallowing of the production site. This took some considerable time to process. The benefits in terms of lice control, arising from this separation, are illustrated (Figure 6) by comparing two complete production cycles, pre- (1995) and post (1997) complete separation of generations. The early harvest of growers, to eliminate two sea-winter fish from the growing cycle, had both mar-

FIG 5: Killary Harbour, licensed salmon sites marked in black

FIG 6: Mean ovigerous lice for the complete production cycle, on 1995 and 1997 generations of salmon in Killary harbour



keting and financial implications, which had to be addressed by the company in their commercial planning. The availability and cost of treatments was also a very serious issue. While new treatments are coming on stream, solving the first half of the problem, the cost issue has not been addressed.

Case Study 2

Kilkieran Bay (Figure 7) is a complex embayment with a well-developed extensive managed fishery for oysters and scallops. There is also a significant salmon farming industry in the bay. There are nine companies operating some twenty fin-fish sites within the bay with a further seven sites in adjacent bays. Single Bay Management plans have been in place since 1994. However, due to the geographical complexity of the area and the number of different commercial entities in the bay there were difficulties both in ensuring full effective separation of generations throughout the bay and in synchronising fallowing of sites. These difficulties arose from the lack of availability of fallowing sites, and the difficulty of achieving harmonisation of production schedules between nine distinct commercial entities operating some twenty sites. Because of the nature of the bay and the location of sites there was a necessity to ensure co-ordination of lice treatments to improve their over-all efficacy. Close co-operation on a range of related fish health issues including harvesting practices, a uniform vaccination protocol for smolts, plankton monitoring for algal blooms and amoeba treatment facilities were also required to facilitate the implementation of Single Bay Management in this bay.

FIG 7: Kilkieran Bay, licensed salmon sites marked in black

In order to address these issues and to offset the costs involved in the early harvest of growers (to facilitate synchronous fallowing) and additional lice treatments, a number of initiatives were brought forward during 1998 and 1999. These included a joint venture approach to smolt management within the bay and the provision of advisory services by the Marine Institute. A number of companies in the bay are in the process of reaching agreement with fishery owners on levels of lice control. This latter development has been facilitated by and is an extension of the development of effective Single Bay Management within the bay.

A joint venture company has been set up to manage smolt inputs for Kilkieran Bay from the year 2000. All of the commercial salmon farms in the bay are shareholders in this joint venture, which will facilitate the adoption of a unified protocol for smolt management, the complete separation of generations and synchronous fallowing of all production sites in the bay. In tandem with this, at the request of the farmers, the Marine Institute has provided a targeted advisory service on lice management. This service currently involves the supply of regular reports on lice levels and infestation pressure on farms in the bay and of advice on synchronised treatments. This is critical to the co-ordination of treatments in a targeted way to maximise efficacy.

Future developments in integrated management

The planning and husbandry practices, which are necessary for Single Bay Management for the purposes of lice control, are also a prerequisite for efficient fish health management. This is particularly true in the case of alternation of generations and fallowing. Wheatley et al (1995) identified annual fallowing and single generation sites as key factors in reducing mortality rates. They also identified sea lice infestations as a possible source of increased mortality. This increase in mortality together with sub-lethal effects such as reduced growth, poorer food conversion ratios (FCRs) and lower quality at harvest, impacts on the efficiency and commercial viability of farms.

While the need for integrated management is clear, because of the heterogeneity within the industry it is not possible to have a single global strategy, which is applicable to all situations. This heterogeneity and the relative efficacy of a range of lice control strategies under differing conditions has been described in previous work (Jackson & Minchin, 1993; Jackson et al, 1997). Effective regimes of sea lice control must take account of the different production cycles in different bays, different species cultivated, the needs of other aquaculture producers in the bays and the needs of other users of the common resource. To do this in a meaningful way requires locally based management committees to develop strategies which are *tailor made* for the local situation. This need is currently being addressed through a new government initiative, C.L.A.M.S. (Co-ordinated Local Aquaculture Management Systems). This system is outlined in an explanatory handbook (O'Carroll & Jackson, 1999) and is being piloted in Killary Harbour and Kilkieran Bay. C.L.A.M.S. will produce the following tangible outputs:-

- A concise description of the bay/area in terms of physical characteristics, history, aquaculture operations, future potential, problems etc.
- Integration of a series of codes of practice for current aquaculture operations and translation of those national codes to the specific circumstances of each bay or coastal region.
- Expansion of the concept of SBM to species other than salmon.
- A development plan for aquaculture in the bay.
- Information on other activities in the bay.
- A local and national communication network, with "bottom up" and "top down" dialogue capacity.

C.L.A.M.S. is seeking to incorporate the best aspects and relevant information of previous work carried out as well as maintaining SBM in its structure. But, further than this, C.L.A.M.S. intends to incorporate the development plans of the local individuals as well as integrating the management practices of the various species sectors that may be operating in the same bay.

Conclusions

- A locally based integrated management strategy is essential for successful management of lice infestations.
- Such a management strategy needs to be integrated as part of a fish health management plan.
- Crucial elements in the success of this plan are separation of generations, annual fallowing, strategic application of treatments and close co-operation between operators.
- Case studies in the west of Ireland have shown that such a plan can be successful in controlling lice infestations and has important spin-off benefits in terms of fish health and efficiency.
- A fundamental characteristic of these plans is the high level of local participation at all stages of their development and associated sense of ownership.

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David Jackson graduated with a BSc from NUI Galway in 1979. He then completed both doctoral and post-doctoral research on Harpacticoid copepods, including an Ireland-France co-operative study which saw over twenty species added to the Irish faunal list and the description of four new species. He spent five years working as a research scientist at the Shellfish Research Laboratory in Carna and now works at the Department of Marine/Marine Institute in Galway. His research interests include harmful and toxic algal events; sealice biology and population dynamics; co-ordinated management of aquaculture operations; and the development and management of inshore fisheries.

Dan Hassett is an honours Zoology graduate who worked for many years in the early expansion of the Irish salmonid aquaculture industry. His present role (since 1993) with the Department of the Marine and Marine Institute, Galway, involves biological monitoring of the salmonid industry in Ireland. His post-graduate interests have ranged from conservation ecology of Natterjack toads in Ireland to parasite ecology of marine fish, birds and mammals (in particular those parasites which utilize fish prey items to transmit their larvae to the final bird or mammal host).

Lorraine Copley has completed two years post-doctoral experience with the Marine Institute in Galway, involving the monitoring of sea-lice, Lepeoptheirus salmonis, on salmon farms in the West of Ireland. She completed her PhD Degree in Zoology at NUI, Galway during the years 1995–1999. Her thesis dealt with the catches of glass eels/elvers (Anguilla anguilla (L.)) at Cathleen's Falls Hydroelectricity Station at Ballyshannon, Co. Donegal, over a period of twenty years, as well as the parasitology of eels from different river systems in Ireland. Lorraine completed a Masters Qualifier in Zoology in UCG during the years 1994–1995. She was awarded her B.Sc. in Science from U.C.G. in 1993.

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Red Tides, Blue-Green Algae and Toxic Fish – An Increasing Threat to Animal and Human Health?

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Abstract

The more significant public and animal health problems associated with abnormal algal blooms are described in this paper. Each is caused by different species of toxic algae occurring in various water bodies, both freshwater and marine, in many parts of the world.

Introduction

Man and animals are exposed to the naturally occurring toxins produced by harmful algae through the consumption of contaminated seafood products or the ingestion of contaminated waters. With the increase in seafood transport worldwide, as well as international travel by seafood consumers, coupled with increased awareness and improved detection techniques, these syndromes are being increasingly recognised both within the UK and worldwide.

Dinoflagellates and Diatoms

When levels of these algae in water reach 10^3-10^6 cells/litre it is termed a bloom. Certain blooms are termed 'red tides' when the tiny pigmented phytoplankton – mostly dinoflagellates – grow in such abundance (>10^6 cells/litre) that they change the colour of seawater to red, brown or even green. Only a few dozen of the many thousands of living phytoplankton species are known to be toxic, so some red tides may not be harmful. Not all toxic algae are coloured and occasionally low levels of dinoflagellates can produce toxic conditions so incidents of poisoning may occur in the absence of red blooms. Environmental fluctuations such as water temperature, salinity, light and nutrient levels profoundly influence the growth and accumulation of algae, and thus their toxicity. The severity and magnitude of these blooms and their geographical occurrence seems to have increased in recent years. Most problems have occurred in near-coastal marine ecosystems. Some researchers believe that a global change in the complex interaction of

climate, ocean and temperature is affecting marine plankton. Water used as ballast in the increasing ship traffic on the world's oceans may also have introduced micro-organisms into new waters.

The toxins represent families of compounds rather than single chemical entities. The reason for their production is still unclear and no specific biochemical role for them has been identified. Some studies have implicated intracellular bacteria present inside algal cells as the primary source of the toxin but recently it has been concluded that toxin production is coded for by nuclear genes in the dinoflagellate. A variety of toxic syndromes in man have been described and these are generally neurological or gastro-intestinal or both. Devastating fish kills may also occur as a result of toxin production.

The main impact on man occurs when bivalve shellfish e.g. mussels, oysters and clams ingest the algae as food and retain the toxins in their tissues. Bivalves may accumulate sufficient toxin to cause human illness after only 24–48 hours filter feeding during a bloom. Both proliferating algal cells and resting cysts can contain toxins and act as sources of shellfish contamination.

(a) Shellfish Poisoning

(i) Paralytic Shellfish Poisoning (PSP)
First recorded in Norway in 1962, outbreaks have since occurred regularly worldwide. Over 1600 cases a year are reported, including an estimated 300 deaths. The last UK outbreak occurred in 1968 and affected 78 people after eating Northumbrian mussels.

Twelve species of dinoflagellate in the genera *Pyrodinium*, *Gymnodinium* and *Alexandrium* have been implicated. These occur regularly throughout Europe and high toxin levels in shellfish are regularly detected.

The toxins of PSP are a group of closely related water-soluble heat-stable compounds based on saxitoxin. Their action is to block the sodium channel linked to neuro-transmission. They are concentrated in the hepato-pancreas of the shellfish. There is extreme variation in individual sensitivity to toxic shellfish but the syndrome can be life-threatening.

The symptoms of PSP are mainly neurological with rapid onset, usually within two hours of ingestion and include tingling, numbness and burning of

perioral region, ataxia, giddiness, fever and muscle paralysis. The most severe cases result in respiratory arrest within 24 hours.

