

Bacterial Fish Pathogens

Diseases of Farmed and Wild Fish

B. Austin and D. A. Austin

Bacterial Fish Pathogens

Diseases of Farmed and Wild Fish

Fourth Edition

 Springer

Published in association with
Praxis Publishing
Chichester, UK



Professor B. Austin
School of Life Sciences
John Muir Building
Heriot-Watt University
Riccarton
Edinburgh
UK

Dr D. A. Austin
Research Associate
Heriot-Watt University
Riccarton
Edinburgh
UK

SPRINGER-PRAXIS BOOKS IN AQUATIC AND MARINE SCIENCES
SUBJECT *ADVISORY EDITOR*: Dr Peter Dobbins Ph.D., CEng., F.I.O.A., Senior Consultant, Marine Devison,
SEA, Bristol, UK

ISBN 978-1-4020-6068-7 Springer Dordrecht Berlin Heidelberg New York

Springer is part of Springer-Science + Business Media (springer.com)

A catalogue record of this book is available from the Library of Congress

Apart from any fair dealing for the purposes of research or private study, or criticism or review, as permitted under the Copyright, Designs and Patents Act 1988, this publication may only be reproduced, stored or transmitted, in any form or by any means, with the prior permission in writing of the publishers, or in the case of reprographic reproduction in accordance with the terms of licences issued by the Copyright Licensing Agency. Enquiries concerning reproduction outside those terms should be sent to the publishers.

© Praxis Publishing Ltd, Chichester, UK, 2007
Printed in Germany

The use of general descriptive names, registered names, trademarks, etc. in this publication does not imply, even in the absence of a specific statement, that such names are exempt from the relevant protective laws and regulations and therefore free for general use.

Cover design: Jim Wilkie
Project management: Originator Publishing Services Ltd, Gt Yarmouth, Norfolk, UK

Printed on acid-free paper

Contents

- Preface** xv
- List of colour plates**. xix
- List of tables** xxi
- List of abbreviations and acronyms** xxiii
- About the authors** xxvii

- 1 Introduction** 1
 - Conclusion 3

- 2 Characteristics of the diseases**. 15
 - Anaerobes 15
 - Eubacteriaceae representative 15
 - Gram-positive bacteria—the “lactic acid” bacteria 16
 - Enterococcaceae representatives 16
 - Streptococcaceae representatives 16
 - Aerobic, Gram-positive rods and cocci 18
 - Bacillaceae representatives 19
 - Corynebacteriaceae representative 20
 - Micrococcaceae representative 20
 - Mycobacteriaceae representatives 20
 - Nocardiaceae representatives 22
 - Planococcaceae representative 23
 - Staphylococcaceae representatives 23
 - Gram-negative bacteria. 24
 - Aeromonadaceae representatives 24
 - Alteromonadaceae representatives 28
 - Campylobacteriaceae representative 28

Enterobacteriaceae representatives	29
Flavobacteriaceae representatives	33
Francisellaceae representative	34
Halomonadaceae representative	35
Moritellaceae representatives	35
Moraxellaceae representatives	35
Mycoplasmataceae representative	36
Neisseriaceae representative	36
Oxalobacteraceae representative	36
Pasteurellaceae representative	37
Photobacteriaceae representatives	37
Piscirickettsiaceae representative	38
Pseudomonadaceae representatives	39
Vibrionaceae representatives	40
Miscellaneous pathogens.	45
“ <i>Candidatus</i> Arthromitus”	45
Unidentified Gram-negative rods	46
3 Characteristics of the pathogens: Gram-positive bacteria	47
Anaerobes	47
Clostridiaceae representative	48
Eubacteriaceae representative	48
Gram-positive bacteria—the “lactic acid” bacteria	49
Carnobacteriaceae representative	49
Gram-positive cocci in chains	53
General comments	53
Enterococcaceae representatives	56
Streptococcaceae representatives	58
Aerobic Gram-positive rods and cocci	63
Bacillaceae representatives	65
Corynebacteriaceae representatives	67
Coryneform bacteria	68
Micrococcaceae representative	69
Mycobacteriaceae representatives	69
Nocardiaceae representatives	73
Planococcaceae representative	78
Staphylococcaceae representatives	78
Miscellaneous Gram-positive bacterial pathogen	79
“ <i>Candidatus</i> Arthromitus”	79
4 Characteristics of the pathogens: Gram-negative bacteria	81
Aeromonadaceae representatives	81
Alteromonadaceae representative	99
Campylobacteriaceae representative	100
Enterobacteriaceae representatives	101

Flavobacteriaceae representatives	112
Francisellaceae representative	122
Halomonadaceae representative	123
Moraxellaceae representatives	123
Moritellaceae representatives	124
Mycoplasmataceae representative	125
Myxococcaceae representative	126
Oxalobacteriaceae representative	126
Pasteurellaceae representative	127
Photobacteriaceae representatives	127
Piscirickettsiaceae representative	131
Rickettsia-like organisms	132
Pseudomonadaceae representatives	132
Vibrionaceae representatives	136
Miscellaneous pathogens	148
Unnamed bacteria	148
5 Isolation/Detection	151
Anaerobes	155
Clostridiaceae representative	155
Eubacteriaceae representative	155
Gram-positive bacteria—the “lactic acid” bacteria	155
Carnobacteriaceae representatives	155
Enterococcaceae representative	155
Streptococcaceae representatives	156
Aerobic Gram-positive rods and cocci	156
Bacillaceae representatives	158
Corynebacteriaceae representative	159
Micrococcaceae representative	159
Mycobacteriaceae representatives	159
Nocardiaceae representatives	160
Planococcaceae representative	160
Staphylococcaceae representatives	161
Gram-negative bacteria	161
Aeromonadaceae representatives	161
Alteromonadaceae representatives	164
Campylobacteriaceae representative	164
Enterobacteriaceae representatives	164
Flavobacteriaceae representatives	167
Francisellaceae representative	168
Halomonadaceae representative	168
Moraxellaceae representatives	169
Moritellaceae representatives	169
Neisseriaceae representative	169
Oxalobacteriaceae representative	169

