

A review of the main bacterial fish diseases in mariculture systems

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Abstract

The aim of this review is to compile some dispersed literature published about different aspects of the most threatening bacterial diseases occurring in fish cultured in marine waters worldwide such as vibriosis, “winter ulcer”, photobacteriosis, furunculosis, flexibacteriosis, “winter disease”, streptococcosis, lactococcosis, BKD, mycobacteriosis and piscirickettsiosis. Therefore, the geographic distribution of each disease and the main host species affected, together with the biochemical and antigenic diversity existing in the aetiological agents are described. In addition, the genetic studies that have been performed to determine the possible existence of intraspecific heterogeneity or clonal lineages within each pathogen are included. We review also in brief the classical methods to isolate the microorganisms from their hosts as well as the serological and/or genetic tools for a rapid diagnosis of the diseases. Finally, the current status in the development of vaccination strategies to prevent these bacterial diseases is also addressed.

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1. Introduction

Aquaculture is an emerging industrial sector which requires continued research with scientific and technical developments, and innovation. The world aquaculture production in 2001 was approximately of 37.9 million tons, which represents around 41% of that obtained from extensive captures for human consump-

tion (FAO, 2003). Marine fish culture is dominated by Atlantic salmon (*Salmo salar*) led by Norway, then Chile, United Kingdom, Canada and Ireland. Other economically important marine fish are gilthead seabream (*Sparus aurata*), seabass (*Dicentrarchus labrax*) and turbot (*Scophthalmus maximus*) in countries such as Greece, Italy, France, Spain and Portugal, and yellowtail (*Seriola quinqueradiata*), ayu (*Plecoglossus altivelis*), flounder (*Paralichthys olivaceus*) and seabream (*Pagrus major*) in Japan.

The appearance and development of a fish disease is the result of the interaction among pathogen, host and environment. Therefore, only multidisciplinary

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studies involving the characteristics of potential pathogenic microorganisms for fish, aspects of the biology of the fish hosts as well as a better understanding of the environmental factors affecting such cultures, will allow the application of adequate measures to prevent and control the main diseases limiting the production of marine fishes. Regarding the infectious diseases caused by bacteria in marine fish, although pathogenic species have been described in the majority of the existing taxonomic groups, only a relatively small number are responsible of important economic losses in cultured fish worldwide (see

Table 1). It is important to point out that diseases classically considered as typical of fresh water aquaculture, such as furunculosis (*Aeromonas salmonicida*), bacterial kidney disease (BKD) (*Renibacterium salmoninarum*) and some types of streptococcosis, are today important problems also in marine culture. Clinical signs (external and internal) caused by each pathogen are dependent on the host species, fish age and stage of the disease (acute, chronic, subclinical carrier). In addition, in some cases, there is no correlation between external and internal signs. In fact, systemic diseases (i.e., pasteurellosis,

Table 1

Aetiological agents of the economically important bacterial fish diseases affecting marine fish cultures

