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REVIEW

Methods for the diagnosis of bacterial fish diseases

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Abstract
The diagnosis of bacterial fish diseases has progressed from traditional culture-dependent methods involving the recovery of pathogens on agar-containing media and identification by examination of phenotypic traits. Newer approaches centre on culture-independent approaches. A problem with culturing is that it lacks sensitivity, tends to be slow, and its success depends on the composition of the media and incubation conditions employed. In contrast, culture-independent methods, now centring on molecular methods, are highly specific and sensitive. This raises an important issue that detection of very low numbers of bacterial cells does not necessarily imply the presence of clinical disease. Positivity could reflect background populations of the pathogen that may be present in the aquatic environment.

Keywords Culturing · Phenotyping · Serology · Molecular methods · Rapid techniques

Introduction

A diverse range of Gram-positive and Gram-negative bacteria have been associated with diseases of marine fish, worldwide (Table 1). Overall, greater attention has been focused on aquaculture rather than wild stocks. The pathogens have been associated with a wide range of clinical manifestations including ulcerations, swellings, erosions and haemorrhagic septicaemias. The diagnostic procedures have been summarised in Table 2 and Fig. 1. Unlike other veterinary and medical counterparts, diagnosis centres on the identification of the pathogen rather than the immediate control of the disease (Austin and Austin 2016). Inevitably, this process takes longer than, for example, a trip to the human physician. Here, diagnosis may result initially from consideration of the gross clinical signs on the patient leading to the rapid implementation of a treatment regime. Certainly, fish disease diagnosis has undergone a transformation. Since the end of the twentieth century, there has been a move away from the traditional histology and culture-dependent approaches. The latter involved the acquisition of cultures and their time-consuming identification. Subsequently, attention has been focused on culture-independent techniques, notably those embracing developments in molecular biology. A topical example is sequencing of the 16S rRNA gene, which does not need intact, viable bacterial cells. The advantage to these culture-independent approaches is speed and accuracy; the bacteria may be studied and identified regardless of whether or not they may be grown in the laboratory. Moreover, there is a very high level of specificity, and this is important when diagnosing disease (Austin 2017).

Culture-dependent techniques

A traditional approach to bacterial fish disease diagnosis centred on attempts to culture the pathogen from pathological material followed by identification using phenotypic data with comparison to published diagnostic schemes, such as those in "Bergey's Manual of Systematic Bacteriology". Where diseased tissues are exposed to the surrounding environment, such as with surface ulcers and/or gills, there is an inherent risk of contamination and difficulties in discerning the relevance of resulting bacterial growth, i.e., which is the pathogen, a secondary invader of already dead or diseased tissue, or a chance contaminant (Fig. 2)? Internal tissues are less likely to be plagued by contaminants providing that they have been derived from freshly dead or moribund fish rather than specimens that have been deceased for numerous hours (Austin and Austin 2016). Personal experience is that the

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Table 1 Dominant bacterial pathogens of marine fish (based on Austin and Austin 2016)