Pasteurellaceae representative	169
Photobacteriaceae representatives	170
Piscirickettsiaceae representative	170
Pseudomonadaceae representatives	170
Vibrionaceae representatives	171
Miscellaneous pathogens	173
“ <i>Candidatus</i> Arthromitus”	173
Unidentified Gram-negative rod	174
Appendix 5.1 Media used for the isolation and growth of bacterial fish pathogens	174
6 Diagnosis	185
Gross clinical signs of disease	186
Sluggish behaviour	186
Twirling, spiral or erratic movement	186
Faded pigment	186
Darkened pigment/melanosis	186
Eye damage—exophthalmia (“pop-eye”)/corneal opacity/rupture	190
Haemorrhaging in the eye	190
Haemorrhaging in the mouth	190
Erosion of the jaws/mouth	190
Haemorrhaging in the opercula region/gills	190
Gill damage	190
White nodules on the gills/skin	191
White spots on the head	191
Fin rot/damage	191
Haemorrhaging at the base of fins	191
Haemorrhaging on the fins	191
Tail rot/erosion	191
Saddle-like lesions on the dorsal surface (columnaris, saddleback disease)	191
Distended abdomen	191
Haemorrhaging on the surface and in the muscle	192
Necrotising dermatitis	192
Ulcers	192
External abscesses	192
Furuncles (or boils)	192
Blood-filled blisters on the flank	193
Protruded anus/vent	193
Haemorrhaging around the vent	193
Necrotic lesions on the caudal peduncle	193
Emaciation (this should not be confused with starvation)	193
Inappetence	193
Stunted growth	193
Sloughing off of skin/external surface lesions	193

Dorsal rigidity	194
Internal abnormalities apparent during post-mortem examination . . .	194
Skeletal deformities	194
Gas-filled hollows in the muscle	194
Opacity in the muscle	194
Ascitic fluid in the abdominal cavity	194
Peritonitis	194
Petechial (pin-prick) haemorrhages on the muscle wall	194
Haemorrhaging in the air bladder	195
Liquid in the air bladder	195
White nodules (granulomas) on/in the internal organs	195
Yellowish nodules on the internal organs	195
Nodules in the muscle	195
Swollen and/or watery kidney	195
False membrane over the heart and/or kidney	195
Haemorrhaging/bloody exudate in the peritoneum	195
Swollen intestine, possibly containing yellow or bloody fluid/ gastro-enteritis	198
Intestinal necrosis and opacity	198
Hyperaemic stomach	198
Haemorrhaging in/on the internal organs	198
Brain damage	198
Blood in the cranium	198
Emaciation	198
Pale, elongated/swollen spleen	198
Pale (possibly mottled/dicoloured) liver	199
Yellowish liver (with hyperaemic areas)	199
Swollen liver	199
Generalised liquefaction	199
The presence of tumours	199
Histopathological examination of diseased tissues	199
Bacteriological examination of tissues	200
Tissues to be sampled	200
Culturing <i>Aeromonas salmonicida</i>	200
A special case for diagnosis—BKD	200
A special case— <i>Piscirickettsia salmonis</i>	201
Identification of bacterial isolates	201
Serology	201
Fluorescent antibody technique (FAT)	202
Whole-cell agglutination	203
Precipitin reactions and immunodiffusion	204
Complement fixation	204
Antibody-coated latex particles	204
Co-agglutination with antibody-sensitised staphylococci	205
Passive agglutination	205

Immuno-India ink technique (Geck)	206
Enzyme-linked immunosorbent assay (ELISA)	206
Immunohistochemistry	207
Immunomagnetic separation of antigens	207
Which method is best?—the saga of BKD	207
Which method is best?—furunculosis	210
Molecular techniques	210
Phenotypic tests	215
Colony morphology and pigmentation	231
The Gram-staining reaction	231
The acid-fast staining reaction	231
Motility	232
Gliding motility	232
Filterability through the pores of 0.45 µm pore size porosity filters	232
The ability to grow only in fish cell cultures	232
Aerobic or anaerobic requirements for growth	232
Catalase production	232
Fluorescent (fluorescein) pigment production	232
Growth at 10, 30 and 37°C	232
Growth on 0% and 6.5% (w/v) sodium chloride and on 0.001% (w/v) crystal violet	232
Requirement for 0.1% (w/v) L-cysteine hydrochloride	233
Oxidation–fermentation test	233
Indole production	233
α-Galactosidase production	233
β-Galactosidase production	233
Production of arginine dihydrolase and lysine decarboxylase	233
Urease production	233
Methyl red test and Voges Proskauer reaction	234
Degradation of blood	234
Degradation of gelatin	234
Degradation of starch	234
Acid production from maltose and sorbitol	234
Production of hydrogen sulphide	234
Coagulase test	235
Other techniques	235
7 Epizootiology: Gram-positive bacteria	237
Anaerobes	237
Clostridiaceae representative	237
Eubacteriaceae representative	238
Gram-positive bacteria—the “lactic acid” bacteria	238
Carnobacteriaceae representative	238
Streptococcaceae representatives	238
Aerobic Gram-positive rods and cocci	239