Agent	Disease	Main marine hosts	Major serotypes/ serogroups	Vaccine availability	PCR-based diagnostic methods
Gram negative					
<i>Listonella anguillarum</i> (formerly <i>Vibrio anguillarum</i>)	Vibriosis	Salmonids, turbot, seabass, striped bass, eel, ayu, cod, red seabream	3	+	+
<i>Vibrio ordalii</i>	Vibriosis	Salmonids	1	+	—
<i>Vibrio salmonicida</i>	Vibriosis	Atlantic salmon, cod	1	+	—
<i>Vibrio vulnificus</i>	Vibriosis	Eels	1	+	+
<i>Moritella viscosa</i> (formerly <i>Vibrio viscosus</i>)	“Winter ulcer”	Atlantic salmon	1	+	—
<i>Photobacterium damsela</i> subsp. <i>piscicida</i> (formerly <i>Pasteurella piscicida</i>)	Photobacteriosis (Pasteurellosis)	Seabream, seabass, sole, striped bass, yellowtail	1	+	+
<i>Pasteurella skyensis</i>	Pasteurellosis	Atlantic salmon	ND	—	—
<i>Aeromonas salmonicida</i> subsp. <i>salmonicida</i>	Furunculosis	Salmonids turbot	1	(+) ^a	+
<i>Tenacibaculum maritimum</i> (formerly <i>Flexibacter maritimus</i>)	Flexibacteriosis	Turbot, salmonids, sole, seabass, gilthead seabream, red seabream, flounder	2 ^b	+	+
<i>Pseudomonas anguilliseptica</i>	Pseudomonadiosis “Winter disease”	Seabream, eel, turbot, ayu	2	(+) ^c	+
Gram positive					
<i>Lactococcus garvieae</i> (formerly <i>Enterococcus seriolicida</i>)	Streptococcosis or lactococcosis	Yellowtail, eel	2	(+) ^d	+
<i>Streptococcus iniae</i>	Streptococcosis	Yellowtail, flounder, seabass, barramundi	2	(+) ^d	+
<i>Streptococcus parauberis</i>	Streptococcosis	Turbot	1	+	+
<i>Streptococcus phocae</i>	Streptococcosis	Atlantic salmon	ND	—	—
<i>Renibacterium salmoninarum</i>	BKD	Salmonids	1	+	+
<i>Mycobacterium marinum</i>	Mycobacteriosis	Seabass, turbot, Atlantic salmon	ND ^e	—	+
<i>Piscirickettsia salmonis</i>	Piscirickettsiosis	Salmonids	1	(+) ^f	+

^a Limited protection in turbot.

^b Further studies are needed to clarify the serotyping scheme.

^c Under development.

^d High protection but the duration is dependent on the fish host.

^e No data reported.

^f Questioned efficacy under field conditions.

piscirickettsiosis) with high mortality rates cause internal signs in the affected fish but they often present a healthy external appearance. On the contrary, other diseases with relatively lower mortality rates (i.e., flexibacteriosis, “winter ulcer syndrome”, some streptococcosis) cause significant external lesions, including ulcers, necrosis, exophthalmia which make fish unmarketable.

The pathogenic agents described in the culture systems are usually present in wild fish populations. However, in natural environments, they rarely cause mortality due to the lack of the stressful conditions that usually occur in the culture facilities.

Despite the review of bacterial diseases described here, marine aquaculture currently offers to the consumers a product of high sanitary quality.

2. Vibriosis

Within the *Vibrionaceae*, the species causing the most economically serious diseases in marine culture are *Listonella (Vibrio) anguillarum*, *Vibrio ordalii*, *V. salmonicida* and *V. vulnificus* biotype 2.

L. anguillarum, aetiological agent of classical vibriosis, possesses a wide distribution causing a typical haemorrhagic septicaemia in a wide variety of warm and cold water fish species of economic importance, including Pacific and Atlantic salmon (*Oncorhynchus* spp. and *S. salar*), rainbow trout (*Oncorhynchus mykiss*), turbot (*S. maximus*), seabass (*D. labrax*), seabream (*S. aurata*), striped bass (*Morone saxatilis*), cod (*Gadus morhua*), Japanese and European eel (*Anguilla japonica* and *Anguilla anguilla*), and ayu (*P. altivelis*) (Toranzo and Barja, 1990, 1993; Actis et al., 1999).

Fish affected by this classical vibriosis show typical signs of a generalized septicaemia with haemorrhage on the base of fins, exophthalmia and corneal opacity. Moribund fish are frequently anorexic with pale gills which reflects a severe anaemia. Oedematous lesions, predominantly centered on the hypodermis, are often observed.

Although a total of 23 O serotypes (O1–O23, European serotype designation) are known to occur among *L. anguillarum* isolates (Sørensen and Larsen, 1986; Pedersen et al., 1999), only serotype O1, O2 and, to a lesser extent, serotype O3 have been

associated with mortalities in farmed and feral fish throughout the world (Tajima et al., 1985; Toranzo and Barja, 1990, 1993; Larsen et al., 1994; Toranzo et al., 1997). The remaining serotypes are considered to be environmental strains and only on rare occasions are isolated as responsible for vibriosis in fish. Whereas serotypes O1 and O2 have a wide distribution, serotype O3 affects mainly eel and ayu.