Pathogen	Disease	Geographical distribution
Gram-positive bacteria		
Mycobacterium spp.	Mycobacteriosis (fish tuberculosis)	Worldwide
Nocardia spp.	Nocardiosis	Worldwide
Renibacterium salmoninarum	Bacterial kidney disease	Europe, Japan, North and South America
Streptococcus iniae	Streptococcosis	widespread
Streptococcus parauberis	Streptococcosis	Europe, USA
Gram-negative bacteria		
Aeromonas salmonicida	Ulcer disease	Baltic Sea, North Sea
Aliivibrio salmonicida	Cold-water vibriosis, Hitra disease	Canada, Norway, Scotland
Aliivibrio wodanis	Winter ulcer disease/syndrome	Iceland, Norway, Scotland
Francisella noatunensis	Francisellosis	Japan, South America, UK, USA
Moritella marina	Skin lesions	Iceland
Moritella viscosa	Winter ulcer disease/syndrome	Iceland, Norway, Scotland
Photobacterium damselae subsp. *damselae*	Photobacteriosis	Asia, Europe, USA
Photobacterium damselae subsp. *piscicida*	Pasteurellosis, pseudotuberculosis	Europe, Japan, USA
Piscirickettsia salmonis	Coho salmon syndrome, salmonid rickettsial septicaemia	Canada, Chile, Greece, Norway, Scotland, USA
Shewanella putrefaciens	Septicaemia	Saudi Arabia
Tenacibaculum dicentrarchi	–	Spain
Tenacibaculum discolor	–	Spain
Tenacibaculum gallaicum	–	Spain
Tenacibaculum maritimum	Bacterial stomatitis, gill disease, black patch necrosis	Europe, Japan, North America
Tenacibaculum soleae	Tenacibaculosis	Spain
Vibrio aestuarianus	–	China
V. alginolyticus	Eye disease, septicaemia	Asia, Europe, Israel
V. anguillarum	Vibriosis	worldwide
V. harveyi	Eye disease, necrotising enteritis, vasculitis, granuloma	Europe, Japan, Taiwan, USA
V. ichthyoenteri	Intestinal necrosis/enteritis	Japan, Korea, USA
V. mimicus	Ascites disease	China
V. ordalii	Vibriosis	Worldwide
V. parahaemolyticus	–	China, India
V. ponticus	Ulcerative disease	China
V. scophthalmi	–	Korea
V. splendidus	Septicaemia, vibriosis	Norway, Spain
V. vulnificus	Septicaemia	Europe, Japan, China, USA

kidneys are an excellent repository for pathogens. Having acquired diseased tissue, a comparatively narrow range of media have been used for culturing (see Table 3). Generally, the media lack imagination in terms of the characteristics of the host and thus the likely nutritional needs of the pathogen, i.e., the nutrients likely to be available in situ to the pathogen. Zobell's marine 2216E agar and/or thiosulphate citrate bile salt agar [TCBS; if vibrios were suspected (Eissa et al. 2019)] are often used with incubation at 15–37 °C for 1–7 days for diseased marine fish. Indeed, scrutiny of recent issues of journals dealing with fish diseases, such as Aquaculture, points to laboratories using very short incubation regimes, i.e. 24 h (e.g. Behera et al. 2018; Yang et al. 2018) at temperatures more appropriate for human bacteria, i.e. incubation at 37 °C (e.g. Behera et al. 2018). The relevance of this approach to fish pathology is questionable insofar as the incubation temperature is inevitably well above the optimum for most fish and their bacterial pathogens; not all fish pathogens grow at 37 °C and may need longer than 24 h to develop visible colonies. Thus, *Aeromonas salmonicida, Aliivibrio wodanis, Francisella noatunensis, Moritella marina, Moritella viscosa, Mycobacterium chelonei* subsp. *piscarium*, *Nocardia salmonicida*, *Photobacterium damselae* subsp. *piscicida, Renibacterium salmoninarum,*

Table 2 Procedures involved in the diagnosis of bacterial fish diseases

Category of information
Gross clinical signs of disease
Examples: behavioural changes, inappetence, emaciation, pigmentation changes, dermatitis/scale loss, erosion, ulceration, abscesses, necrosis, haemorrhaging, exophthalmia, gill damage, nodules on the surface, abdominal distension, protrusions, paralysis
Internal abnormalities
Examples: skeletal deformities; muscle damage, ascites, peritonitis; haemorrhaging, swollen organs, granulomas, nodules on organs, necrosis, liquefaction, swollen intestine/gastro-enteritis, emaciation
Histopathology
Pathogens may be visualised in diseased tissue sections
Microbiology
Culture-dependent: culturing, phenotypic, serological, molecular-based identification
Culture-independent: serology, molecular techniques