	Corynebacteriaceae representative	242
	Mycobacteriaceae representatives	242
	Nocardiaceae representatives	242
	Staphylococcaceae representatives	243
	“ <i>Candidatus</i> Arthromitus”	243
8	Epizootiology: Gram-negative bacteria	245
	Aeromonadaceae representatives	245
	Alteromonadaceae representative	268
	Enterobacteriaceae representatives	268
	Flavobacteriaceae representatives	272
	Halomonadaceae representative	275
	Moraxellaceae representatives	275
	Mycoplasmataceae representative	275
	Oxalobacteriaceae representative	276
	Pasteurellaceae representative	276
	Photobacteriaceae representatives	276
	Piscirickettsiaceae representative	277
	Pseudomonadaceae representatives	277
	Vibrionaceae representatives	279
	Miscellaneous pathogen	282
	Causal agent of <i>Varracalbmi</i>	282
9	Pathogenicity	283
	Anaerobes	283
	Eubacteriaceae representative	283
	Gram-positive bacteria—the “lactic acid” bacteria	284
	Carnobacteriaceae representatives	284
	Enterococcaceae representatives	284
	Streptococcaceae representatives	284
	Aerobic Gram-positive rods and cocci	285
	Bacillaceae representatives	287
	Corynebacteriaceae representative	288
	Coryneforms	288
	Micrococcaceae representative	288
	Mycobacteriaceae representatives	288
	Nocardiaceae representatives	289
	Planococcaceae representative	289
	Staphylococcaceae representatives	290
	Gram-negative bacteria	290
	Aeromonadaceae representatives	290
	Alteromonadaceae representatives	312
	Campylobacteriaceae representative	312
	Enterobacteriaceae representatives	313
	Flavobacteriaceae representatives	319

Francisellaceae representative	322
Halomonadaceae representative	322
Moraxellaceae representatives	322
Moritellaceae representatives	323
Neisseriaceae representative	323
Oxalobacteriaceae representative	323
Pasteurellaceae representative	323
Photobacteriaceae representatives	324
Piscirickettsiaceae representative	326
Pseudomonadaceae representatives	326
Vibrionaceae representatives	328
Miscellaneous pathogens	334
“ <i>Candidatus</i> Arthromitus”	334
Unknown Gram-negative rod	335
10 Control	337
Wild fish stocks	337
Farmed fish	338
Husbandry	338
Genetically resistant stock	339
Adequate diets/dietary supplements	341
Vaccines	344
Composition of bacterial fish vaccines	345
Methods of vaccine inactivation	345
Methods of administering vaccines to fish	346
Vaccine development programmes: Gram-positive bacteria	347
Streptococcaceae representatives	347
Vaccine development programmes: Aerobic Gram-positive rods and cocci	348
Mycobacteriaceae representatives	349
Nocardiaceae representatives	349
Vaccine development programmes: Gram-negative bacteria	350
Aeromonadaceae representatives	350
Alteromonadaceae representative	365
Enterobacteriaceae representatives	365
Flavobacteriaceae representatives	368
Moritellaceae representative	370
Photobacteriaceae representative	370
Piscirickettsiaceae representative	371
Pseudomonadaceae representatives	371
Vibrionaceae representatives	372
Non-specific immunostimulants	378
Antimicrobial compounds	379
Chemotherapy development programmes: Anaerobes	385
Eubacteriaceae representative	385

Chemotherapy development programmes: Gram-positive bacteria . . .	386
Carnobacteriaceae representatives	386
Enterococcaceae representatives	386
Streptococcaceae representatives	386
Chemotherapy development programmes: Aerobic Gram-positive rods and cocci	387
Bacillaceae representatives	388
Corynebacteriaceae representative	388
Micrococcaceae representative	389
Mycobacteriaceae representatives	389
Nocardiaceae representatives	389
Planococcaceae representative	389
Staphylococcaceae representatives	390
Chemotherapy development programmes: Gram-negative bacteria . . .	390
Aeromonadaceae representatives	390
Campylobacteriaceae representative	393
Enterobacteriaceae representatives	393
Flavobacteriaceae representatives	395
Moraxellaceae representatives	397
Moritellaceae representative	397
Oxalobacteriaceae representative	397
Photobacteriaceae representative	398
Piscirickettsiaceae representative	398
Pseudomonadaceae representatives	398
Vibrionaceae representatives	399
Miscellaneous pathogens	400
Unknown Gram-negative rod	400
Disinfection/water treatments	401
Preventing the movement and/or slaughtering of infected stock	402
Probiotics/biological control	403
Inhibitors of quorum-sensing	404
11 Conclusions	405
Recognition of emerging conditions	405
Taxonomy and diagnosis	405
Isolation and selective isolation of pathogens	406
Ecology (epizootiology)	406
Pathogenicity mechanisms	406
Control measures	407
The effects of pollution	407
Zoonoses	408
Bibliography	413
Index	545

Preface

This fourth edition of *Bacterial Fish Pathogens* is the successor to the original version, first published by Ellis Horwood Limited in 1987, and was planned to fill the need for an up-to-date comprehensive text on the biological aspects of the bacterial taxa which cause disease in fish. The impetus to prepare a fourth edition stemmed initially from discussion with Chinese colleagues when it became apparent that the book was particularly well used and cited (>1,600 citations in China since 1999). Since publishing the third edition, there has been a slowing down in the number of new fish pathogens. However, there has been a steady increase in the number of publications about some aspects of bacterial fish pathogens, including the application of molecular techniques to diagnosis and pathogenicity studies. Consequently, we considered that it is timely to consider the new information in a new edition. The task was made immeasurably easier by the ready availability of electronic journals, which could be accessed from the office. Weeks of waiting for inter-library loans did not feature during the research phase of the project. Our strategy was to include information on new pathogens and new developments on well-established pathogens, such as *Aeromonas salmonicida* and *Vibrio anguillarum*. Because of the deluge of new information, we have needed to be selective, and in particular, we have once again condensed details of the pathology of the diseases, because there are excellent texts already available that cover detailed aspects of the pathological conditions. Nevertheless, this fourth edition will hopefully meet the needs of the readership. As with all the preceding editions, it is emphasised that most of the information still appertains to diseases of farmed, rather than wild, fish.

The scope of the book covers all of the bacterial taxa that have at one time or another been reported as fish pathogens. Of course, it is realised that some taxa are merely secondary invaders of already damaged tissues, whereas others comprise serious, primary pathogens. Shortcomings in the literature or gaps in the overall understanding of the subject have been highlighted.