In contrast to the serotype O1 which is antigenically homogeneous, serotypes O2 and O3 display antigenic heterogeneity and the existence of two subgroups within each serotype, named respectively O2a and O2b and O3A and O3B, has been demonstrated (Olsen and Larsen, 1993; Santos et al., 1995). Interestingly, whereas subgroup O2a occurs both in salmonid and non-salmonid fish, subgroup O2b has only been detected in strictly marine fish. In the case of serotype O3, the subgroup O3A is recovered from diseased fish and subgroup O3B comprises only environmental strains.

Genetic studies have been also performed to study the intraspecific variability within the major pathogenic serotypes of *L. anguillarum* (O1 and O2) (Pedersen and Larsen, 1993; Skov et al., 1995; Tiainen et al., 1995; Toranzo et al., 1997). A homogeneity was detected by rRNA gene restriction analysis (ribotyping) within the serotype O1 strains regardless of the geographic area or fish host, and using pulsed-field gel electrophoresis (PFGE) Scandinavian strains and southern European isolates could be separated into two clonal lineages. A greater genetic heterogeneity was demonstrated within *L. anguillarum* serotype O2 with 32 distinct ribotypes being reported. However, a genetic difference between north European and south European O2 isolates could be also detected.

These serological and genetic studies are of epidemiological value to determine the possible origin of the *L. anguillarum* infections, as well as to implement adequate vaccination programs in one particular country.

L. anguillarum can be presumptively diagnosed on basis of standard biochemical tests. However, a serological confirmation employing serotype-specific polyclonal antisera is necessary (Toranzo et al., 1987). Although commercial diagnostic kits based on slide agglutination or in ELISA test have been developed for a fast diagnosis of vibriosis, they do not allow the

distinction of serotypes (Romalde et al., 1995) and therefore are not useful for epidemiological purposes. From 1989, several DNA probe-based detection protocols were developed, but they were not specific and/or sensitive enough to be used in the diagnosis of vibriosis in the field. Only recently, a PCR-based approach was described for the accurate detection of *L. anguillarum* in infected fish tissues (Osorio and Toranzo, 2002). The target gene was *rpoN*, a gene that codes for the sigma factor σ 54.

Although there are a large number of commercial *L. anguillarum* vaccines devised to be used mainly by bath or injection (Newman, 1993; Toranzo et al., 1997), the majority of them include in their formulations only serotype O1 or a mixture of serotypes O1 and O2a. To our knowledge, only one licenced bacterin (GAVA-3) developed by the University of Santiago (Spain) covers the three antigenic entities of *V. anguillarum* responsible of most epizootics (O1, O2a and O2b) in marine aquaculture (Toranzo et al., 1997).

In the case of strictly marine fish such as turbot or seabass, aqueous *L. anguillarum* bacterins are being employed by bath exposure for 1–2 g fish. Two treatments are necessary in the vaccinal bath at monthly intervals. However, for salmonids cultured in Nordic countries, different polyvalent oil-based vaccines including distinct combinations of *L. anguillarum* with other pathogens such as *V. ordalii*, *Vibrio salmonicida*, *A. salmonicida*, *Moritella viscosa* and infectious pancreatic necrosis virus are also available on the market to be used by the i.p. route (Toranzo et al., 1997; Greger and Goodrich, 1999).

The species *V. ordalii*, which has been established to accommodate strains formerly classified as *V. anguillarum* biotype 2 (Schieve and Crosa, 1981), has been isolated mainly in North America, Japan and Australia affecting salmonids (Toranzo and Barja, 1993; Austin and Austin, 1999). Recent phenotypic and molecular studies performed by our research group indicated that this species is also present in Atlantic salmon cultured in Chile (unpublished results). Although this vibriosis can be categorized as a haemorrhagic septicaemia, *V. ordalii* bacteremia develops later than the infections with *L. anguillarum*. This explains the lower number of bacterial cells in the blood of infected fish (Ransom et al., 1984).