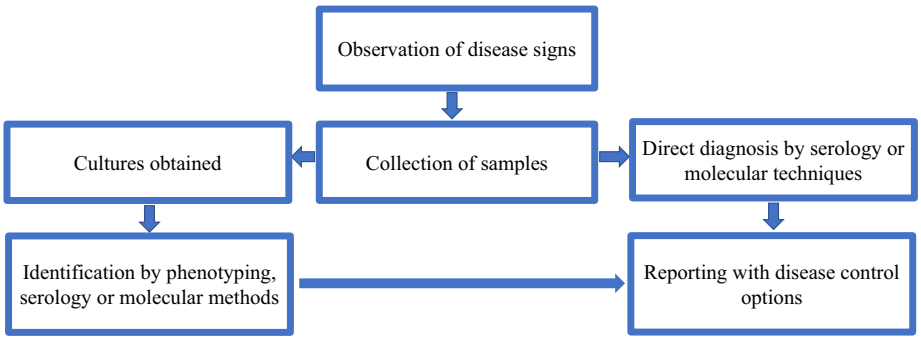

Fig. 1 Stages in the diagnosis of bacterial fish disease

Fig. 2 The TCBS plate was inoculated with swabbed material from an ulcer on a marine flatfish. Following incubation at 25 °C for 7 days, a dense diverse array of bacterial colonies was observed. The issue for the microbiologist concerned the choice of colony to subculture for the development of one or more pure cultures for further study

Tenacibaculum maritimum and *Vibrio tapetis* do not grow at 37 °C (Austin and Austin 2016). Moreover, *A. salmonicida,* mycobacteria, nocardias and streptococci need longer than 24 h to develop colonies on solid media (Austin and Austin 2016). Some specialised media exist for the recovery of more fastidious pathogens, such as *Mycobacterium* and *R. salmoninarum.* In other cases, media may be supplemented with blood, e.g. 5–7.5% (v/v) sheep or horse blood (Austin and Austin 2016). The desired outcome after incubation is the presence of dense bacterial growth; scant growth of colonies being indicative only of the presence of contaminants. Conventionally, pure dense growth from diseased tissues was considered to be indicative of the recovery of the actual pathogen. Yet, there is an awareness that some diseases may result from microbial consortia working together or sequentially, as will be discussed later. Nevertheless, the immediate goal has been the recovery of pure cultures, although the reasons for the choices of which colonies/growth to use from the isolation plates may well be highly subjective (Fig. 2). There is not any easy answer to this problem—does one take representatives of the dominant colony types? If so, how are the representative colonies chosen for purification. An alternative is to adopt a random approach by which the back of the inoculated agar plates is marked in numbered squares, and colonies removed for purification according to random number generators. In any case, the experience of the microbiologist is of paramount importance to ensure that meaningful colonies are chosen for further study. Then,

Table 3 Examples of media used to recover marine bacterial fish pathogens

Medium	Pathogen
Non-selective medium	
Brain heart infusion agar + 3% (w/v) NaCl	*Shewanella putrefaciens*
Cytophaga agar prepared in seawater	*Tenacibaculum* spp.
Flexibacter maritimus medium (FMM)	*Tenacibaculum* spp.
Marine 2216E agar	*Photobacterium damselae*, *Vibrio* spp.
Tryptone soya agar + 1–2% (w/v) NaCl	*Aeromonas salmonicida*, *Aliivibrio salmonicida*, *V. harveyi*, *V. splendidus*, *V. vulnificus*
Non-selective medium—enriched	
AUSTRAL-Salmonid Rickettsial Septicaemia (SRS) broth + L-cysteine	*Piscirickettsia salmonis*
Blood agar + 0.5–1.5% (w/v) NaCl	*Aliivibrio wodanis*, *Moritella marina*, *Moritella viscosa*, *Photobacterium damselae* subsp. *piscicida*, *V. splendidus*, *V. tapetis*
Cystine heart agar supplemented with 1% (w/v) haemoglobin	*Francisella* spp.
Glucose asparagine agar	*Nocardia* spp.
Kidney disease medium 2 (KDM2)	*R. salmoninarum*
Löwenstein–Jensen medium/dorset egg medium	*Mycobacterium* spp., *Nocardia* spp.
Middlebrook 7H10 medium	*Mycobacterium* spp.
Ogawa egg medium	*Mycobacterium gordonae*
Selective medium	
Coomassie brilliant blue agar	*Aeromonas salmonicida*
Selective kidney disease médium (SKDM)	*R. salmoninarum*
Thiosulphate citrate bile salt sucrose agar (TCBS)	*Photobacterium damselae*, *Vibrio* spp.