In preparing the text, we have sought both advice and material from colleagues. We are especially grateful to the following for the supply of photographs:

Dr. J.W. Brunt
Dr. H. Daskalov
Dr. G. Dear
Dr. T. Itano
Dr. V. Jencic
Dr. D.-H. Kim
Dr. A. Newaj-Fyzul
Dr. N. Pieters
Professor M. Sakai
Professor X.-H. Zhang

B. and D. A. Austin
Edinburgh, 2007

To Aurelia Jean

Colour plates

(see colour section between pp. 236 and 237)

- 4.1 *Aer. salmonicida* subsp. *salmonicida* producing brown, diffusible pigment around the colonies on TSA
- 6.1 The rainbow trout on the left has bilateral exophthalmia caused by *Ren. salmoninarum*. The second fish is a healthy specimen
- 6.2 A rainbow trout displaying haemorrhaging in the eye caused by infection with *Lactococcus garvieae*
- 6.3 A rainbow trout displaying extensive haemorrhaging in the mouth caused by ERM
- 6.4 A tilapia displaying haemorrhaging around the mouth caused by infection with *Aeromonas* sp.
- 6.5 Erosion of the mouth of a ghost carp. The aetiological causal agent was *Aer. bestiarum*
- 6.6 Erosion of the mouth of a carp. The aetiological causal agent was *Aer. bestiarum*
- 6.7 Erosion and haemorrhaging of the mouth of a ghost carp. The aetiological causal agent was *Aer. bestiarum*
- 6.8 A tilapia displaying haemorrhaging on the finnage caused by infection with *Aeromonas* sp.
- 6.9 Extensive erosion of the tail and fins on a rainbow trout. Also, there is some evidence for the presence of gill disease. The aetiological agent was *Aer. hydrophila*
- 6.10 A saddleback lesion characteristic of columnaris (causal agent = *Fla. columnare*) on a rainbow trout
- 6.11 A distended abdomen on a rainbow trout with BKD
- 6.12 Surface haemorrhaging and mouth erosion on a carp which was infected with *Aer. bestiarum*
- 6.13 Haemorrhagic lesions on the surface of a carp which was infected with *Aer. hydrophila*
- 6.14 Surface haemorrhaging on a tongue sole (*Cynoglossus semilaevis*) infected with *Edw. tarda*
- 6.15 Petechial haemorrhages on the surface of an eel with Sekiten-byo

- 6.16 Surface haemorrhaging on a grayling infected with BKD
- 6.17 Extensive surface haemorrhaging on a turbot with vibriosis
- 6.18 Haemorrhaging on the fins and around the opercula of a sea bass. The aetiological agent was *V. anguillarum*
- 6.19 An ulcer in its early stage of development on a Koi carp. The aetiological agent was atypical *Aer. salmonicida*
- 6.20 A well-developed ulcer on a Koi carp. The aetiological agent was atypical *Aer. salmonicida*
- 6.21 An ulcerated goldfish on which the lesion has extended across the body wall, exposing the underlying organs. The aetiological agent was atypical *Aer. salmonicida*
- 6.22 Carp erythrodermatitis. The aetiological agent is likely to be atypical *Aer. salmonicida*
- 6.23 An ulcer, caused by *Vibrio* sp., on the surface of olive flounder
- 6.24 Limited tail erosion and an ulcer on the flank of rainbow trout. The casual agent was considered to be linked to ultramicrobacteria
- 6.25 An extensive abscess with associated muscle liquefaction in the musculature of rainbow trout. The aetiological agent was *Aer. hydrophila*
- 6.26 A dissected abscess on a rainbow trout revealing liquefaction of the muscle and haemorrhaging. The aetiological agent was *Aer. hydrophila*
- 6.27 A furuncle, which is attributable to *Aer. salmonicida* subsp. *salmonicida*, on the surface of a rainbow trout
- 6.28 A dissected furuncle on a rainbow trout revealing liquefaction of the muscle
- 6.29 A blood blister on the surface of a rainbow trout with BKD
- 6.30 Extensive skin erosion around the tail of a rainbow trout. The cause of the condition was not proven
- 6.31 Mycobacteriosis in yellowtail. Extensive granulomas are present on the liver and kidney
- 6.32 Nocardiosis in yellowtail. Extensive granulomas are present on the liver and kidney
- 6.33 Swollen kidneys associated with BKD
- 6.34 Generalised liquefaction of a rainbow trout associated with infection by *Aeromonas*
- 6.35 An API-20E strip after inoculation, incubation and the addition of reagents. The organism was a suspected *Aeromonas*
- 6.36 An API-zym strip after inoculation, incubation and the addition of reagents. The organism is the type strain of *Ren. salmoninarum*
- 11.1 Red mark disease syndrome (= winter strawberry disease) in rainbow trout. The skin lesions do not usually penetrate to the underlying muscle
- 11.2 Red mark disease syndrome (= winter strawberry disease) in rainbow trout. With this form of the condition, scales and epidermal cells have been sloughed off
- 11.3 Red mark disease syndrome (= winter strawberry disease) in rainbow trout. The reddening is often seen in fish of >500 g in weight
- 11.4 The reddened area associated with red mark disease syndrome (= winter strawberry disease) in >500 g rainbow trout
- 11.5 The reddened area around the vent associated with red mark disease syndrome (= winter strawberry disease) in >500 g rainbow trout.