In contrast to *L. anguillarum*, *V. ordalii* is antigenically homogeneous with no serotypes being detected. Cross-reactions can exist between *V. ordalii* and *L. anguillarum* serotype O2 using polyclonal antisera, but immunoblot analysis with absorbed antisera demonstrate that LPS of both species do not have identical antigenic properties (Mutharia et al., 1992). In fact, commercial bacterins including as antigens *L. anguillarum* serotype O1 and *V. ordalii* elicit very poor protection against infections by *L. anguillarum* serotype O2 (Toranzo et al., 1997).

Intraspecific genetic studies performed in *V. ordalii* shows that three ribotypes were discernible within this pathogen. However, the genetic homology among the strains was more than 95% which supports the clonality of this species (Tiainen et al., 1995).

V. salmonicida is the aetiological agent of the “Hitra disease” or “cold water vibriosis”, which affects salmonids and cod cultured in Canada and Nordic countries of Europe (mainly Norway and UK) (Bruno et al., 1986; Egidius et al., 1986; Sørsum et al., 1990). Cold water vibriosis is characterized by severe anaemia and extensive haemorrhage, especially in the integument surrounding the internal organs of fish including the caeca, abdominal fat and kidney. A generalized septicaemia with large numbers of bacteria is usually found in the blood of affected fish.

As the name of the disease indicates, *V. salmonicida* only grow at temperatures below 15 °C and in media supplemented with blood. This pathogen is biochemically and antigenically homogeneous being a hydrophobic protein, called VS-P1, present in the surface layer, the dominant antigen in all the strains (Espelid et al., 1987; Hjelmeland et al., 1988). A confirmative serological identification of this species based on the slide agglutination tests using a specific commercial polyclonal antiserum is usually employed for routine purposes. Despite the economic importance of this type of vibriosis in nordic Countries, to our knowledge, no PCR based approach have been developed for an accurate detection of this pathogen in the field.

Epidemiological studies of cold water vibriosis have been focused only on the plasmid content of *V. salmonicida* from salmon and cod (Sørsum et al., 1990). Although different profiles have been observed, all of them contain a 21–24 Md plasmid. A 61 Md plasmid was only present in the cod isolates

originating from northern Norway, which could indicate the existence of a particular subtype within the cod strains of this species. However, vaccination experiments demonstrated that there are no major antigenic differences between different strains of *V. salmonicida* that have any impact on protective immunity (Lillehaug et al., 1990). As stated above, salmonids in nordic countries are systematically vaccinated with oil-adjuvanted bacterins containing at least two pathogenic vibrios *V. anguillarum* and *V. salmonicida* (Toranzo et al., 1997).

Vibrio vulnificus comprises two biotypes. Biotype 1 is an opportunistic human pathogen causing disease generally associated with handling or ingestion of raw shellfish, and the biotype 2 strains are virulent for eel (Tison et al., 1982; Biosca et al., 1991; Dalsgaard et al., 1998). However, this biotype 2 may also cause in some occasions infection in human representing a potential health hazard for fish farmers (Amaro and Biosca, 1996). Biotype 2 is biochemically homogeneous, indole production being the main trait which distinguishes both biotypes (Amaro et al., 1992; Biosca et al., 1997). Whereas biotype 1 is antigenically diverse, biotype 2 strains constitute a homogeneous O serogroup regardless of their geographic origin. It is now considered that this biotype is a new serotype of *V. vulnificus* that is adapted to infect eel and thus nominated serotype E (Biosca et al., 1997). Therefore, to avoid possible misidentification with strains of biotype 1, the confirmative identification of the eel pathogen *V. vulnificus* serotype E must be based in the use of an agglutination tests using the specific antiserum. In addition, the use of a selective medium for *V. vulnificus* (VVM) was proved to be useful for a preliminary differentiation of the eel pathogen in mixed bacterial populations (Marco-Noales et al., 2001). Genetic techniques such as ribotyping, randomly amplified polymorphic DNA (RAPD) and amplified fragment length polymorphism (AFLP) have been also described as powerful tools to discriminate eel-pathogenic strains from clinical and environmental isolates (Aznar et al., 1993; Arias et al., 1997).