(ii) Diarrhetic Shellfish Poisoning (DSP)
This intoxication follows consumption of shellfish containing toxins produced by one of several species of dinoflagellate, including *Dinophysis* spp. and *Prorocentrum* spp. Mussels are most frequently implicated but scallops, oysters and cockles may also be affected. At cell densities as low as 200 cells/litre seawater, shellfish can accumulate sufficient toxins to cause poisoning in humans.

Affected areas remain predominantly Japan and Europe but also include America and Australia. Ten thousand cases were reported worldwide between 1976-1990. Toxins were first detected in UK shellfish in 1991 and the first recorded case occurred when two people became ill after eating imported mussels. The second and latest outbreak in 1997 involved 49 people after eating illegally harvested mussels produced in the UK. These patients presented with acute (within 30 minutes) onset of vomiting, diarrhoea, abdominal pain and fever. All had eaten UK mussels in two London restaurants. The dinoflagellates have been regularly detected in UK waters since 1994, especially in Scotland.

The toxins of DSP consist of a group of 12 polyether carboxylic acids including okadaic acid (OA), dinophysis toxins (DTX-4), pectenotoxins and yessotoxin. DTX-4 is unusual in being water soluble. OA inhibits protein phosphatases. The toxins are heat stable and freezing preserves the toxicity.

Symptoms of diarrhoea, nausea, vomiting and abdominal pain may be experienced up to twelve hours after eating shellfish, but normally within two hours, with a maximum duration of three to four days. They are usually self-limiting and not life-threatening.

(iii) Neurotoxic Shellfish Poisoning (NSP)
The causative dinoflagellate, *Ptychodiscus breve* (formerly *Gymnodinium breve*), is not native to EU waters and no UK cases have been recorded. Poisoning incidents have occurred mainly in the USA and Mexico. A large outbreak occurred in New Zealand in 1993. Third countries must be authorised to export molluscan shellfish to the EU and neither the USA nor Mexico has such an authorisation at present.

The toxins of NSP consist of a family of nine brevetoxins which bind to a site on the sodium channel and trigger the release of synaptic neurotransmitters.

Symptoms may be experienced one to three hours after ingestion of toxin and include numbness or tingling in the mouth, progressing to the extremities, followed by ataxia and gastro-intestinal symptoms. Recovery is generally complete in a few days. Contact dermatitis and conjunctivitis in bathers has been reported due to formation of toxin aerosols by wave action. The toxins may also be responsible for fish kills, sometimes massive.

(iv) Amnesic Shellfish Poisoning (ASP)
This syndrome was first seen in Canada in 1987 when over 100 people became ill after eating blue mussels. No cases have been recorded in the UK and outbreaks remain confined to the USA and Canada. The causative algal species are not dinoflagellates but diatoms in the genus *Pseudonitzschia*. These have been detected in UK waters since 1996 when monitoring began, and ASP toxin was found in Scottish waters in 1999 at levels which led to closure of scallop grounds. Trace levels were also found in several other areas but at well below the maximum permitted level.

In addition to shellfish, it is now known that toxin accumulates in fish including anchovies, sardines and mackerel, and in crab viscera. The deaths of Californian seabirds have been tentatively linked to the consumption of fish.

The toxins of ASP consist of domoic acid, and a range of derivatives, which are potent neurotransmitters with receptor sites in the CNS, where they mimic the naturally neurotransmitter glutamate. Memory loss results from lesions in the hippocampus.

Symptoms of ASP occur within 24 hours of ingestion of toxin. Initial gastro-intestinal symptoms progress to a range of neurological effects. Deaths have been reported, although these may not have been due to direct toxicity. The loss of short-term memory is a significant feature and is sometimes permanent.

(v) Monitoring for shellfish toxins
Because of their potential threat to human health, monitoring programmes are in operation around the world to ensure that shellfish on sale for human consumption are safe.

In the UK an annual monitoring programme for marine biotoxins in shellfish has been in operation since the outbreak of PSP intoxication of humans in 1968. An intensive programme has been maintained since 1991. From April 2000 this programme will be co-ordinated by the Food Standards Agency. Detection methods are based on mouse bioassay using shellfish flesh, although new techniques such as high performance liquid chromatography (HPLC) and immunoassay are being developed. In addition, a programme of phytoplankton monitoring for significant marine algae was instituted in 1995. This measures the density of cells in seawater and is undertaken by CEFAS, Lowestoft.

The monitoring programme which is a requirement of the EU Shellfish Hygiene Directive 91/492/EEC is co-ordinated by MAFF and tests bivalve molluscs for the presence of PSP and DSP toxins. Samples are also analysed as part of an R & D project on ASP toxin in order to provide data on which to base a monitoring programme. This programme will be operated on a trial basis during 1999-2000. The Directive is implemented in England and Wales by the Food Safety (Fishery Products & Live Shellfish) (Hygiene) Regulations 1998, as amended (SI 994/1998). The Regulations lay down the levels of toxin permitted in shellfish as follows:

PSP – 80μg/100g tissue
DSP – no detectable toxin
ASP – 20μg/g tissue

Mussels are sampled as the bivalves of choice as they concentrate algal toxins more readily than others. If action levels of toxin are found then other bivalve and crustaceans are sampled. All shellfish samples are collected by local authority Environmental or Port Health Officers. From the period April 1998 to March 1999 shellfish from 25 primary inshore sampling sites in 22 separate local authority areas and five offshore scallop fishing grounds were examined. Testing is carried out throughout the year, although more intensively during spring and summer months, at the Marine Laboratory, Aberdeen. In areas where toxins are detected, warning notices are posted and a voluntary closure of the harvesting area instituted. In addition notices may be placed in local newspapers to warn casual gatherers.

Occasional occurrences of both PSP and DSP are recorded by the monitoring programme in both Scottish and English waters. Between April 1998 to

March 1999 PSP toxins were found in 19 samples from six separate areas, mainly the North East and South West of England, and DSP toxins were detected in eight samples from three areas.

The phytoplankton monitoring programme provides an effective warning system for the possible presence of marine biotoxins in shellfish and serves as a valuable indicator of when additional shellfish flesh sampling is needed. However, in England and Wales, the precise relationship between the presence of toxic algae and the presence of toxins has not yet been established.

(b) Ciguatera Poisoning

Globally this is the most common fish-borne intoxication with an estimated 50 000 cases a year. Cases in the UK are rare but there is increased importation of tropical reef-feeding fish which may pose a health risk to UK consumers. Over 400 species of such fish are potentially ciguatoxic and these include the larger predators, such as grouper, sea bass, red snapper and barracuda.

(i) Toxin

Certain dinoflagellates, e.g. *Gambierdiscus toxicus*, live in association with seaweeds on coral reefs and produce ciguatoxin. This toxin targets specific receptors on protein channels in muscle and nerve cells. Small reef-dwelling herbivorous fish ingest the toxin, which becomes concentrated and may be modified in large predators further up the food chain. Four toxin groups have been implicated – ciguatoxin, maitotoxin, scaritoxin and palytoxin.

(ii) Symptoms

These toxins produce gastrointestinal and neurological symptoms. Diarrhoea, vomiting and abdominal pain are followed by dizziness, anxiety, sweating, muscle aches and a numbness and tingling of the mouth and digits. Reversal of temperature sensation has been described. Paralysis and death ensues only rarely. Symptoms usually resolve within two to five days but neurological symptoms may persist for weeks or even months.

(iii) Control

There is no antidote and currently no easy way to measure these toxins in any seafood product prior to consumption. They resist cooking and other processing procedures. Some protection is afforded by avoiding unusually large

Photographs of some toxic algae

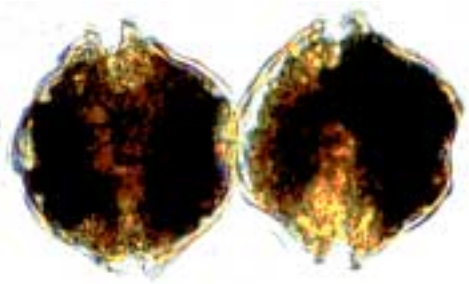
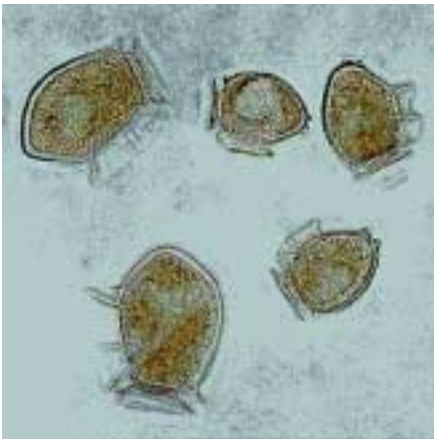


FIG 1: *Alexandrium* sp.

FIG 2: *Dinophysis* sp.



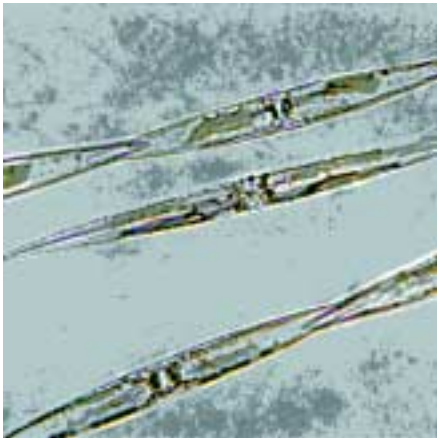


FIG 3: *Pseudo-nitzschia* sp.

reef fish and excluding internal organs and roe from the diet. Third countries must be approved and operate to equivalent standards to the EU before they can export fish and fishery products to the EU. When evaluating such standards account would be taken of controls on this toxin.

Cyanobacteria

Sometimes called blue-green algae, or more colloquially, pond scum, their toxic effects have been known for over 100 years, and have been associated with spectacular kills of wild and domestic animals around the world. At least 50–70% of samples of cyanobacterial bloom and scum samples from hundreds of water bodies worldwide have been found to be toxic. They occur in freshwater lakes, ponds, reservoirs, slow-flowing rivers, estuaries and even marine waters. Cyanobacteria grow more successfully than true algae in nutrient rich waters. As concentrations of nitrogen and phosphorus increase in our water supplies as a result of industrial and agricultural run-off, blooms are promoted, with the increased likelihood of human and animal exposure to toxins. They often float to the surface of waters, due to gas vacuoles in their cells, where they may drift to the lee shore and accumulate as scum. As cells senesce and die, toxins are released into the water. Animal toxicity follows the ingestion of whole cells and toxic effects depend on the type and amount of toxin, the concentration of cells, and the species, size, sex and age of animal.