Tables

1.1	Bacterial pathogens of freshwater and marine fish,	4
3.1	Comparison of <i>Eubacterium limosum</i> with <i>Eu. tarantellae</i>	50
3.2	Characteristics of fish-pathogenic lactobacilli	51
3.3	Characteristics of fish-pathogenic lactobacilli and streptococci.	54
3.4	Characteristics of <i>Renibacterium salmoninarum</i>	66
3.5	Characteristics of nocardias	75
4.1	Characteristics of <i>Aeromonas salmonicida</i>	87
4.2	Characteristics of <i>Edwardsiella tarda</i> and <i>Paracolobactrum anguillimortiferum</i>	104
4.3	Differential characteristics of <i>J. lividum</i> recovered from moribund and dead rainbow trout fry	128
5.1	Methods of isolation for bacterial fish pathogens	152
6.1	External signs of disease associated with the bacterial fish pathogens	187
6.2	Internal signs of disease.	196
6.3	Profiles of fish pathogens obtained with the API 20E rapid identification system	217
6.4	Differential characteristics of some fish pathogens obtained with the API 20NE rapid identification system	219
6.5	Distinguishing profiles of Gram-positive bacteria as obtained with API zym	220
6.6	Characteristics of selected taxa by Biolog-GN	222
6.7	Diagnostic traits of the Gram-positive bacterial fish pathogens	225
6.8	Diagnostic traits of the Gram-negative bacterial fish pathogens.	227
8.1	Experimental data concerning the survival of <i>A. salmonicida</i> in water	250
10.1	Methods of controlling bacterial fish diseases.	338
10.2	Composition of the purified basal medium to which different concentrations of vitamin C at 0–150 mg/kg were added.	342
10.3	Vaccines for <i>A. salmonicida</i>	354
10.4	Methods for application of antimicrobial compounds to fish.	381
10.5	Methods of administering commonly used antimicrobial compounds to fish .	382

Abbreviations and acronyms

<i>Aer.</i>	<i>Aeromonas</i>
AFLP	Amplified Fragment Length Polymorphism
AHL	Acylated Homoserine Lactone
A-layer	The additional surface layer of <i>Aer. salmonicida</i>
<i>Arc.</i>	<i>Arcobacter</i>
ARISA	Automated Ribosome Intergenic Spacer Analysis
ATCC	American Type Culture Collection, Rockville, Maryland
BHI	Brain Heart Infusion
BHIA	Brain Heart Infusion Agar
BKD	Bacterial Kidney Disease
BLIS	Bacteriocin-Like Substance
BMA	Basal Marine Agar
bp	base pair
<i>Car.</i>	<i>Carnobacterium</i>
CBB	Coomassie Brilliant Blue agar
CDC	Centers for Disease Control and Prevention, Atlanta, Georgia
CE	Carp Erythrodermatitis
CFU	Colony-Forming Unit
CgP	Cytidine-phosphate-Guanosine
<i>Chrys.</i>	<i>Chryseobacterium</i>
CHSE-214	CHinook Salmon Embryo 214 cell line
<i>Cit.</i>	<i>Citrobacter</i>
<i>Cl.</i>	<i>Clostridium</i>
CLB	<i>Cytophaga</i> -Like Bacteria
CLED	Cystine Lactose Electrolyte-Deficient agar
<i>Cor.</i>	<i>Corynebacterium</i>
CpG	Cytidine-phosphate-Guanosine

xxiv **Abbreviations and acronyms**

<i>Cyt.</i>	<i>Cytophaga</i>
DNA	DeoxyriboNucleic Acid
ECP	ExtraCellular Product
EDTA	Ethylene Diamine Tetraacetic Acid
<i>Edw.</i>	<i>Edwardsiella</i>
ELISA	Enzyme-Linked ImmunoSorbent Assay
<i>En.</i>	<i>Enterococcus</i>
<i>Ent.</i>	<i>Enterobacter</i>
EPC	Epithelioma Papulosum Cyprini (cell line)
ERM	Enteric RedMouth
<i>Esch.</i>	<i>Escherichia</i>
<i>Eu.</i>	<i>Eubacterium</i>
FAME	Fatty Acid Methyl Ester
FAT	Fluorescent Antibody Test
FCA	Freund's Complete Adjuvant
FIA	Freund's Incomplete Adjuvant
<i>Fla.</i>	<i>Flavobacterium</i>
<i>Fle.</i>	<i>Flexibacter</i>
G + C	Guanine plus Cytosine
GCAT	Glycerophospholipid : Cholesterol AcylTransferase
GFP	Green Fluorescent Protein
GMD	Glucose Motility Deeps
<i>H.</i>	<i>Haemophilus</i>
<i>Haf.</i>	<i>Hafnia</i>
HG	Hybridisation Group
hsp	heat shock protein
i.m.	intramuscular
i.p.	intrapерitoneal
iFAT	indirect Fluorescent Antibody Test
IROMP	Iron-Regulated Outer Membrane Protein
ISR	Intergenic Spacer Region
IU	International unit
<i>J.</i>	<i>Janthinobacterium</i>
kb	kilobase
kDa	kiloDalton
KDM2	Kidney Disease Medium 2
LAMP	Loop-mediated isothermal AMPLification
LD ₁₀₀	Lethal Dose 100%
LD ₅₀	Lethal Dose 50%, i.e. the dose needed to kill 50% of the population
<i>Lis.</i>	<i>Listeria</i>
LPS	LipoPolySaccharide
MDa	megaDalton
MHC	Mueller–Hinton agar supplemented with 0.1% (w/v) L-cysteine hydrochloride