Several PCR based methods for the diagnosis of this vibriosis have been developed using as target sequences the 23S ribosomal gene (23S rDNA) or the cytolysin gene of *V. vulnificus* (Arias et al., 1995; Coleman and Oliver, 1996; Osorio and Toranzo,

2002) allowing the successfully detection of the pathogen in eel tissues, tank water and sediments.

Until recently, no vaccines had been manufactured to prevent vibriosis caused by the serovar E of *V. vulnificus*; however, a specific bacterin named Vulnivaccine was developed by the University of Valencia (Spain), which proved to be effective in field conditions (Fouz et al., 2001). A triple exposure to the vaccine in a short space of time (approx. 1 month) by prolonged immersion is needed to ensure an acceptable level of protection for a 6 month period. After that, an oral booster with Vulnivaccine-supplemented food is recommended to achieve a long lasting protection period (Esteve-Gassent et al., 2004). However, as no cross-protection among serotypes exists, it was recently demonstrated that vaccinated eels with serovar E of *V. vulnificus* can be infected by other less frequent serovars of the pathogen possessing low degree of virulence which act as secondary pathogens (Fouz and Amaro, 2003).

3. “Winter ulcer”

“Winter ulcer” is a disease affecting sea-farmed Atlantic salmon reared at cold temperatures and, therefore, it occurs mainly during the winter season. The disease is characterised by skin ulcers confined to scale-covered parts of the body surface and often diffuse or petechial haemorrhage in internal organs are also present (Lunder et al., 1995; Benediktsdóttir et al., 1998; Bruno et al., 1998a). This disease was observed in the 1990s in Norway (Salte et al., 1994; Lunder et al., 1995), Iceland (Benediktsdóttir et al., 1998) and, more recently, in Scotland (Bruno et al., 1998a; Laidler et al., 1999). Although the fish mortality is limited, the disease has economic significance due to lowered quality of affected salmon.

Although several causes of “winter ulcer” were postulated (Salte et al., 1994), bacteriological studies demonstrated that a new psychrotrophic *Vibrio* species termed *Vibrio viscosus* (because of its thread-forming, adherent colonies in conventional media) is the main causative agent of this condition (Lunder et al., 2000). Further characterization using 16S rRNA sequencing analysis showed that *V.*

viscosus should be reclassified as *M. viscosa* (Benediktsdóttir et al., 2000).

Interestingly, in association with *M. viscosa*, other new psychrotrophic *Vibrio* species classified as *Vibrio wodanis* (closely related to *Vibrio logei*) (Lunder et al., 2000) has been isolated from winter ulcers in Norway, Iceland and Scotland. Experimental infection in Atlantic salmon with *M. viscosa* strains induced a disease similar to “winter ulcer”, while inoculation with *V. wodanis* isolates have no effect, which strongly support the important role of *M. viscosa* as primary pathogen in the disease (Benediktsdóttir et al., 1998; Bruno et al., 1998a; Lunder et al., 1995). However, the possible role of *V. wodanis* by suppressing the healing process of skin ulcers infected primarily by *M. viscosa* cannot be ruled out.

M. viscosa is rather inert biochemically and often requires prolonged incubation times on test media (from 4 to up to 10 days). The key biochemical properties that enable *M. viscosa* to be distinguished from other pathogenic vibrios recorded from salmonids, such as *V. anguillarum* and *V. salmonicida*, are a positive lysine decarboxylase and negative citrate, mannitol and sucrose reactions (Bruno et al., 1998a; Lunder et al., 2000; Benediktsdóttir et al., 2000).

Although *M. viscosa* is considered as a phenotypically and genetically homogeneous species (Lunder et al., 2000; Benediktsdóttir et al., 2000), using the highly discriminative fingerprinting method AFLP, *M. viscosa* strains grouped into distinct subgroups according to their geographical origin: one subgroup contained the isolates from Norway, while the strains from Iceland grouped into two closely related clusters corresponding respectively to the south-west and north Iceland isolates (Benediktsdóttir et al., 2000). This finding indicates a common clonal origin of *M. viscosa* within a particular geographical area. By contrast, *V. wodanis* can be defined as a biochemically and genetically heterogeneous species (Lunder et al., 2000; Benediktsdóttir et al., 2000), not showing any signs of clonal spread that is often characteristic of primary pathogens. This result is consistent with the lack of capacity of this species to induce disease in the challenge tests.