(i) **Toxins**
Cyanobacteria produce a wide range of bioactive compounds linked to human health problems and animal deaths. No confirmed human death has been attributed to the toxins. It has been postulated that they are synthesised as a defence against predatory zooplankton, and are thought to arise as secondary metabolites. Some evidence suggests that certain toxins are carcinogenic.

"New" toxins are continually emerging but over 60 have already been characterised. They fall into two groups.

- neurotoxins, e.g. anatoxin-a, which mimics acetylcholine but is not degraded by acetylcholine esterase, and anatoxin-a(s), which is an organophosphate (OP) and may be the only natural OP so far discovered. The neurotoxins anatoxin-a and -a(s) are thought to be unique to

cyanobacteria but two others, saxitoxin and neosaxitoxin, occur in marine algae. Why freshwater cyanobacteria should produce the same chemicals as marine eukaryotes is still an unsolved riddle.

- hepatotoxins, eg microcystins, of which there are more than 40 structural variants, and nodularin. Microcystins are cyclic heptapeptides and are the most widespread and frequently implicated causes of toxicosis. They are approximately ten times more toxic than strychnine.

(ii) Detection Methods

Laboratory techniques employed to detect cyanobacterial toxins include mouse bioassays, immunoassays, HPLC and cell line assays. Finnish workers have also described a simple bioassay using *Artemia* brine shrimp larvae. Whole animal assays are becoming increasingly unacceptable and alternatives are being actively developed.

Within an individual water body, cyanobacterial toxin levels may vary widely from week to week at the same sampling station. In addition a mixed pattern of high and low toxicity per unit cyanobacterial biomass, when sampled at an individual lake on a single occasion, has been found. These spatial and temporal variations obviously present sampling problems.

(iii) Disease Syndromes

Neurotoxins have been linked to animal deaths in N. America, UK, Australia and Scandinavia. Hepatotoxins have been linked to incidents in all parts of the world. Mortalities in a variety of mammals, birds and fish have all been described, usually in periods of warm temperatures and high sunshine levels in spring or early summer.

The three most important genera in veterinary medicine are *Microsystis* (hepatoxic), *Anabaena* and *Aphanizomenon* (both neurotoxic). Hepatoencephalopathy occurs consistently in lethally poisoned animals, and in ruminants poisoning may be accompanied by hepatogenous photosensitization. Histopathologically the hepatotoxins cause hepatic centrilobular necrosis and intrahepatic haemorrhage, and acute exposure has resulted in deaths of birds, mice, dogs, sheep and cattle. Fish kills in eutrophic water bodies (rich in organic and mineral nutrients) at times of cyanobacterial blooms have been reported for many years. These are traditionally ascribed to oxygen depletion due to bloom respiration at night, or to high microbial oxygen

demand due to bloom die-off. Physical gill blockage by cyanobacterial cells and colonies, high pH levels and high ammonia levels during bloom senescence may also be significant. However reports of mass fish mortalities showing severe liver pathology have been linked with microcystin production from decaying blooms of *Anabaena flos-aquae*.

In 1989, 20 sheep and 15 dogs died after entering Rutland Water and ingesting scum containing *Microcystis aeruginosa*. Sailboarders reported skin rashes, blistering inside the mouth, and severe thirst, and young children playing at the edge of the scum developed vomiting and diarrhoea. Also in 1989 canoeists on Rudyard Lake, Staffordshire, who had been performing eskimo rolls through a scum of *M. aeruginosa* developed symptoms including sore throats, headaches, blistered mouths, dry cough, abdominal pains, vomiting and diarrhoea. Two became severely ill and were hospitalised. Laboratory tests confirmed cyanobacterial poisoning.

In 1990 three dogs died showing neurotoxic signs following ingestion of *Oscillatoria* spp scum in Loch Insh, Scotland. Strains of this species produce anatoxin-a. The first report of sheep deaths exposed to cyanobacterial neurotoxins appeared in 1995. Fourteen sheep died in Australia after drinking water with a heavy bloom of *Anabaena circinalis*. Toxins were found in the gut contents of the dead sheep. The same toxins have been shown to bioaccumulate in Australian freshwater mussels fed on the same neurotoxic species. Australian strains of *Anabaena circinalis* have been shown to produce all three groups of paralytic shellfish poisons, namely the mildly toxic C-toxins, the more potent gonyautoxins and the highly neurotoxic saxitoxin. Each has the same mode of action, blocking sodium channels and causing death by respiratory failure.

In 1993–94 the death of wild birds on lakes in Denmark coincided with marine cyanobacterial blooms dominated by *Anabaena lemmermannii* var. *minor*, which produces anatoxin-a(s), a cholinesterase inhibitor. These were the first confirmed wild animal deaths linked to this toxin.

Conclusion

Our level of knowledge about each of the many harmful algal species varies significantly, but even the best-studied remain poorly characterised with respect to bloom or population dynamics. In the past two decades the inci-

dence of toxic blooms has increased. The impact of human activities is not yet clear and much work remains to be done to unravel the influence of environmental variables on algal growth and toxin production. However it is hoped that the controls in place within the EU should at least help protect the consumer from contaminated shellfish.

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PROCEEDINGS

Fish Veterinary Journal (2002) (6) 51–68

The European Union and Fish health – The Brussels Process explained

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Abstract

This paper attempts to explain the legislative process involved in the development of the current EU fish health regime. It begins by briefly looking at sources of national and EU law. It goes on to describe the key EU institutions involved in the legislative process and the legislative process itself, using, where possible, concrete examples drawn from the fish health area. It explains the important role played by committees, such as the Standing Veterinary Committee in developing new legislation. It attempts to summarise the main contents of the most important fish health Directives and Decisions. Finally it offers a critique of the legislation in the light of recent fish disease developments. Only animal health issues are dealt with in this paper. EU Legislation dealing with other issues such as veterinary medicines, public health and animal welfare issues etc. are not covered.

Introduction

It has been said that 'the European Community is nothing if not a Community based on law'. The EU fish health regime consists of a body of EU legislation which lays down the rules governing such matters as the movement of live fish and eggs within the Community, the classification of diseases, zoning of the of the Community territory based on the presence or absence of some of these diseases and measures to be taken in the event of certain of these diseases breaking out.

To understand this regime of legislation it is useful to have some basic knowledge of the background to this legislation such as the sources of law in the Community and the Member States, how EU legislation comes into being, the role of the Community institutions in making legislation and the significance of EU legislation and how it becomes inserted into the national legal order of the Member States. It is also useful to be aware of the important role of committees of technical experts, drawn from the Member States, in the creation of legislation. The paper deals only with animal health issues. There are many Directives and

Regulations, which deal with other important veterinary issues such as licensing of veterinary medicines, public health, animal welfare, veterinary checks etc. However space would not allow such issues to be dealt with in this paper.

Sources of law in the UK and Ireland

The principle sources of law in both Ireland and the UK can be summarised as follows:

- **Constitution**.
  Ireland has a written constitution, whereas the UK has an unwritten one (although Tom Kettle, MP in the early 1900s remarked "in dealing with England you are dealing not with an unwritten, but with a badly written, constitution"!)

- **Acts and Statutes of Parliament**

- **Common Law**
  'The commonsense of the community crystallised and formulated by our forefathers.' At its simplest it can be considered to be judge-made law.

- **Equity**
  The body of rules based on principles of fairness formulated and administered by the Court of Chancery to make up for certain deficiencies which developed over time in the Common Law.

- **European Convention of Human Rights**

- **European Union Law**

In this paper only EU law will be considered. Where necessary the relationship between EU and national law will be pointed out.

Sources of EU Law

The principal sources of EU law can be summarized as follows:

- ***Primary Legislation*** *– Treaty law and amendments e.g. EC Treaty, European Coal and Steel Treaty (ECSC), TEU (Maastricht) etc.*

- ***General Principles of Law*** *e.g. Proportionality, Legal certainty, Legitimate expectation, right to a fair hearing etc*

These general legal principles of Law are drawn from the legal traditions of the Member States. For example the concept of proportionality comes principally from German law. The right to a fair hearing (*audi alteram partem*) comes from English Common law

- ***International Agreements*** *e.g. GATT*

These agreements, although involving non-member states have been declared an integral part of Community law. These agreements can be between the Community and non-member states or Member States. International agreements concluded by the Community are binding on the Community, e.g. the GATT.

- ***Secondary legislation*** *- Regulations, Directives, Decisions*

EU Fish health legislation is secondary legislation, primarily Directives and Decisions. International treaties such as the GATT have important legislative implications also. International organisations such as the OIE (World Animal Health Organization), of which all 15 Member States are members, also play a role in influencing EU legislation, the OIE, principally through its Aquatic Animal Disease Code and Diagnostic Manual.

An example:

The application of *Treaty law* and *legal principles* to fish health
The European Court of Justice (ECJ) decides a case involving fish health

Commission – v – Germany (Case C-131/93)

In 1989 Germany prohibited the importation of crayfish for commercial purposes from other Member States in order to protect native crayfish stocks against crayfish plague caused by Aphanomyces. The Commission brought an action against Germany on the grounds that the import of live crayfish from another Member State was impeded and thus in breach of Article 30 of the Treaty of Rome, which prohibits quantitative restrictions or equivalent restrictions to intra-community trade. Germany sought to justify its action on animal health grounds, a derogation allowed

for in Article 36. The key question for the Court rested on the principle of proportionality. Was the total ban on imports disproportionate? The Court supported and upheld the Commission view that the objective of preventing crayfish plague could have been achieved by measures having less restrictive effects on intra-community trade than a total ban on imports, e.g. health checks.

Secondary EU Legislation

These 'Acts' of the Community are *Regulations*, *Directives*, *Decisions*, *Recommendations* and *Opinions*.

Regulations
Regulations are legally binding in their entirety, in the same way, throughout the Community territory. A good example of this type of legislation is Regulation 2377/90, which contains the rules on Maximum Residue Levels (MRLs) for veterinary medicines. This regulation became part of the national legal order of each Member State without any need for national implementing legislation. Because of this, Regulations are often described as being *directly applicable*.

Directives
Directives are legally binding as to the effects to be achieved. Directives set out objectives. They are legally binding on the Member States but must be incorporated into the law of each Member State. How they are incorporated into law and the form of legislation used is left up to the individual Member States to decide. In common law jurisdictions such as the UK and Ireland where the principal of 'duality' applies, Directives are usually transposed into national law as either Statutory Instruments or sometimes as Statutes. For example in the UK the fish health Directive 91/67/EEC was transposed into UK law as the Fish health Regulations, 1992 and in Ireland together with Directive 93/53/EEC as European Communities (Aquaculture Animals and Fish) Placing on the Market and Control of Certain Diseases) Regulations, 1996, S.I. 253 of 1996.