MIC	Minimum Inhibitory Concentration
MIS	Microbial Identification System
<i>Mor.</i>	<i>Moraxella</i>
mRNA	messenger RNA
MRVP	Methyl Red Voges Proskauer
<i>msa</i>	major soluble antigen (gene)
MSS	Marine Salts Solution
<i>Myc.</i>	<i>Mycobacterium</i>
NCBV	Non-Culturable But Viable
NCIMB	National Collection of Industrial and Marine Bacteria, Aberdeen, Scotland
<i>Nec.</i>	<i>Necromonas</i>
<i>Noc.</i>	<i>Nocardia</i>
ODN	OligoDeoxyNucleotide
OMP	Outer Membrane Protein
ORF	Open Reading Frame
p57	57 kDa protein (of <i>Ren. salmoninarum</i>)
<i>Pa.</i>	<i>Pasteurella</i>
PAGE	PolyAcrylamide Gel Electrophoresis
PAP	Peroxidase–AntiPeroxidase enzyme immunoassay
PBS	Phosphate-Buffered Saline
PCR	Polymerase Chain Reaction
PFGE	Pulsed-Field Gel Electrophoresis
PFU	Plaque Forming Unit
<i>Ph.</i>	<i>Photobacterium</i>
PMSF	PhenylMethyl–Sulphonyl Fluoride
<i>Pr.</i>	<i>Providencia</i>
<i>Ps.</i>	<i>Pseudomonas</i>
QPCR	Quantitative Polymerase Chain Reaction
RAPD	Randomly Amplified Polymorphic DNA
<i>Ren.</i>	<i>Renibacterium</i>
RFLP	Restriction Fragment Length Polymorphism
RLO	<i>Rickettsia</i> -Like Organisms
ROS	Reactive Oxygen Species
RPS	Relative Percent Survival
rRNA	ribosomal RiboNucleic Acid
RT-PCR	Reverse Transcriptase Polymerase Chain Reaction
RTFS	Rainbow Trout Fry Syndrome
RTG-2	Rainbow Trout Gonad-2 cell line
<i>Sal.</i>	<i>Salmonella</i>
SBL	Striped Bass Larvae
S _D	Dice coefficient
S-layer	Surface layer
SDS	Sodium Dodecyl Sulphate
<i>Ser.</i>	<i>Serratia</i>

xxvi Abbreviations and acronyms

SKDM	Selective Kidney Disease Medium
SSH	Suppression Subtractive Hybridisation
<i>Sta.</i>	<i>Staphylococcus</i>
<i>Str.</i>	<i>Streptococcus</i>
TCBS	Thiosulphate Citrate Bile Salts Sucrose Agar
TCID	Tissue Culture Infectivity Dose
TSA	Tryptone Soya Agar
TSB	Tryptone Soya Broth
<i>V.</i>	<i>Vibrio</i>
<i>Vag.</i>	<i>Vagococcus</i>
VAM	<i>Vibrio Anguillarum</i> Medium
<i>vapA</i>	virulence array protein gene A
VHH	<i>Vibrio harveyi</i> Haemolysin
VHML	<i>Vibrio harveyi</i> Myovirus-Like (bacteriophage)
<i>Y.</i>	<i>Yersinia</i>

About the authors

Brian Austin is Dean of the University (Science and Engineering) and Professor of Microbiology in the School of Life Sciences, Heriot-Watt University. From 1975 to 1978 he was Research Associate at the University of Maryland, U.S.A., and from 1978 to 1984 he was Head of Bacteriology at the Fish Diseases Laboratory in Weymouth, U.K. He joined Heriot-Watt University as a Lecturer in Aquatic Microbiology in 1984.

Professor Austin gained a B.Sc. (1972) in Microbiology, a Ph.D. (1975) also in Microbiology, both from the University of Newcastle upon Tyne, and a D.Sc. (1992) from Heriot-Watt University. He was elected F.R.S.A. and Fellow of the American Academy of Microbiology, and is a member of the American Society of Microbiology, Society of Applied Bacteriology, Society of General Microbiology, European Association of Fish Pathologists, and the U.K. Federation of Culture Collections; and has written previous books on bacterial taxonomy, marine microbiology, methods in aquatic bacteriology, methods for the microbiological examination of fish and shellfish, and pathogens in the environment.

Dawn Austin is a Research Associate at Heriot-Watt University, a position she has held since 1986. Prior to this she was Research Assistant at the University of Maryland (1977–1979), Lecturer in Microbiology, University of Surrey (1983–1984), and Research Fellow of the Freshwater Biological Association, The River Laboratory, Dorset (1984–85).

Dr Austin gained a B.S. (1974) from City College, The City University, New York; an M.S. (1979) and a Ph.D. (1982) both from the University of Maryland.

1

Introduction

Representatives of many bacterial taxa have, at one time or another, been associated with fish diseases. However, not all of these bacteria constitute primary pathogens. Many should be categorised as opportunistic pathogens, which colonise and cause disease in already damaged hosts. Here, the initial weakening process may involve pollution or a natural physiological state (e.g. during the reproductive phase) in the life cycle of the fish. There remains doubt about whether some bacteria should be considered as fish pathogens. In such cases, the supportive evidence is weak or non-existent. Possibly, such organisms constitute contaminants or even innocent saprophytes. However, it is readily apparent that there is great confusion about the precise meaning of disease. A definition, from the medical literature, states that:

“... a disease is the sum of the abnormal phenomena displayed by a group of living organisms in association with a specified common characteristic or set of characteristics by which they differ from the norm of their species in such a way as to place them at a biological disadvantage ...”

(Campbell *et al.*, 1979)

This definition is certainly complex, and the average reader may be excused for being only a little wiser about its actual meaning. Dictionary definitions of disease are more concise, and include “an unhealthy condition” and “infection with a pathogen [= something that causes a disease]”. One conclusion is that disease is a complex phenomenon, leading to some form of measurable damage to the host. Yet, it is anticipated that there might be profound differences between scientists about just what constitutes a disease. Fortunately, infection by micro-organisms is one aspect of disease that finds ready acceptance within the general category of disease.

For his detailed treatise on diseases of marine animals, Kinne (1980) considered that disease may be caused by:

2 Introduction

- genetic disorders;
- physical injury;
- nutritional imbalance;
- pathogens;
- pollution.