identification of the pure cultures may be achieved phenotypically, serologically or by molecular techniques (Austin and Austin 2016). The relevance of this approach to the host and its environment was rarely considered. Moreover, it has been unclear what proportion of bacterial cells actually produces colonies on solid media. It should be emphasised that the acquisition of one or more pure cultures does not necessarily mean that the actual pathogen has been recovered, but could reflect recovery of secondary invaders of already diseased tissue or even contaminants from the aquatic environment. Bacteriological examination on single occasions within a disease cycle would not identify possible microbial succession whereby an organism initiates an infection but subsequently become outcompeted by others. Yet, pure cultures do have their uses, serving for reference purposes, and enabling associated studies of epizootiology, pathogenicity factors and disease control (e.g. the determination of antibiotic sensitivity patterns).

From the conventional approach of using phenotypic traits together with diagnostic schemes, such as the tables contained in "Bergey's Manual of Systematic Bacteriology", many modern laboratories have subsequently adopted commercial systems, namely API 20E, API 20NE, API 50CH, API 50L, API-ZYM, Biolog-GN, Biolog-GP, Enterotubes and RapidID 32 systems, most of which were originally designed for use with medical bacteria. Therefore, their use for bacterial fish pathogens may well have led to erroneous identifications when comparisons of the test results were made with schemes designed for medical bacteria that grow well overnight at 37 °C. Nevertheless, it has been reported that results with Biolog-GP have been comparable with 16S rRNA gene sequencing for some Gram-positive bacterial fish pathogens, correctly identifying 18 *Lactococcus garvieae* and 10 *Streptococcus iniae* cultures. Unfortunately, *S. parauberis* was misidentified as *Enterococcus faecalis*; the type strain of *L. piscium* did not grow on the recommended medium (Verner-Jeffreys et al. 2011). The alternative has been to use serology or molecular identification procedures with the cultures. This has enabled more accurate identifications, and, in the case of serology, quick results.

Certainly, there are many issues with culturing methods, and it is apparent that for the majority of known bacterial fish pathogens to grow in the laboratory a diverse range of media and incubation conditions are necessary. This means that laboratories need to encompass a wide range of approaches to ensure the best chance of recovering the pathogens in culture. Moreover, there is a major concern with the time needed to obtain and identify cultures when the real thrust should be on controlling the disease.

Serology

Before the current interest in molecular techniques, serodiagnosis opened the possibility of rapid diagnoses directly from infected fish tissues even on the fish farm. Polyclonal antisera were effective at recognising the presence of pathogens when used in the fluorescent antibody test, whole cell agglutination, antibody-coated latex particles (= latex test), the immuno-India ink technique (= Geck test) or the enzyme linked immunosorbent assay (ELISA) (e.g. Saeed and Plumb 1987). The availability of highly specific monoclonal antibodies, such as those marketed by Aquatic Diagnostics Limited (Stirling; Goerlich et al. 1984), reduced the chance of misidentification; with polyclonal antisera there was always the risk of cross-reactions with resultant erroneous conclusions. Techniques include:

(1) Whole cell agglutination using polyclonal antisera has been used extensively for identifying cultures with accuracy depending on the specificity of the antiserum (e.g. Kitao 1982). A culture is mixed with antiserum in the presence of saline on a microscope slide and a positive result indicated by clumping of the bacteria observed within 2 min.
(2) Latex agglutination involved antisera/antibodies that were absorbed onto latex particles. Pure or mixed cultures or pathological material containing pathogens are mixed with the reagent on a glass or plastic surface. A positive reaction as indicated by clumping of the latex particles develops within 2 min. The technique has been demonstrated to be successful for the diagnosis of Hitra disease/cold-water vibriosis (e.g. McCarthy 1975; Sakai et al. 1986). Commercial kits have been developed, e.g. by BioNor in Norway, and used extensively in diagnostic laboratories (Romalde et al. 1995). A variation in the technique involves the use of antibody-sensitised cells of *Staphylococcus aureus* instead of latex particles, and has been used successfully for the diagnosis of *Aeromonas salmonicida* and *R. salmoninarum* (e.g. Kimura and Yoshimizu 1984).
(3) Immuno-india ink technique (= Geck technique) involved the use of india ink and antiserum, which was reacted with the bacterial suspension on a microscope slide. A positive result, which developed within 15 min, was the observation by microscopy of bacterial cells clearly outlined in india ink. Unfortunately, negative results, i.e. unstained bacterial cells, were difficult to visualise. The technique was described for *A. salmonicida* (McCarthy and Whitehead 1977) and has had only limited use in diagnostics.
(4) The fluorescent antibody technique permitted the observation of bacterial cells directly in tissues (Kawahara and Kusuda 1987). The approach utilised antiserum, fluorescein isothiocyanate, a suspension of the pathogen or pathological material and a fluorescence microscope. A positive response was the presence of fluorescing cells, which were observed with the fluorescence microscope. However, fluorescence faded after 20–30 min, which necessitated rapid observation.
(5) The enzyme-linked immunosorbent assay (ELISA) was both specific and sensitive, and opened up the possibility of field diagnoses (e.g. Austin et al. 1986). The technique involved antibody-coated plastic or glass surfaces, on which were captured bacterial cells from cultures or pathological material. In sequence, there was addition of an antibody–enzyme conjugate (typically, alkaline phosphatase or horseradish peroxidase) and an enzyme–substrate (*o*-phenylenediamine for alkaline phosphatase). A positive reaction in terms of a colour change would develop within 60 min. Developments included indirect ELISA, indirect blocking ELISA and competitive ELISA (e.g. Swain and Nayak 2003).
(6) Immunohistochemistry found use for recognising pathogens, e.g. *Aliivibrio salmonicida* and *Photobacterium damselae* subsp. *piscicida* in fresh and fixed tissues (Abu-Elala et al. 2015; Evensen et al. 1991).
(7) A refinement to the ELISA involved polyclonal antibody-coated gold nanoparticles in an immunoassay, which enabled the rapid, sensitive and specific detection of pathogens directly in tissues within 45 min (Saleh et al. 2011).

To some extent, serological techniques have declined in use since the developments in molecular biology. However, serology is used routinely for the identification of cultures in the laboratory, and some ELISA kits are still on the market. For example, the Central Institute of Freshwater Aquaculture in India developed two agglutination and ELISA kits for use by fish farmers in field conditions.

Culture-independent methods—molecular biology

Molecular methods with their high sensitivity and specificity have become the favoured options in many laboratories for the diagnosis of fish diseases (e.g. Abu-Elala et al. 2015; Bartkova et al. 2017; Fernandez-Alvarez et al. 2016; Keeling et al. 2013; Mooney et al. 1995; Yan et al. 2018). Techniques capable of detecting virtually single cells have been described. For example, Hiney et al. (1992) reported a polymerase chain reaction (PCR) that was capable of detecting ~ 2 cells of *A. salmonicida,* whereas Høie et al. (1997) detected 10^3 and 10^4 colony forming units (CFUs) of *A. salmonicida* in 100 ml of kidney suspension with 16S rRNA sequencing and plasmid primers, respectively. Subsequent developments included nested PCR (Taylor and Winton 2002), terminal-restriction fragment length polymorphism

(RFLP) (Nilsson and Strom 2002), PCR–RFLP (Puah et al. 2018), multiplex PCR (Chapela et al. 2018), real-time PCR (Keeling et al. 2013), quantitative real-time-PCR (Du et al. 2017), real-time recombinase polymerase amplification (Pang et al. 2019) and reverse transcription-multiplex PCR (Rattanachaikunsopon and Phumkhachorn 2012). All these techniques reported extremely high levels of sensitivity, detecting numbers of cells well below the level associated with occurrences of clinical disease (Austin and Austin 2016). This raises the issue about the significance of positive results. If overt disease signs have been observed, then positivity with molecular tools provides strong indication of the identity of the pathogen. If clinical signs are absent, then positivity may suggest the presence of asymptomatic or carrier fish or background populations of the pathogen present in the aquatic environment. In short, the data need to be interpreted critically.