Decisions
Decisions, like Regulations, are legally binding and directly applicable on those to whom they are addressed. Decisions may be directed to Member States or companies and even to individuals. Much Community fish health legislation is in the form of Commission Decisions. For example the detailed

diagnostic tests and sampling plan for fish diseases are contained in Commission Decision 92/532/EEC.

Opinions and Recommendations
Since these are not legally binding they will not be dealt in this paper.

Besides being *directly applicable* (see section on Regulations), EU Law has been found by the European Court of Justice (ECJ) to be *directly effective*, which means that it confers rights on individuals, which must be upheld in national courts. Finally, and most importantly, EU Law is supreme over national law.

A Note on the Legislative Institutions of the European Union

The European Parliament
The European Parliament originally played a weak consultative role in the legislative process. Since the Single European Act onwards its role has been greatly enhanced and now it virtually has a joint role with the Council in enacting legislation in some areas e.g. Public Health. Parliament also has an important supervisory role in relation to other EU institutions.

The Council
Traditionally the Council was considered the supreme law-making body of the EU. It is made up of the representatives of the Member States at Ministerial level. Council meetings are arranged by subject matter, e.g. Agriculture, Transport, Fisheries, etc. At these meetings legislation proposed by the Commission is adopted. The Council is assisted by two important committees at ambassadorial (Permanent Representative) and deputy ambassadorial level, known as COREPER I (dealing mainly with internal and technical matters) and, the more important, COREPER II (dealing mainly with external and political matters) as well as technical working parties and working parties of attachés.

The Commission
The Commission plays a central role in the law making process. It has the sole right of initiating legislation. It can also make laws itself; exercising power delegated to it by the Council by a process sometimes termed 'Comitology'. The Commission also develops legislation and supervises its implementation. In addition the Commission defends the Treaty and represents the Community

interest at international level (e.g. at OIE, WTO etc). There are currently 20 Commissioners, at least one and no more than two come from each Member State. The governments of the Member States nominate Commissioners. The European Parliament (EP) must approve all nominees.

The Commission bureaucracy is organized into *Directorates General*. Previously these were numbered but more recently have been named according to their subject matter of responsibility e.g. 'Health and Consumer Protection Directorate-General' or, abbreviated from the French, as 'DG SANCO'.

The Court of Justice
The Court of Justice (ECJ) has played an enormous role in 'legislating' through its judgments, interpreting EU Law in cases referred to it from the national courts of the Member States under the EC Treaty.

The EU Legislative Process

The EU has no single legislative procedure. The legal base for Community legislation depends on the particular Treaty article. There are seven principal procedures involved in creating new EU legislation. Those in italics are commonly used in creating new veterinary legislation, including fish health legislation.

1. **Council acting alone**
   Quite rare

2. **Council and Commission acting alone**

3. ***Council, Commission and consultation with European Parliament***
   This is known as the 'old procedure'. All fish health Directives were enacted under Article 37(previously Article 43) using this procedure.

4. **Council, Commission and Co-operation procedure with the European Parliament**
   Introduced by Single European Act. It enhanced the role of the European Parliament to some extent. With the support of one Member State, Parliament could block the adoption of a measure. It is largely replaced by the Co-Decision procedure.

5. ***Council, Commission with the European Parliament: The Co-Decision Procedure***
   This procedure further enhanced the power of the European Parliament by giving it the power of a negative final say in relation to certain legislation. The Council retains a positive final say. Public Health legislation must be enacted using this procedure (Article 251).

6. **Council, Commission and the European Parliament: Assent**

7. ***Exercise of Delegated Power of Legislation by the Commission***
   The Standing Veterinary Committee (SVC) procedure is a good example of this method of legislating. The European Parliament is not involved.

Much veterinary legislation was enacted under Article 37 (previously Article 43) by the 'Old Procedure' involving a proposal by the Commission to the Council with the European Parliament being consulted. The other principal way is by Decision of the Commission using powers delegated to it by the Council, e.g. The Standing Veterinary Committee procedure. If there is a public health or consumer protection aspect to the legislation then the Co-Decision procedure applies (Article 251).

An example:

EU Fish Health Legislation
How the fish health Directives came about – The 'Old Procedure'. Article 37(previously Article 43)

All the fish health Directives have their legal basis in Article 37 (previously Article 43) used in conjunction with qualified majority voting, using the 'old procedure' meaning a proposal from the Commission to the Council and consultation with the European Parliament and the Economic and Social Committee (ECOSOC). Consultation with the European Parliament and ECOSOC means just that. There was no obligation on the Council to heed this opinion (nor did it in many instances).

Under qualified majority voting the different Member States have a certain number of votes each, depending on their population For example Ireland has 3 votes whilst the UK has 10 votes. To obtain a qualified majority for a legislative measure requires a minimum of 62 votes out of a total of 87. This is not easily achieved and to be adopted a proposal requires the support of many large Member States and several smaller ones also. The least number of votes needed to defeat a proposal is 26 votes and is known as a 'blocking minority'.

The Role of Committees and Working Parties in Creating Fish Health Legislation

It would be difficult to exaggerate the role of working parties and committees in developing legislation in the EU. Many committees and working parties of government and private experts assist the Commission and The Council in their work

Working Parties
The Commission submits virtually every new legislative proposal to a working party of experts drawn from the civil service of the Member States, before it is sent to the Council or Parliament. The experts assist the Commission by proposing improvements and useful changes to the legislation. The myth that the Commission is awash with functionaries is just that. Without the advice of technical and administrative civil servants from the Member States the Commission would be unable to do its work of initiating and implementing legislation. The Commission usually accepts some, but not necessarily all, of the comments and proposals from the experts.

On receiving a legislative proposal from the Commission, the Council, likewise, submits the proposal to a technical working party drawn from the civil service of the Member States. These are often the same people who advised the Commission on the proposal except their role, in theory at least, is somewhat different in that at Council meetings they are acting in the interests of the Member State they represent.

Committees
Committees play a more formalized role than working parties in the legislative process. Certain areas of Community policy are highly regulated and require numerous decisions, which must be adopted quickly by the Commission. When The Commission exercises its delegated power of legislation, committees of representatives from Member States assist it in this task. The purpose of a committee, representing the interests of the Member States, inserted into the legislative process is to create a check on the powers delegated to the Commission. When the Council delegates legislative powers to the Commission it rarely gives it *carte blanche*, hence the existence of committees. These committees function in virtually all the areas covered by the EC Treaty. 260 committees assist the Commission in the exercise of its implementing powers. In the Agriculture sphere alone, 30 committees assist the Commission.

There are three basic types of Committee involved in the legislative process. These consist of *Advisory Committees*, *Management Committees* and *Regulatory Committees*. Each operates under a different procedure, known as Procedure I, II and III. From a veterinary and fish health point of view, the Advisory and, more especially, the Regulatory Committees play an important role in developing legislation. The *Advisory Veterinary Committee* is an example of an advisory committee, which is used by the Commission to obtain opinions of trade and professional interests and consumers in problems of harmonization of veterinary legislation. Another is the *Scientific Veterinary Committee*. This committee acts in a consultative capacity to the Commission on all scientific and technical problems concerning animal health, veterinary public health and animal welfare. It is composed of three sections corresponding to these three fields.

However, from a legislative point of view the most important type of Committee is the *Regulatory Committee* because of its role in the exercise of delegated power of legislation by the Commission (Procedure III). The 'sting in the tail' of Procedure III is that the Commission can only adopt a measure *if it is in accord* with the opinion of the Committee i.e. the support of a qualified majority of the Committee is required. If there is not sufficient support, the power of decision reverts to the Council. If the Council fails to act the Commission may adopt the measure. The three procedures all have a number of variants, which will not be detailed here.

An example:

The Standing Veterinary Committee (SVC)
The Commission exercising Delegated Power of Legislation from the Council

The animal health area is a good example of a highly regulated area requiring numerous decisions and regulations, which often have to be adopted quickly. Much fish health legislation has been created through the SVC procedure. Decision 92/532/EEC laying down the fish disease sampling plans and diagnostic methods is an example of fish disease legislation being created by the Commission assisted by the Standing Veterinary Committee. There are many other such fish health examples.

In reality fish health matters are rarely discussed in detail by the SVC, mainly because fish disease experts are rarely present at the meetings, as the number of fish items is usually small. Generally, an informal committee of fish disease experts from the

Member States discusses the matter in advance of an SVC meeting. If agreed by them, and having briefed their SVC delegates, the decisions are adopted formally by the SVC. Membership of the SVC is not fixed and Member States will send different representatives to the meetings, depending on the topics on the agenda. Occasionally a SVC meeting will be taken up by a totally fish health agenda, but this is unusual. The Committee meets about once a fortnight and meetings frequently last about two days. Up to forty or more agenda items may be discussed and some voted upon. Voting is along the lines appertaining in meetings of the Council, being by qualified majority.

EU Fish Health Legislation and the Single Market

The background to the current fish health and public health legislation was the White Paper *Completing the Single Market*, published in 1985. This document had great implications for veterinary legislation generally and put forward certain radical veterinary legislative principles, which became common to most animal health legislation in the years that followed. These principles can be summarised as follows:

- All veterinary controls to be limited to the place of departure. Frontier controls to be abolished
- Common standards to be established for trade between Member States
- The role of the veterinary certificate to be drastically reduced
- Removal of frontiers by 1988
- Approximation of legislation by 1992

In the veterinary sector the principal effect of the White Paper was to establish the principle of allowing the free movement of live animals and animal products within the EU unless, for animal or public health reasons, EU legislation itself prevented or restricted it. The deadlines set out in the White Paper were generally achieved but fish disease legislation tended to lag behind that of other types of livestock production and, to some extent, suffered as a result. The models of legislation used in the fish health area were based largely on those used for diseases of terrestrial animals and the differences between disease control in the aquatic and terrestrial environments were not always sufficiently considered.

An Overview of the EU Fish Health Legislation

The principal Fish health Directives and Decisions are listed below, although the list is not intended to be an exhaustive one. It is not the intention of this paper to

go into great detail on the content of the individual Directives and Decisions. From a practical point of view the most important are Directive 91/67/EEC (as amended), Directive 93/53/EC (as amended) and Directive 95/70 EC.

Directives

The various fish health Directives are detailed and complicated, particularly Directive 91/67/EEC, and require careful study. The foregoing only attempts to highlight the main points contained in the legislation.