This list of possible causes illustrates the complexity of disease. An initial conclusion is that disease may result from biological (= *biotic*) factors, such as pathogens, and *abiotic* causes, e.g. the emotive issue of pollution. Disease may also be categorised in terms of epizootiology (Kinne, 1980), namely as:

- *Sporadic* diseases, which occur sporadically in comparatively small numbers of a fish population.
- *Epizootics*, which are large-scale outbreaks of communicable disease occurring temporarily in limited geographical areas.
- *Pan-zootics*, which are large-scale outbreaks of communicable disease occurring over large geographical areas.
- *Enzootics*, which are diseases persisting or re-occurring as low-level outbreaks in certain areas.

The study of fish diseases has concentrated on problems in fish farms (= aquaculture), where outbreaks either begin suddenly, progress rapidly often with high mortalities, and disappear with equal rapidity (= *acute* disease) or develop more slowly with less severity, but persist for greater periods (= *chronic* disease).

This text will deal with the diseases caused by bacteria. However, it is relevant to emphasise that disease is not necessarily caused by single bacterial taxa. Instead, there may well be synergistic interactions between two or more taxa. This possibility is often ignored by scientists. Then, there are the situations in which infectious diseases are suspected but not proven. An example includes red mark syndrome/disease (also known as winter strawberry disease) of rainbow trout in the U.K. where the causal agent is suspected—but not proven—to be bacterial of which *Fla. psychrophilum* or *Aer. hydrophila* are suspected to be the possible aetiological agent.

Disease is usually the outcome of an interaction between the host (= fish), the disease-causing situation (= pathogen) and external stressor(s) (= unsuitable changes in the environment; poor hygiene; stress). Before the occurrence of clinical signs of disease, there may be demonstrable damage to/weakening of the host. Yet all too often, the isolation of bacteria from an obviously diseased fish is taken as evidence of infection. Koch's Postulates may be conveniently forgotten.

So, what are the bacterial fish pathogens? A comprehensive list of all the bacteria, which have been considered to represent fish pathogens, has been included in Table 1.1 (see p. 4). Some genera, e.g. *Vibrio*, include many species that are acknowledged to be pathogens of freshwater and/or marine fish species. Taxa (highlighted by quotation marks), namely "*Catenabacterium*", "*H. piscium*" and "*Myxobacterium*" are of doubtful taxonomic validity. Others, such as *Pr. rettgeri* and *Sta. epidermidis*, are of questionable significance in fish pathology insofar as their recovery from diseased

animals has been sporadic. A heretical view would be that enteric bacteria (e.g., *Providencia*), comprise contaminants from water or from the gastro-intestinal tract of aquatic or terrestrial animals. Many of the bacterial pathogens are members of the normal microflora of water and/or fish. Others have been associated only with clinically diseased or covertly infected (asymptomatic) fish. Examples of these “obligate” pathogens include *Aer. salmonicida* and *Ren. salmoninarum*, the causal agents of furunculosis and bacterial kidney disease (BKD), respectively. In later chapters, it will be questioned whether or not bacteria should be considered as obligate pathogens of fish, at all. It is a personal view that the inability to isolate an organism from the aquatic environment may well reflect inadequate recovery procedures. Could the organism be dormant/damaged/senescent in the aquatic ecosystem; a concept which has been put forward for other water-borne organisms (Stevenson, 1978)?

It is undesirable that any commercially important species should suffer the problems of disease. Unfortunately, the aetiology of bacterial diseases in the wild is often improperly understood. Moreover, it seems that little if anything may be done to aid wild fish stocks, except, perhaps, by controlling pollution of the rivers and seas, assuming that when environmental quality deteriorates this influences disease cycles. In contrast, much effort has been devoted to controlling diseases of farmed fish.

Conclusion

- The list of fish pathogens has extended substantially since 1980. Current interest focuses on the vibrios, CLBs, mycobacteria and streptococci–lactococci.
- A question mark hangs over the significance of some organisms to fish pathology—are they truly pathogens or chance contaminants?
- There has been considerable improvement in the taxonomy of some groups (e.g., vibrios).
- There has been a shift from emphasis on culture-dependent to culture-independent techniques.
- Molecular methods have become commonplace in laboratories involved in the study of fish diseases.

Table I.1. Bacterial pathogens of freshwater and marine fish

Pathogen	Disease	Host range	Geographical distribution
ANAEROBES "Catenabacterium" sp.	—	Grey mullet (<i>Mugil auratus</i>)	U.S.A.
Clostridiaceae representative <i>Clostridium botulinum</i>	Botulism	Redfish (<i>Sebastes</i> sp.) Salmonids	Denmark, England, U.S.A.
Eubacteriaceae representative <i>Eubacterium tarantellae</i>	Eubacterial meningitis	Striped mullet (<i>Mugil cephalus</i>)	U.S.A.
GRAM-POSITIVE BACTERIA— THE "LACTIC ACID" BACTERIA Carnobacteriaceae representative <i>Carnobacterium piscicola</i>	Lactobacillosis, pseudokidney disease	Salmonids	North America, U.K.
Enterococcaceae representatives <i>Enterococcus</i> (<i>Streptococcus</i>) <i>faecalis</i> subsp. <i>liquefaciens</i> <i>Vagococcus salmoninarum</i>	—	Rainbow trout (<i>Oncorhynchus mykiss</i>), catfish Atlantic salmon (<i>Salmo salar</i>), brown trout (<i>Salmo trutta</i>), rainbow trout	Italy Australia, France, North America
Lactobacillaceae representative <i>Lactobacillus</i> spp.	Lactobacillosis, pseudokidney disease	Salmonids	North America, U.K.
Streptococcaceae representatives <i>Lactococcus garvieae</i> (= <i>Enterococcus</i> <i>seriolicida</i>)	Streptococcicosis/ streptococcosis	Many fish species	Australia, Europe, Israel, Japan, Saudi Arabia, Red Sea, South Africa, Taiwan, U.S.A.