An inactivated oil-adjuvanted vaccine against *M. viscosa* has been shown to give protection in Atlantic salmon (Greger and Goodrich, 1999). Today, *M. viscosa* has been incorporated in the oil based multi-

valent vaccines employed routinely in the salmon industry of Norway, Faroe Islands and Iceland.

Interestingly, *M. viscosa* was also recovered in scarce occasions from plaice (*Pleuronectes platessa*) and rainbow trout suffering skin ulcers (Lunder et al., 2000; Benediktsdóttir et al., 2000), which indicates that this species is not necessarily restricted to causing disease in Atlantic salmon. However, the spread of this bacterium among fish reared in marine waters remains to be determined in the future.

4. Photobacteriosis

Photobacteriosis, described also as pasteurellosis, is caused by the halophilic bacterium *Photobacterium damsela* subsp. *piscicida* (formerly *Pasteurella piscicida*), which was originally isolated from mortalities occurring in natural populations of white perch (*Morone americanus*) and striped bass in 1963 in Chesapeake Bay. Since 1969, pasteurellosis has been one of the most important diseases in Japan, affecting mainly yellowtail (*S. quinquerediata*), and from 1990 it has caused economic losses in the marine culture of gilthead seabream, seabass and sole (*Solea senegalensis* and *Solea solea*) in the Mediterranean countries of Europe and hybrid striped bass (*M. saxatilis* × *M. chrysops*) in the USA (Toranzo et al., 1991a,b; Magariños et al., 1996, 2001, 2003; Romalde and Magariños, 1997; Zorrilla et al., 1999; Romalde et al., 1999a). Fish pasteurellosis was also known as pseudotuberculosis, because it is characterized by the presence of white nodules in the internal viscera, particularly, spleen and kidney. Severe mortalities occur usually when water temperatures are above 18–20 °C. Below this temperature, fish can harbour the pathogen as subclinical infection for long time periods (Magariños et al., 2001).

Regardless of the geographic origin and source of isolation, all strains of this pathogen are biochemically and serologically homogeneous (Magariños et al., 1992a,b, 1996, Bakopoulos et al., 1997). However, DNA fingerprinting methods as ribotyping (Magariños et al., 1997), AFLP (Thyssen et al., 2000; Kvitt et al., 2002) and RAPD (Magariños et al., 2000, 2003; Hawke et al., 2003) proved to be valuable epidemiological tools since they allowed to detect two clear separate clonal lineages within *Ph. damsela* subsp.

piscicida. Whereas a clonal lineage comprises all European isolates, the second clonal lineage includes the Japanese and USA isolates.

Despite the great phenotypic homogeneity exhibited by all *Ph. damsela* subsp. *piscicida* strains, differences in the degree of virulence was observed depending of their source of isolation. Thus, the LD50 values of the sole and hybrid striped bass isolates was considerably lower than those exhibited by strains recovered from other fish hosts (Magariños et al., 2003; Thune et al., 2003).

Differences in susceptibility to pasteurellosis on the basis of fish age have been demonstrated in gilthead seabream, for example fish above 50 g are resistant to infection. Histological observations and “in vitro” killing assays demonstrated that neutrophils and macrophages of seabream of this size efficiently phagocytize and kill the bacteria, while these cell types are not functional in small fish (Noya et al., 1995; Skarmeta et al., 1995).

The presumptive identification of the pathogen is based on standard biochemical tests. In addition, although *Ph. damsela* subsp. *piscicida* is not included in the API-20 E code index, this miniaturized system can be also useful for a rapid presumptive diagnosis of the disease because all strains display a characteristic profile (2005004) (Magariños et al., 1992a). A slide agglutination test using specific antiserum is needed for a confirmative identity of the microorganism.