❑ **Council Directive 91/67/EEC**
Concerning the Animal Health Conditions governing the placing on the market of aquaculture animals and products

This Directive lays down the rules governing the placing on the market of aquaculture animals and products. The aim of the Directive is to help bring about a Single Market in aquaculture animals and products, whilst, at the same time, avoiding the spread of disease. The Directive contains 30 articles in 4 separate Chapters and has 4 annexes. The Directive has been amended on several occasions, particularly by Directives 93/54/EEC and Directives 95/22/EC.

Firstly, the Directive categorizes the important diseases of fish and shellfish into three disease lists; List I, II and III depending on their cause, economic importance, and presence or absence and distribution in Community territory. The susceptible fish species for each disease were also specified. Interestingly, the Directive does not define the meaning of a list I, II or III disease but the following definitions, based on an early unpublished working document, reasonably describe them:

List I diseases
These are compulsorily notifiable, diffusible epizootic diseases absent from the Community, which pose a serious threat to the Community economy. Only one disease is currently present on this list, namely Infectious Salmon Anaemia (ISA)

List II diseases
These are contagious diseases which are present in parts but not all of the community territory and which pose a serious threat to the Community economy. Two diseases of fish and two diseases of molluscan shellfish were included in this category, Infectious Haematopoietic Necrosis (IHN) and Viral Haemorrhagic Septicaemia (VHS) of fish and Bonamiosis and Marteiliosis of native oysters.

List III diseases
These are diseases, which are widely distributed in the Community and normally controlled at farm level or eradicated on a voluntary health scheme basis.

Secondly the Directive introduced into fish health legislation for the first time the concept of 'zones', by which the Community territory could be classified according to the presence or absence of List II diseases. Zones in which one or more of the List II diseases were shown to be absent were designated as 'approved zones' for one or all of the diseases. Zones in which one or other of the List II diseases were known to be present were classified as 'unapproved zones'. Two types of zones are described in the Directive, namely 'continental' and 'coastal' zones. As the names imply 'continental zones' describe the inland freshwater situation where zones are based on individual river catchments or groups of catchments. 'Coastal zones' refer to distinct parts of the coastline. The Directive describes in detail how 'approved zone' status can be achieved by a Member State and how, once granted, this status can be maintained. The basic principle laid down in the Directive for the placing on the market of aquaculture animals is that only movement of live fish and shellfish between zones of equivalent zone status is permitted or from zones of a higher to one of lower health status.

The Directive also contains rules on trade with third countries although the details of these rules have yet to be drawn up but will be developed by the Commission.

❑ Council Directive 93/54/EEC and Directive 95/22/EC

These Directives amend the disease list and list of 'susceptible species' in Directive 91/67/EEC. It also amends the procedure for obtaining approved zone status and definitions of various types of zones.

❑ Council Directive 98/45/00

Amends Directive 91/67/EEC in several ways but principally by changing the time scale needed to obtain approved zone status.

❑ Council Directive 93/53/EEC
Introducing Minimum Community measures for the control of certain fish diseases

This Directive describes the minimum Community measures for the control of the List I and List II diseases. List I and II diseases must be made notifiable in all Member States. All farms must keep certain records of fish movements and mortalities.

In the event of a List I being suspected and reported, the Directive lays down that the suspect farm must be placed under official surveillance and steps taken to pre-

vent further spread of the disease. These steps involve controls and prohibitions on movement of fish, feed, equipment or persons on to or from the farm. Following confirmation of the disease, all fish must be slaughtered. The Directive goes on to require cleaning and disinfection prior to any re-stocking of the farm. An epizootic investigation is also mandatory.

In the event of an outbreak of a List II disease, slaughter of all fish on an infected farm is also mandatory to regain approved zone status for the zone involved.

❑ Council Directive 2000/27/EC

This amends Directive 93/53/EEC by dropping the requirement for immediate withdrawal of infected fish from an infected farm. In future fish shall be withdrawn 'in accordance with a scheme established by the Official Service and approved by the Commission assisted by the Standing Veterinary Committee' (note another example of the delegated power of the Commission to legislate, assisted by the SVC).

❑ Council Directive 95/70
Introducing minimum community measures for the control of certain diseases affecting bivalve molluscs

This Directive establishes minimum Community measures for the control of the diseases affecting certain bivalve molluscs. It is analogous to Directive 93/53 and applies some of the requirements of this Directive to molluscan shellfish, such as the requirement for reporting of disease and mortality in cultivated molluscs.

List II diseases and abnormal mortalities must be reported to the official services. Member States are required to monitor and sample bivalve mollusc farms and restrict movements of molluscs in the event of disease or abnormal mortalities.

Decisions

❑ Placing on the market of *Crassostrea gigas*

Commission Decision 93/55/EEC

❑ Additional guarantees for the placing on the Market

Commission Decision 93/44/EEC (Spring Viraemia of Carp)

❑ Sampling Plans and Diagnostic methods

Commission Decision 92/532/EEC (fish Diseases)
Commission Decision94/306/EC (mollusc diseases)

❑ **Approved zones with regard to VHS and IHN**

Commission Decision 92/538/EEC (GB and NI)
Commission Decision 93/39/EEC (Guernsey)
Commission Decision 93/40/EEC (Isle of man)
Commission Decision 93/73/EEC (Ireland)
Commission Decision93/74/EEC (Denmark)
Commission Decision 95/125/EC (France)

❑ **Approved farms in non-approved zones**

Commission Decision 95/124/EC (Germany)
Commission Decision 95/336/EC Germany
Commission Decision 95/470/EC (Belgium)
Commission Decision 95/473/ EC (France)

❑ **Movement Documents**

Commission Decision 93/22/EEC

❑ **IHN and VHS Programmes**

Commission Decision94/862/EC (Spain)
Commission Decision94/863/EC (France)
Commission Decision 95/479/EC (Finland)
Commission Decision 96/221/EC (Denmark)
Commission Decision 97/185/EC (UK)

❑ **Bonamiosis and Marteiliosis programmes**

Commission Decision 92/528/EEC (UK)
Commission Decision 93/56/EEC (Ireland)
Commission Decision 93/57/EEC (Jersey)
Commission Decision 93/58/EEC (Guernsey)
Commission Decision 93/59/EEC (Isle of man)
Commission Decision 94/722/EC (France)

❑ **Imports from third Countries**

Commission Decision 95/597/EC (Crassostrea gigas)

❑ **Safeguard measure**

Commission Decision 97/587/EC (ISA Norway)
Commission Decision 1999/766/EC (ISA Norway)
Commission Decision 2000/431/EC (ISA Norway)
Commission Decision 96/490/EC (Gyrodactylus salaris)
Commission Decision 98/24/EC (Gyrodactylus salaris)

Discussion

The first thing that must be said is that the legislation has contributed to maintaining the *status quo* disease-wise, at least in relation to the distribution of some important freshwater diseases in the EU, such as VHS. However, the principal purpose of the fish health legislation was twofold: *to contribute to the completion of the internal market, avoiding the spread of infectious or contagious disease*. To what extent both of these twin objectives have been or indeed could have been achieved is questionable. It could be argued that the legislation has been used by some Member States to simply maintain the *status quo* in relation to imports of live salmonids and, as such, has worked well for them. Such countries like the UK and Ireland, being islands, have had a strong policy of restricting the importation of live salmonids into their territories and both countries were quick to steal a march on other Member States, using historical data to obtain *approved zone status* for the whole of their territories for the diseases IHN and VHS. This effectively prohibited any importations of live salmonids into either country from the rest of the EU for several years.

However, this situation is changing as approved zone status has been obtained by other Member States for at least part of their territories which has opened up the possibility of some intracommunity trade in live salmonids between Ireland, the UK and other parts of the EU. On the other hand, the imposition of a *safeguard measure* to protect the territories of the UK and Ireland from the introduction of the parasite *Gyrodactylus salaris* has further reduced the prospect of a true single market developing. But in the last analysis, it must be said that the White paper on *Completing the Internal Market* did concede that certain import restrictions would still be justified on grounds of protection of animal health.

The *zone* concept introduced by *Directive 91/67/EEC* has led to some interesting and surprising results. The purpose of creating *zones* based on geography was, presumably, to move away from the idea of a market based on national territories and frontiers. If so, then this aim has not been altogether successful. For example, the UK and Ireland by combining all their river catchments into single large zones comprising the whole of their respective national territories would seem to have undermined this notion. On the other hand, it must also to be said that if a Member State were obliged to consider each river catchment as a separate zone this would create an administrative nightmare in relation to the movement of live fish between approved zones.

The other extreme has been the recent trend of some Member States applying for *approved zone status* for what amounts to little more than individual farms in areas where farms infected with list II diseases are known to occur (*unapproved zones*). If such applications were allowed, then the expressed aim of avoiding the spread of disease would seem to be placed at risk. In retrospect, it might have been preferable to place an upper and lower limit on the size of *approved zones* thus facilitating the creation of a single market truly based on geographical zones rather than national territories and also preventing the creation of zones so small that they pose a real disease risk to larger *approved zones*. In this way, *approved zones* could be composed of several catchments but not so large as to be synonymous with the whole of the national territory and one or two farms would not be considered of sufficient size to warrant being considered an *approved zone*.

Recent disease occurrences and scientific developments have placed a large question mark over the *zone* system as it applies to the marine environment as well as the diseases included in list I and II. The isolation of marine rhabdoviruses indistinguishable from classical rainbow trout VHS strains on all but the most sophisticated of tests, has led many veterinarians specializing in fish medicine to call for a re-examination of the application of the *zone* system to the marine environment. Two outbreaks of VHS in marine turbot farms in approved zones, one in the UK and the other in Ireland, resulting in the destruction of surviving fish to regain approved zone status, has probably curtailed significant investment in marine flatfish farming in both countries. In addition, in work funded by the EU Commission, the current knowledge about marine VHS isolates makes the concept of *approved coastal zones* for VHS, to say the least, extremely questionable. Nearly 40,000 marine fish have been examined in Europe alone and it is known that there are now about 121 North European marine VHS virus isolates (4 from turbot farms) reported. Also, there are 14 susceptible species reported and probably more to come. Most isolates are from herring and sprat. These marine VHS virus isolates from wild fish are either non-pathogenic or have low pathogenicity for rainbow trout and salmon, although they are pathogenic for turbot. The EU Commission cannot ignore these scientific findings for much longer. It is no longer possible to consider coastal areas to be *approved* (free of VHS) when it is known that so many wild marine species actually carry this virus.