The ability to discriminate between two or more pathogens even from different genera was an obvious improvement for diagnosticians. Thus, the triplex loop-mediated isothermal amplification (LAMP) method detected and discriminated *V. alginolyticus*, *V. anguillarum* and *V. harveyi* with a high level of sensitivity from experimentally infected fish (Yu et al. 2013). Similarly, other techniques have mirrored the ability to differentiate between various bacterial pathogens. Thus, González et al. (2004) used a multiplex PCR and DNA microarray for the simultaneous and differential diagnosis of *A. salmonicida, Photobacterium damselae* subsp. *damselae*, *V. anguillarum*, *V. parahaemolyticus* and *V. vulnificus* with a minimum detection limit of the equivalent of four to five bacterial cells. Moreover, Matsuyama et al. (2006) developed a low-density oligonucleotide DNA array for the detection and discrimination of multiple *Photobacterium* and *Vibrio* spp. Similarly, DNA microarrays detected *Aeromonas hydrophila*, *Nocardia seriolae*, *S. iniae*, *V. alginolyticus*, *V. anguillarum* and *V. harveyi* (Shi et al. 2012); another publication described the detection of *A. hydrophila*, *Edwardsiella tarda*, *Flavobacterium columnare*, *L. garvieae*, *Photobacterium damselae*, *Pseudomonas anguilliseptica*, *S. iniae* and *V. anguillarum* with sensitivities of 10^3 CFU/ml for pure cultures (Chang et al. 2012). The simultaneous recognition of *Photobacterium damselae*, *Pseudomonas baetica*, *Tenacibaculum maritimum*, *T. soleae* and *V. harveyi* was achieved with reverse line blot hybridisation with the sensitivity ranging from 1 to 100 pg of genomic DNA of the pure culture (López et al. 2012). Another example was the multiple PCR-RNA polymerase that differentiated *Photobacterium damselae*, *V. harveyi* and *V. ichthyoenteri* in olive flounder with detection limits in kidney of 2.5×10^6 CFU/g, 2.5×10^4 CFU/g and 2.5×10^5 CFU/g, respectively (Kim et al. 2014). In addition, a real-time fluorogenic LAMP detected *N. seriolae*, *Pseudomonas putida*, *S. iniae*, *V. alginolyticus*, *V. anguillarum*, *V. fluvialis*, *V. harveyi*, *V. parahaemolyticus*, *V. rotiferianus* and *V. vulnificus* with a reaction time of < 30 min (Zhou et al. 2014). The question about which method is best largely reflects personal choice. However, all the techniques described to date are noted for their specificity and sensitivity. It is hoped that future developments will enhance the applicability of molecular methods to field use thereby enabling both rapid and accurate diagnoses, which would facilitate the instigation of meaningful disease control strategies.

The issue of possible co-infections

The possibility that disease may be attributed to two or more different pathogens in the same host either occurring together or sequentially has slowly gained recognition, and complicates diagnostic procedures. Certainly, co-infection may affect the severity of disease as the susceptibility to different pathogens may be changed with mixed infections. Of course, there will be implications for diagnosis and immunoprophylaxis (Kotob et al. 2016). In particular, it has been considered to be highly likely that co-infection may well reduce the beneficial effect of vaccination (Figueroa et al. 2017). Examples of co-infection have reached the scientific literature, and incidences appear to be increasing possibly as scientists become aware of, and actively look for, evidence. Thus, Loch et al. (2012) reported that diseased Chinook salmon (*Oncorhynchus tshawytscha*), that were returning to spawn in tributaries of Lake Michigan, USA, contained numerous bacterial pathogens. Unfortunately, many of the current approaches to diagnoses would be unlikely to recognise co-infections or cases of microbial succession whereby one organism initiates an infection with others developing and/or exacerbating the condition. The primary reason may be that the presence of more than one organism in diseased animals would be interpreted by many diagnosticians as evidence of contamination. A possible explanation would be that secondary invaders or saprophytes were colonising already diseased tissue. This may have been the case with *Aeromonas. hydrophila*, which was isolated from fish with columnaris that were recovered from lakes in Saskatchewan, Canada (Scott and Bollinger 2014). Certainly, it is speculative how many cases of co-infections have been missed and not reported. Nevertheless, there are examples of co-infections involving bacteria with other bacteria, or parasites or viruses. Loach (*Misgurnus anguillicaudatus*), which were farmed in China, harboured a new disease that was associated with two bacterial taxa, namely *Shewanella putrefaciens* and *V. anguillarum* (Qin et al. 2014). In addition, *Pseudomonas anguilliseptica* was found with *Delftia acidovorans* in European eels (Andree et al. 2013). *Moritella viscosa* and *Aliivibrio wodanis* occurred in salmon with winter ulcer disease (Hjerde et al. 2015). It was considered from cell culture evidence involving use of culture supernatants