<i>Lactococcus piscium</i>	Lactobacillosis, pseudokidney disease	Rainbow trout	North America
<i>Streptococcus dysgalactiae</i>	—	Amberjack (<i>Seriola dumerili</i>), yellowtail (<i>Seriola quinqueradiata</i>)	Japan
<i>Streptococcus difficilis</i> (= <i>Str. agalactiae</i>)	Meningo-encephalitis	Carp (<i>Cyprinus carpio</i>), rainbow trout, silver pomfret (<i>Pampus argenteus</i>), tilapia (<i>Oreochromis</i> spp.)	Israel, Kuwait, USA
<i>Streptococcus iniae</i> (= <i>Str. shiloi</i>)	Meningo-encephalitis, streptococcosis/streptococcosis	Various freshwater and coastal fish species	Australia, Bahrain, Europe, Israel, Japan, Saudi Arabia, South Africa, U.S.A.
<i>Streptococcus milleri</i>	—	Koi carp (<i>Cyprinus carpio</i>)	U.K.
<i>Streptococcus parauberis</i>	Streptococcosis/streptococcosis	Turbot (<i>Scophthalmus maximus</i>)	Spain
AEROBIC GRAM-POSITIVE RODS AND COCCI			
<i>Renibacterium salmoninarum</i>	Bacterial kidney disease (BKD; Dee disease; corynebacterial kidney disease)	Salmonids	Europe, Japan, North and South America
Bacillaceae representatives <i>Bacillus</i> spp.	Septicaemia; bacillary necrosis	Various freshwater fish species including catfish (<i>Pangasius hypophthalmus</i>)	Nigeria, Vietnam
<i>Bacillus cereus</i>	Branchio-necrosis	Carp (<i>Cyprinus</i> sp.), striped bass (<i>Morone saxatilis</i>)	U.S.A.
<i>Bacillus mycooides</i>	Ulceration	Channel catfish (<i>Ictalurus punctatus</i>)	Poland, U.S.A.
<i>Bacillus subtilis</i>	Branchio-necrosis	Carp	Poland

(continued)

Table 1.1 (cont.)

Pathogen	Disease	Host range	Geographical distribution
Corynebacteriaceae representatives <i>Corynebacterium aquaticum</i>	Exophthalmia	Striped bass	U.S.A.
Coryneform bacteria	“Corynebacteriosis”	Salmonids	England
Micrococcaceae representative <i>Micrococcus luteus</i>	Micrococcosis	Rainbow trout	England
Mycobacteriaceae representatives <i>Mycobacterium</i> spp. (<i>Myc. abscessus</i> , <i>Myc. anabanti</i> , <i>Myc. chelonae</i> subsp. <i>piscarium</i> , <i>Myc. fortuitum</i> , <i>Myc. goodii</i> , <i>Myc. marinum</i> , <i>Myc. montefiorensis</i> , <i>Myc. neoaurum</i> , “ <i>Myc. piscium</i> ”, “ <i>Myc. platypocillus</i> ”, <i>Myc. pseudoshottisii</i> , “ <i>Myc. ranae</i> ”, “ <i>Myc. salmoniphilum</i> ”, <i>Myc. shottisii</i> , <i>Myc. scrofulaceum</i> , <i>Myc. simiae</i> , <i>Myc. smegmatis</i> , <i>Myc. ulcerans</i>)	Mycobacteriosis (fish tuberculosis)	Most fish species	Worldwide
Nocardiaceae representatives <i>Nocardia</i> spp. (<i>Noc. asteroides</i> , <i>Noc.</i> <i>salmonicida</i> , <i>Noc. seriolae</i>)	Nocardiosis	Most fish species	Worldwide
<i>Rhodococcus</i> sp.	Ocular oedema	Chinook salmon (<i>O. tshawytscha</i>)	Canada
<i>Rhodococcus erythropolis</i>	?	Atlantic salmon	Norway, Scotland
Planococcaceae representative <i>Planococcus</i> sp.	—	Salmonids	England

Staphylococaceae representatives					
<i>Staphylococcus aureus</i>	Eye disease	Silver carp (<i>Hypophthalmichthys molitrix</i>)	India		
<i>Staphylococcus epidermidis</i>	—	Gilthead sea bream (<i>Sparus aurata</i>), red sea bream (<i>Chrysophrus major</i>), yellowtail (<i>Seriola quinqueradiata</i>)	Japan, Turkey		
<i>Staphylococcus warneri</i>	Ulcerations	Rainbow trout	Spain		
GRAM-NEGATIVE BACTERIA					
Aeromonadaceae representatives					
<i>Aeromonas allosaccharophila</i>	—	Elvers	Spain		
<i>Aeromonas bestiarum</i>	—		U.S.A.		
<i>Aeromonas caviae</i>	Septicaemia	Atlantic salmon (<i>Salmo salar</i>)	Turkey		
<i>Aeromonas hydrophila</i> (= <i>Aer. liquefaciens</i> , <i>Aer. punctata</i>)	Haemorrhagic septicaemia, motile aeromonas septicaemia, redsores disease, fin rot	Many freshwater fish species	Worldwide		
<i>Aeromonas jandaei</i>	—	Eel (<i>Anguilla</i> sp.)	Spain		
<i>Aeromonas salmonicida</i> (subspecies <i>achromogenes</i> , <i>masoucida</i> , <i>salmonicida</i> and <i>smithia</i>) (= <i>Haemophilus piscium</i>)	Furunculosis, carp erythrodermatitis, ulcer disease	Salmonids, cyprinids, and marine species (dabs, cod)	Worldwide		
<i>Aeromonas sobria</i>	—	Perch (<i>Perca fluviatilis</i>), gizzard shad (<i>Dorosoma cepedianum</i>)	Switzerland, U.S.A.		
<i>Aeromonas veronii</i> biovar <i>sobria</i>	Epizootic ulcerative syndrome	African catfish (<i>Clarias gariepinus</i>), rajputi (<i>Puntius gonionotus</i>), rui (<i>Labeo rohita</i>), catla (<i>Catla catla</i>), shole (<i>Channa striatus</i>)	Bangladesh		

(continued)