The outbreak of Infectious Salmon Anaemia (ISA) has further called the rationale of the EU fish health regime into question. Since the disease was

first identified in Norway in 1994, it has also since been found in New Brunswick, Canada in 1996, Nova Scotia in 1998, Scotland also in 1998 and the Faeroe Islands and Chile in 2000. The virus has also been isolated from several species of wild fish in the marine environment. Given the worldwide geographic distribution of this disease, it is now widely believed by many reputable scientists that this virus is endemic in the marine environment and that the policy of eradication is no longer realistic or desirable. Eradication of a disease can only be realistic if the possibility of re-infection can be eliminated. This cannot be achieved in the marine environment. Furthermore, any question of eradication of fish diseases must be coupled with compensation or else the necessary support needed for eradication from the aquaculture industry will not be forthcoming. The EU policy of persisting with an eradication policy for this disease in the face of the growing scientific knowledge on the prevalence of the disease in the marine environment on a worldwide basis seems increasingly difficult to defend.

Further criticisms of the legislation are that it is overly complicated; that it fails to deal with the question of imports from third countries or List III diseases; that it concentrates on problems of northern European aquaculture; and that it does little to improve the overall health of EU aquaculture.

Nonetheless, one can be optimistic that the review of the current legislation recently commenced by the Commission will lead to improved legislation which better achieves the aims which were originally set out for it and thus benefits the aquaculture industry of the EU, provided that the review is realistic, practical and thorough and takes account of the new scientific and epidemiological data which has become available since the first legislation was agreed in 1991.

Some Further Reading

All EU legal textbooks tend to become out of date quickly because of Treaty changes and case law. For those interested in further information on the subject, the following textbooks are recommended.

Craig, P and G. de Burca. *EU Law. Text Cases and Materials*. 2nd edition. Oxford University Press. 1998. *An outstanding up to date university level textbook. For the seriously interested reader.*

Stephen Weatherill. *Law and integration in the European Union*. Claerendon

Press. Oxford. 1995. *A very readable, discursive and sympathetic book from one of the foremost UK authorities on EU law. However, unless recently updated, the 1995 edition is probably now out of date*.

Josephine Shaw. *European Community law*. Macmillan. I993. *An excellent book also. Only buy a copy if recently updated*.
T.C. Hartley. *Foundations of European Community law*. 4th edition. Clarendon Press. Oxford. 1999. *Very clear, well written and up to date. A standard textbook for undergraduate students*.

Mike Cuthbert. *Nutshells European Union law*. 3rd edition. Sweet and Maxwell. 2000. *Short and accurate. Contains more than enough information for the general reader*.

John McArdle has degrees in Veterinary Medicine (MVB) as well as History and Economics (BA) from the National University of Ireland, Dublin. On a scholarship from the Nuffield Foundation he studied at Stirling University and was one of the first veterinarians to obtain the MSc in Aquatic Veterinary Studies from the Institute of Aquaculture in 1973. He also holds a Diploma in Legal Studies and Certificate in EU Law from the Dublin Institute of Technology. He worked as a Veterinary Research Officer in the Irish Dept, of Agriculture for 4 years and later as Fish Pathologist in the Dept. of the Marine and Natural Resources and Marine Institute for 20 years. He represented Ireland on various EU Commission and Council technical working groups and committees, including the Standing Veterinary Committee, for many years. He availed of voluntary early retirement from the public service at the end of 1999. He is currently consultant veterinarian to the Central Fisheries Board. The Board operates 3 freshwater fish farms in Ireland. He also acts as a consultant to the Shannon Regional Fisheries Board and from time to time to the Marine Institute.

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Atlantic salmon broodstock and vertical transmission of Infectious Pancreatic Necrosis virus

L.A. Laidler
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Infectious Pancreatic Necrosis (IPN) virus is responsible for an acute disease of juvenile salmonids. Transmission of the virus to progeny has been shown to occur vertically via eggs in brook trout (*Salvelinus fontinalis*), (Bootland et al, 1991) and rainbow trout (*Onchorhynchus mykiss*), (Wolf et al, 1963). Dorson and Torchy (1985) used artificially infected rainbow trout sperm to fertilise virus-free eggs and showed clinical disease in the progeny fry.

A number of experiments to determine the occurrence of vertical transmission in Atlantic salmon have been undertaken by Smail and Munro (1989, 1993).

Infection of eggs via virus adsorbed to sperm was investigated using eggs from IPN virus negative females and artificially infected milt at one virion per sperm (Smail & Munro, 1989). Virus was detected inside the egg, showing that sperm can cause virus entry into the egg but there was a drop in titre by 60 minutes after water hardening and virus was not detected two weeks later.

Smail & Munro (1989) attempted to artificially infect eggs by injecting hens with virus prior to spawning and by egg bathing. Virus was not detected in the eggs of hens which had received a low injected dose of up to $4x\ 10^6$ plaque forming units (pfu)/kg or were bathed with up to 9×10^6 pfu per egg. At higher injected doses of up to 8×10^9 pfu/kg or bathing at 1×10^9 pfu per egg, virus was detected five minutes post-fertilisation but not two weeks later.

In a study of broodstock naturally infected with IPN virus at a commercial salmon farm, Smail & Munro (1993) examined 40 female fish which showed virus positivity rates in kidney and ovarian fluid of 22.5% and 17.5% respectively. Virus titres in kidney were up to 5.4×10^6/g in kidney and up to 3.7×10^6/ml in ovarian fluid. The eggs were fertilized with milt from males shown to be virus free. Eggs from three spawnings were followed through hatching to first feeding fry in mid-July but virus was not detected at any sampling.

Although vertical transmission in Atlantic salmon has not been shown experimentally or in naturally infected fish, this may be due to the limited amount of work done. Although the study by Bootland et al (1991) demonstrated vertical transmission in brook trout it was noted that there was a low probability of it being observed in laboratory studies.

It has been suggested that infected eggs are likely to exist at a very low frequency. In brook trout, vertical transmission only occurred in fish with high virus titres in ovarian fluid (Wolf et al, 1963). Anti-viral activity within the egg may be another mechanism for decreasing the incidence (Smail & Munro, 1989). Carriage on the egg surface ('false' vertical transmission) was suggested by Smail & Munro (1993) as a means of viral spread to yolk-sac fry.

Surveying the incidence of IPN virus infection in Norway, Krogsrud et al (1989) found that the virus was widespread in both fresh and seawater fish farms. Methods to control vertical transmission of the virus, including discarding the eggs of virus positive parents and disinfecting eggs with iodophor, have apparently been ineffective. Such circumstantial evidence for vertical transmission must always be examined critically. For example, the possibility exists that fish may have become infected horizontally from incoming water or through inadequate disinfection of the facility after the presence of previously infected fish.

To prevent vertical transmission of IPN virus in their breeding programme, Marine Harvest (Scotland) follow the procedures required by the regulatory authorities i.e. disinfecting eggs with iodophor and discarding those from parents shown to be virus positive. This protocol was introduced for the 1983-year class fish and to date, no incidence of IPN virus infection in freshwater stocks has been detected by the government inspectorate. In seawater however, the virus (but not the disease) has been regularly recorded. In an attempt to follow the course of infection, a trial was set up to sample fish before and after transfer to seawater (Laidler, unpublished data). Following usual practice, eggs were disinfected post-fertilisation with iodophor containing 100ppm active iodine. A freshwater population was chosen for study from which the parental-positive discard rate due to infection with IPN virus was 18%. One hundred and fifty fish were sampled two weeks prior to seawater transfer and then at four, twelve and twenty weeks post-transfer. Previous investigations at the receiving seawater site had shown that mussels

(*Mytilus edulis*) on the shore adjacent to the salmon pens were positive for IPN virus and 100 were sampled in parallel with the salmon. None of the salmon or mussels sampled yielded IPN virus in cell culture. Thus, no information was gained regarding the nature or timing of horizontal infection. The results suggest, for this trial at least, that for a parent population with a relatively high infection rate, the current methods to control vertical transmission are effective.

Although the salmon industry has to bear the significant costs of screening broodstock and egg disinfection, no other control strategy is currently available to prevent spread of the virus and a potentially damaging increase in clinical disease.

Because of the circumstantial evidence that vertical transmission can occur, albeit probably at very low frequency, further studies are indicated. From a salmon industry standpoint, understanding how vertical transmission occurs may lead to more effective strategies for prevention.

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Smail, D.A. & Munro, A.L.S. (1993) Vertical transmission studies on IPNV in Atlantic salmon (*Salmo salar* L.) ICES, Maricult. Comm., CM 1993/F: 36, Copenhagen.

Wolf, K., Quimby, M.C. & Bradford, A.D. (1963) Egg-associated transmission of IPN virus of trouts. *Virology* **2**, 317–321.

Tony Laidler's interest in fish diseases began while working at the MAFF Veterinary Laboratories Agency Penrith laboratory. While there, he had the opportunity to gain membership of the Institute of Biology and obtain an MSc by research into the isolation and detection of Renibacterium salmoninarum. Moving to Marine Harvest in 1989, he is now Laboratory Manager in charge of laboratories in Lochailort and Stornoway. Apart from fish diseases his professional interests encompass quality assurance microbiology and salmon flesh quality analysis.

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Infectious Pancreatic Necrosis (IPN) of Atlantic salmon

A Summary of the Fish Veterinary Society (FVS) Seminar, 22nd June 2000 Edinburgh

Vets from the UK, Norway and Ireland, together with representatives from the salmon farming industry, vaccine companies and government official services participated in a timely seminar on IPN in Atlantic salmon organised by the FVS in Edinburgh

Background

Infectious pancreatic necrosis (IPN) is a contagious disease of fish caused by infectious pancreatic necrosis virus (IPNV). IPNV is an aquatic birnavirus with a worldwide distribution (Reno 1999). The disease was first recognised in North America in farmed brook trout in 1941, but the causative agent IPNV was not isolated until 1957 (Wolf et al. 1960). Numerous IPNV serotypes have now been isolated and characterised. The most pathogenic strain appears to be the Sp serotype which has been consistently associated with serious disease outbreaks in fresh and sea water. Until recently the disease was regarded mainly as a problem of young salmonids in the first 2-3 months post first feeding in freshwater. However over the last decade serious disease outbreaks in Atlantic salmon post smolts in sea water associated with IPNV have been reported from Faroe Islands, Shetland Islands (Smail et al. 1992 & 1995) and Norway (Jarp et al. 1995, Taksdal et al. 1995 and Jarp 1996).

IPN: The disease

IPNV causes mortality in fry and fingerling salmonids and Atlantic salmon post smolts and is characterised by behavioural changes, gross external, internal lesions and histopathological changes. In post smolts in sea water erratic swimming, sluggishness, some loss of appetite and emaciation can occur 1–3 months after transfer to sea. Non-specific external signs include hyper-pigmentation, exophthalmia and petechial haemorrhages on the ventral surface. Internal gross lesions are visceral petechiae and an empty gut containing a yellow exudate. Microscopically there is focal to diffuse necrosis of the pancreatic acinar cells and a catarrhal inflammation of the gut.