that *Aliivibrio wodanis* secreted toxins and influenced the development of infection by *Moritella viscosa* (Karlsen et al. 2014).

There are examples of bacteria causing infections in conjunction with parasites. For example, *R. salmoninarum* and *Nanophyetus salmincola* occurred in wild juvenile Chinook salmon (Sandell et al. 2015). *Yersinia ruckeri* and *Neoparamoeba perurans*, the latter of which causes amoebic gill disease, have been reported to co-infect Atlantic salmon in Tasmania, Australia (Valdenegro-Vega et al. 2015). The freshwater trematode *Nanophyetus salmincola* impaired the immune function of juvenile Chinook salmon, reducing resistance to *V. anguillarum* (Roon et al. 2015). Experiments revealed that co-infection of Atlantic salmon with *Caligus rogercresseyi* and *Piscirickettsia salmonis* led to decreased survival and reduced specific growth rate among vaccinates compared to infection with the bacterial pathogen alone. Moreover, the bacterial loading and clinical signs of disease were significantly increased in co-infected fish (Figueroa et al. 2017).

Examples of infections involving bacteria and viruses include co-infection of *Edwardsiella tarda* with aquabirnavirus that led to higher mortalities in Japanese flounder (Pakingking et al. 2003). *A. salmonicida* together with infectious salmon anaemia virus caused secondary infections to infectious pancreatic necrosis virus in Atlantic salmon (Johansen and Sommer 2001). *V. harveyi* and *Edwardsiella tarda* co-infected olive flounder with aquatic birnavirus leading to heavy mortalities in Korean farms (Oh et al. 2006).

Diseases may involve bacteria, viruses and parasites. For example, bathing of salmon in Chile to treat *C. rogercresseyi* resulted in increased stress and immunosuppression, which in turn led to chronic caligidosis and a higher prevalence of disease caused by *N. perurans, P. salmonis* and infectious salmon anaemia virus (Gonzalez et al. 2016).

Conclusions

For routine diagnostics, it is questionable whether culture-dependent approaches will continue in widespread use if the aim is purely to equate a disease with its pathogen. Certainly, reliance on culturing has not always been successful for the recovery of pathogens, due to the lack of or use of suitable media and appropriate incubation conditions. It is argued that with the move away from culturing and phenotyping, diagnostics lost much of the previous subjectivity and inaccuracies caused by reliance on inappropriate diagnostic schema, notably those developed for medically important bacteria. Clearly, molecular techniques have improved the accuracy of bacterial identification, and progressed from use only in specialised laboratories to those involved with routine diagnostics. However, it is prudent to remember that serology currently offers the possibility of rapid, field-based diagnoses, as exemplified by ELISA kits. Yet for both molecular and serological systems, there are concerns about positive reactions for what are ultrasensitive methods, especially if overt disease signs are absent. The possibility exists that some positive results may reflect the recognition of natural background populations of pathogens that are not necessarily relevant for disease diagnoses. Also, there are concerns about false positive results, which could elicit economically costly responses. To be effective, diagnoses need to consider all available information including the clinical signs of disease before making firm conclusions. Diagnoses need to help, not hinder the management of fish diseases.

Compliance with ethical standards

Conflict of interest The author declares that there is no conflict of interest.

Human and animal rights This article does not contain any studies with human participants or animals performed by the author.

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