Diagnosis is made on the basis of the presence of typical histopathological lesions, immunohistochemistry and virus isolation. The epizootiology of IPN is poorly understood, but the spread and severity of the disease in sea water in Scotland is mirroring the disease pattern observed over the past 5 years in Norway.

Aims of the IPN Seminar

1. To get an update on the current prevalence and severity of IPN in Atlantic salmon.
2. To summarise the current state of knowledge of the transmission & natural history of IPN in salmon.
3. To identify gaps in the knowledge and identify key areas for further research.
4. To make recommendations to government and industry on how to minimise the impact of IPN

Salmon Broodstock and vertical transmission of IPNV
Tony Laidler, Marine Harvest (Scotland) Ltd (MH)

Tony Laidler of MH presented the current state of knowledge on the vertical transmission of IPNV in salmon. There is evidence of covertly infected fish shedding IPNV through seminal and ovarian fluids and that IPNV adheres to sperm and to the egg shell. However the conclusion from a review of the literature and monitoring MH broodstock since 1981 was that true vertical transmission, i.e. IPNV infection and transmission within the egg, is unproven in salmon. Vertical transmission has been shown in other salmonid species and there is circumstantial evidence in salmon that the virus can be passed to progeny. Further research is clearly necessary to determine if true vertical transmission occurs in salmon. Despite the significant costs of broodstock screening and egg disinfection, no other control strategy is currently available. Further research is clearly necessary to determine the mechanism of vertical transmission in salmon.

IPN Challenge Models
Dr. Simon Wadsworth, Marine Harvest (Scotland) Ltd (MH)

Dr. Simon Wadsworth, R&D officer for MH reviewed the development of successful *Aeromonas salmonicida* challenge models as an introduction to the development of a successful IPN challenge model at MH. Despite the

isolation of IPNV in 1957, it has until recently been extremely difficult to consistently experimentally reproduce IPN disease in the different developmental stages of various salmonid species. The development of reproducible challenge models is essential not only to the understanding of the disease, but as a vital part of vaccine development and other control strategies. MH has now achieved a successful cohabitant model. Naïve low grilse, photoperiod 2 smolts (PP2 smolts put to sea in January & February) are infected intraperitoneally with 0.1 ml of a high titre single passage Shetland IPNV Sp strain. These heavily infected fish cohabited with naïve uninfected fish at 7-9°C. Forty percent mortality was recorded in injected fish and 40% in the cohabitant fish. His work also indicated that susceptibility to IPN disease was related to strain of fish, stress, salinity and water temperature i.e. smolts were more susceptible at low water temperatures <12°C. The latter observation may partly explain why farms in the Shetland Islands experienced more severe IPN outbreaks due to lower water temperatures at transfer. He suggested that smolts were most susceptible at or around the transfer to seawater, which would explain the high incidence of disease shortly after transfer.

Based on his observations he made the following recommendations:
- Select resistant stocks for high risk sites (Sites where clinical IPN detected)
- Transfer fish to high risk sites at high or increasing temperature profiles
- Avoid PP2 transfers in January & February
- Minimise stress e.g. grading, long transport times in and around transfer
- Good sea water tolerance essential at transfer
- Dietary modulation around transfer may affect impact of IPN
- Effective vaccines essential

The subsequent discussion confirmed that virus shedding from infected fish was rapid and much still remained to be resolved on when smolts became infected and why IPN infection and disease occurred on fallowed marine sites. Further research is required to consolidate the IPN challenge model and on the identification of sources and reservoirs of IPN virus in the wild.

Status of IPN in Scottish salmon farms

T S Hastings, D A Smail and R M Stagg
FRS Marine Laboratory, Victoria Road, Aberdeen AB11 9DB

IPN is regarded internationally as a significant disease of salmonid fish with a wide geographical distribution. In Canada, it is a Schedule 2 disease with

certification required for movement of live fish and ova between Provinces. In the Maritimes, IPNV infection is fairly widespread in seawater but disease outbreaks are rare and live fish movements are generally permitted between farms of similar status. In Norway, IPN is a reportable list 2 disease. The disease is widespread in freshwater and seawater with significant losses reported in recent years.

Vertical transmission of IPN has been demonstrated in several salmonid species including brook trout, rainbow trout and Arctic char. Although vertical transmission has not been proven in Atlantic salmon, studies at FRS Marine Laboratory have demonstrated the presence of IPNV in gonads and gonadal fluids of maturing broodstock, and shown experimentally that virus can enter the ovum adsorbed to spermatozoa and can survive with the ovum. IPN is a list III disease under EC Directive 91/67 and is a notifiable disease (except in trout) in the UK under the Diseases of Fish Act 1937. Where IPN is suspected or confirmed, controls are placed on the movement of live fish and ova onto and off a farm. Movement of live fish may be permitted from an infected site to a site of similar status as part of a clearance program and if there is no significant risk to other stocks in the vicinity of the receiving site. On infected broodstock sites, all broodstock must be tested and ova from positive parents must be destroyed. As an additional precaution, ova must be surface-disinfected after fertilization.

In Scotland there has been a low incidence of infection in fresh water salmon farms between 1995-99 and no reported cases of clinical disease. During the same period there has been a high and increasing incidence of infection in seawater with a number of cases of clinical disease. The majority of cases of clinical disease have been reported in Shetland. Outbreaks of disease have also been recorded in juvenile farmed halibut. There is ongoing surveillance of wild fish for IPN. Virus is occasionally isolated from wild salmonids but no cases of clinical disease have been recorded.

In conclusion, although IPN is widespread in the marine environment, the control policy has been effective to date in maintaining a low incidence of disease in Atlantic salmon in the fresh water environment.

The impact of IPN on Salmon hatchery & smolt producers.
Alan Dykes, Lakeland Smolts Ltd

This speaker highlighted the cost and difficulties of the current IPN control policy to smolt producers. Those producers who were forced to carry out 100% broodstock testing estimated that the cost can be up to 10% of egg sale price. He cited examples of how parallel tests carried out on duplicate samples at different laboratories gave very different results and examples of where positive broodstock produced negative fry and vice versa. He indicated serious gaps in our knowledge on the use of disinfectants to aid control and the absence of any requirement to treat contaminated water effluent. IPN in fry and parr in Norway was closely correlated with water reuse. Transport stress was an important contributory factor. He asked what would happen in the UK if IPN occurred in sea-reared trout? He sees the need for a complete review of current IPN controls, given the endemic nature of the disease in the marine sites and the high cost of the freshwater broodstock testing. The introduction of effective vaccines should be encouraged and more research effort is required to fully understand and control IPN in the U.K.

A report of recent IPN problems in freshwater salmon fry.
Tom Turnbull, Hydro Seafood GSP

Tom Turnbull reported a serious clinical outbreak of IPN in freshwater salmon fry in Scotland earlier this year. Stocks were from 4 egg sources all of which were certified IPN free. Mortalities ranged from 60 to 100% and severe pathological lesions were recorded. Preliminary epidemiological investigations did not reveal a link to egg producers, any illegal action, local IPN infection or the source of the infection. Water re-circulation may have played a role in the outbreak. Subsequent investigations by Hydro Seafood revealed that the stock with the highest mortalities had come from broodstock with a 20% discard rate because of positive broodstock testing. This surely calls into question the efficacy of the broodstock testing used. All the infected stock were culled, the sites were cleaned and disinfected (very difficult). Estimated losses were over £350,000 and there are no guarantees that IPN virus will not reappear if the sites are restocked.

The Shetland experience of IPN in salmon production.
Jim Nicolson, Westside Veterinary Clinic, Shetland

Over the past decade, IPN virus has caused significant mortalities in farmed salmon in Shetland. This has resulted in serious economic loss; the estimated cost to the industry exceeds £2 million annually, based on the value of fish alone. There are also serious welfare issues for affected fish. The present number of salmon smolts put to sea in Shetland is around 12 million. The majority of smolts are reared in hatcheries on the West coast of Scotland. These are then transported, mostly in April, by well boat to sea sites in Shetland. Clinical IPN usually occurs 6–10 weeks post transfer, with mortality of one to fifty percent being recorded 3–4 weeks later. S0's seem to be more severely affected. On average around 10% of all fish transferred die as result of IPN virus. There is no recognised treatment for this disease. Various attempts to reduce the level of losses by feeding management and the use of immuno-stimulants have been inconclusive. With the advent of IPN vaccines which can be administered to smolts at the hatchery stage, there is now a possibility of taking preventative action to protect against the virus.

The speaker described the devastation which IPN can cause to small and larger salmon farms on Shetland using case studies to demonstrate the severe losses incurred. Jim Nicolson would strongly recommend that the new IPN vaccines, which are readily available and extensively used in Norway, should be made available as soon as possible to help control a very serious economic disease.

IPN Recombinant vaccines
Dr Tony Ellis, Marine Lab Aberdeen

Dr Ellis presented his studies on a prototype recombinant IPN vaccine being developed in collaboration with Aberdeen University, SSGA, SSRA and AVL. Due to the lack of a reproducible experimental challenge model for IPN, vaccine efficacy trials were based on comparative antibody responses and the ability of vaccinated fish to clear IPN virus after challenge. He has demonstrated that a yeast recombinant IPN VP2 protein vaccine appeared to be the most immunogenic of those studied, in that it induced antibody responses in a higher proportion of fish than other test vaccines and this vaccine also neutralised the IPNV in a significant number of fish. It also appeared to enable vaccinated fish to clear the IPN virus after challenge. This work is ongoing.

Results from laboratory & field trials, five years after the introduction of the world's first IPN vaccine
Dag Knappskog, Intervet Norbio

Results from laboratory and field trials, 5 years after the introduction of a recombinant IPN vaccine in 1995 in Norway were presented. Epidemiological studies in Norway revealed the most significant cause of smolt losses from 1994–1999 was clinical IPN with an annual mortality of 6–12%. After the introduction of the first IPN vaccine in 1995 there was a significant drop in IPN losses up to 1997, but due to concerns about local reactions, this vaccine was withdrawn. Subsequently in 1998–99 a third generation recombinant IPN VP2 protein product was introduced, which is currently used in 50% of smolts in Norway. This vaccine has been shown in extensive laboratory and field trials to be efficacious against both sub-clinical and clinical IPN infections as demonstrated by a reduced frequency of infection and clinical outbreaks and reduced mortality after challenge. In 21 out of 26 clinical IPN outbreaks, rVP2 vaccinated fish had a 50% better survival than fish vaccinated without the rVP2 protein. The average mortality was less than 10% compared to fish that received other IPN vaccines which had an average mortality of 30%.

Report of the use of IPN vaccines in Norway
Bernt Melgard, Alpharma

Development of efficacious IPN vaccines was been hampered for many years by the lack of a validated challenge model. Field trials with an inactivated IPN vaccine in 1999 by Alpharma in Norway revealed a mortality rate of 46–48% compared to 15–16% in fish vaccinated with this vaccine. In similar cohabitant trials IPN vaccinated fish had a mortality of 4% with unvaccinated fish having 9% mortality. In dose response studies increasing the antigen or viral component of the vaccine did not exhibit any advantage. The addition of IPN to multivalent bacterial vaccines did not appear to diminish the protection to the bacterial diseases. It was emphasised that while IPN vaccines were very promising in aiding the control of clinical IPN that good husbandry procedures and attention to minimising stress at seawater transfer was very important.

A wide ranging discussion took place after the presentations, which highlighted the lack of accurate information on the economic significance, prevalence of disease or distribution of IPNV infection in salmon in the UK. A consensus was not reached on all issues but the Fish Veterinary Society has drafted the following recommendations which urgently need to be addressed by the salmon industry, official authorities and fish vets.

Fish Veterinary Society Recommendations

- A full review of current distribution and economic impact of IPN virus in salmon.
- Cost-benefit analysis to determine if current control procedures are cost effective.
- Review of broodstock testing procedures to ensure consistent and validated sampling and test results.
- Comparative assessment of other diagnostic techniques available.
- The use of efficacious IPN vaccines should be encouraged to reduce viral challenge and economic losses (Parallels with furunculosis vaccines can be drawn and Norwegian industry gaining advantage of vaccine use.)
- Code of Practice on disinfection for infectious diseases at all stages of production should be drawn up and widely distributed.
- Code of husbandry practice should be drawn up: e.g.
  1. Selection of resistant stocks for high risk sites (Sites where clinical IPN detected)
  2. Transfer fish to high risk sites at high or increasing temperature profiles
  3. Avoid PP2 transfers in January & February
  4. Minimise stress e.g. grading, long transport times in and around transfer
  5. Good sea water tolerance essential at transfer
  6. Dietary modulation around transfer may affect impact of IPN
- Research
- A comprehensive review of the current state of knowledge on IPN in salmonids.
- Continue work on experimental challenge model.
- A detailed epidemiological study of IPN in fresh and sea water is required to identify contributing factors, reservoirs and methods of reducing challenge.
- Vaccine development.

Fish Veterinary Society would like to acknowledge generous sponsorship of the seminar lunch by Hydro Seafood GSP and mail shot by Bayer plc.

Summary Report compiled by Dr. Marian McLoughlin & Belinda Weigall on behalf of Fish Veterinary Society October 2000

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IPN seminar participants

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Julian Braidwood – Vericore
Edward Branson – FVS
Chris Brodie – Loch Duart Salmon
Lydia Brown – Vetrepharm
Stephen Campbell – VSD Stormont
Donald Campbell – Vetrepharm
Richard Collins – IOA Stirling
David Cox – Fish Vet Group
Alan Dykes – Lakeland Smolts Ltd
Tony Ellis – Marine Lab Aberdeen
John Finlay – Nutreco
Trevor Hastings – Marine Lab Aberdeen
Andy Holliman – VLA Penrith
Mr P Ingeborgvik – AVL
Colin Johnstone – Marine Harvest (Scotland) Ltd
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John McArdle – Aquaculture Veterinarian
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Mr R Wardle – AVL
Amanda Wigens – Intervet UK
Belinda Weigall – FVS

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Applied Fish Pharmacology
Keith M.Treves-Brown (2000)
324 pages, Hardback, £99.00
Kluwer Academic Publishers, Dordrecht. ISBN 0-412-62180-0

This unassuming book is impressive. The author has worked at the Veterinary Medicines Directorate and is a member of the Fish Veterinary Society. He has also been a sub-editor for this journal and his attention to detail has not lapsed throughout this substantial reference book. Consequently, he is ideally suited to have written a book that fills a great void by providing easily available and reliable information on fish medicines. It is written at a level suitable for a newly qualified veterinarian with a familiarity of pharmacology in other species but is aimed at all readers with an interest in fish health care.

Although most of our knowledge about fish therapeutics is based on work with farmed species, there are pertinent comments relating to carp and some ornamental fish. In the past, and due to the anecdotal nature of treatments for pet fish, my own rule of thumb was to use at least two different sources for dose rates. However, as a general practitioner treating ornamental fish, I now feel more confident about making informed decisions based on current knowledge contained in this book. It is deceptively informative and in the course of reading, the book it is clear how very different some aspects of pharmacology in fish is by comparison to mammals. Even between groups and species of fish, there are some significant differences. This book should be read thoroughly and carefully.

It is an opinionated review of the literature, emphasised by the periodic use of an exclamation mark! In some places, the author has highlighted inconsistencies and conflicting information by quoting verbatim from some papers. He has also summarised many published reports and condensed whole papers into a few concise sentences. The style is precise and to the point: essential for a vast subject that covers a large volume of literature.

The book is in four parts. Part one discusses methods of administration, safety of fish medicines and the law as separate chapters. The first chapter, on administration, outlines the various methods used to treat fish and includes water medication, in-feed, injection and briefly mentions gavage and topical application. The merits and practical aspects of each method are discussed. The increasingly important issue of safety relating to the target species, the operator, the consumer and the environment are highlighted in chapter 2. The

third chapter is a substantial review of the legislation and covers 28 pages. It explains in clear terms the aims of the legislation, labelling requirements, off-label use, in-feed medication, environmental and consumer safety, and marketing authorisations across the world.

Part two discusses several aspects of the commonly used antibacterial drugs, namely tetracyclines, penicillins, macrolides, sulfonamides and quinolones. There is also a short chapter on other systemic antibacterial agents including nitrofurans, chloramphenicol and florfenicol. Each chapter provides a concise introduction to pharmacological characteristics of the group and I found this a useful refresher of information that I last read about at vet school! The specific indications for use of each drug are outlined together with the disadvantages and dose regimens. There is a detailed review of data on the pharmacokinetics in many different species and includes information where available on the palatability, bioavailability, absorption, distribution, elimination and withdrawal periods. Similarly, the pharmacodynamics of some drugs is reviewed: for oxytetracycline, one of the most studied antibacterials used in fish, it includes comments on immuno-suppression, inhibition of erythropoiesis, growth promotion and the results of over-dosage. In recognition of the increasing awareness of the effects of drugs in the environment, the section also summarises the information currently known about the contamination of wildlife and sediments, and inactivation of the drug.

Part three covers other chemotherapeutic agents and includes systemic antiprotozoal agents (fumagillin, nitroimidazoles), externally applied antimicrobial agents, ectoparasiticides and anthelmintics. A thorough review of scientific literature relating to the uses and safety aspects of commonly used chemicals such as formalin, malachite green, chloramine-T and copper sulphate is given. The ectoparasiticides include organophosphates (trichlorfon, dichlorvos, azamethiphos), hydrogen peroxide, ivermectin, cypermethrin and benzyl-ureas (teflubenzuron, diflubenzuron). The short chapter on anthelmintics discusses treatments for trematodes, nematodes and cestodes, and highlights the difficulty of treating some of these parasites.

The concluding part discusses pharmacodynamic agents such as anaesthetics, breeding induction agents, sex hormones, immuno-stimulants, vaccines, osmoregulators and disinfectants. Each of these topics could fill a book in their own right but they have been condensed into a manageable size for practical use. Disinfection is an important aspect of disease prevention and hygiene but rarely discussed so thoroughly in other fish health texts. I was

interested to read that vaccination by anal intubation was more effective than oral administration and I was amused at the thought of the practical aspects of integrating this technique into a smolt production line!

Throughout the book, there are many tables and chemical formulae but only a few illustrations, one of which graphically demonstrates how to remove the pituitary from fish. At the back of the book, there are three appendices, which include a useful index containing the manufacturers and trade names of products formulated for use in fish, scientific and common names of cultured species, and a glossary of terms. An extensive index that spreads across 13 pages is also provided.

The broader the subject becomes, the more exposed a book is to criticism. However, I found very few faults. Although there are references given at the end of each chapter, only a few are inserted into the text itself (including one from an earlier issue of the Fish Veterinary Journal!). There may be sound reasons for this format but readers will occasionally want to go to the original article without having to search through titles in the further reading list. I had to think hard about chemicals that had been omitted from the book and eventually came up with Receptal® (buserelin) licensed in the UK to reduce mortality due to egg binding and to facilitate stripping of rainbow trout. Vitamin C is mentioned as one of the 'most extensively studied' immunostimulants but is not discussed. Similarly, there are only a few passing remarks about potassium permanganate and methylene blue. The glossary could have been expanded to include terms that may be unfamiliar to general practitioners such at T_{max}, C_{max}, $t^{1/2}\gamma$ and several recurring abbreviations.

At first glance, it is difficult to fully appreciate the scale of this book. This is an impressive review to have been written by a sole author and I suspect that future editions will be, by necessity, a multi-author effort. As in other areas of the aquaculture industry, our knowledge of fish pharmacology is expanding rapidly and it is difficult to keep up to date with the constant changes. As a result, some minor details are already outdated, partly because this book was over two years in press, but this does not detract from its long-term value. I would therefore thoroughly recommend this book to anyone who is responsible for prescribing medication to fish.

William H. Wildgoose

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FVS Web Site

The Fish Veterinary Society has a 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.

FISH veterinary SOCIETY

SPONSOR

The Fish Veterinary Society would like to thank the following company which has generously contributed to the cost of publishing this Journal.

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 a result of interested 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 its 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. 4 (September 1999)

Aerobic microflora of imported tropical ornamental fish from Singapore and South America. Part 1: Characterisation. *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)
- Color guide of Tropical Fish Diseases: on freshwater fish (G. Bassleer)
- Self-Assessment Colour Review of Ornamental Fish (G.A. Lewbart)

Issue No. 5 (June 2000)

Dental overgrowth and trimming in a pufferfish. *R. Rees Davies*
Mycobacteriosis: detection and identification of aquatic *Mycobacterium* species. *S. Puttinaowarat, K.D. Thompson & A. Adams*
Fish surgery: an overview. *W.H. Wildgoose*
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An overview of carp diseases in the UK. *D. Bucke*
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Slice®: good news for salmon, bad news for sea lice. *J. McHenery & J.D. Johnson*
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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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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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