The Norwegian company Bionor AS has marketed kits based not only on direct bacterial agglutination, but also on ELISA tests which allow a rapid detection of *Ph. damsela* subsp. *piscicida* in fish tissues. The evaluation of these ELISA kits in the field, demonstrated that the sensitivity of the magnetic beads-EIA based method (Aquaeia-Pp) was 100 to 1000 times higher than the standard ELISA Kit (Aquarapid-Pp) (Romalde et al., 1999b), which indicates its usefulness for the detection of asymptomatic fish.

From 1997, a variety of DNA-based protocols have been also developed for a fast, and specific detection of the causative agent of pasteurellosis. However, only a multiplex PCR approach that target the 16S ribosomal RNA (16S rDNA) and *ureC* genes allowed the specific discrimination between *Ph. damsela* subsp. *piscicida* and *Ph. damsela* subsp. *damsela* (Osorio et al., 1999, 2000; Osorio and Toranzo, 2002).

The application of fast, specific and sensitive serological and molecular tools such as those based on ELISA or PCR is of crucial importance in the case of pasteurellosis since it has been demonstrated that the pathogen can be transmitted vertically through the ovarian and seminal fluids from apparently healthy broodstock (Romalde et al., 1999b), and that this bacterium undergoes a viable but no culturable state (Magariños et al., 1994) which makes its detection difficult in the farm environment.

Several commercial vaccines against *Ph. damsela* subsp. *piscicida* are available on the market, but their efficacy is dependent of the fish species, fish size, vaccine formulation and use of immunostimulants (Magariños et al., 1996; Romalde and Magariños, 1997). However, only in the case of the licenced bacterin (DI vaccine) patented by the University of Santiago (Spain) and available commercially was its effectiveness demonstrated in gilthead seabream larvae of only 50 days old (Magariños et al., 1999). As the majority of the pasteurellosis outbreaks occur from larval stages to fingerlings of 10–30 g, a vaccination programme which comprises a first dip immunization at the larval stage and a booster vaccination when fish reach a size of about 1–2 g is recommended to avoid high economic losses caused by this disease.

Recently, different stable siderophore deficient and *aro-A* deletion mutant strains have been constructed using an allelic replacement technique, which in experimental trials proved to be useful candidates as live vaccines for striped bass hybrids (Thune et al., 2003).

5. Furunculosis

A. salmonicida subsp. *salmonicida* is the causative agent of the so-called “typical” furunculosis, which causes economically devastating losses in cultivated salmonids in fresh and marine waters. It also affects a variety of non-salmonid fish and shows a widespread distribution (Toranzo et al., 1991a,b; Toranzo and Barja, 1992; Austin and Adams, 1996; Bernoth, 1997; Ellis, 1997; Bricknell et al., 1999; Hiney and Oliver, 1999). The impact of the “typical” furunculosis may even become a limiting factor in the continued survival among certain threatened populations of fish, such as the wild Atlantic salmon in some areas of USA and

Spain. In fact, it has been demonstrated that Atlantic salmon harbour covert *A. salmonicida* infections when they return from ocean migrations (Cipriano et al., 1996; Barja and Dopazo, 2003). Typical furunculosis develops as a chronic or acute haemorrhagic septicaemia, often with extensive liquefactive necrosis. In the acute cases, deep ulcerative lesions usually appear. The atypical strains of *A. salmonicida* are included within three subspecies, *masoucida*, *achromogenes* and *smithia* and cause ulcerative diseases in a variety of fish species such as goldfish (*Carassius auratus*), carps (*Cyprinus* spp.), eel, marine flat fish and salmonids mainly in Europe and Japan.

Although *A. salmonicida* subsp. *salmonicida* can be isolated in conventional microbiological media, the appearance of the typical brown pigmented colonies generally take more than 48 h. For the primary recovery from fish tissues especially in the case of carrier fish, a pre-enrichment of the samples in culture broth is recommended. It has been demonstrated that the mucus is an useful site for a non-lethal detection of *A. salmonicida* from asymptomatic fish (Cipriano et al., 1994). To recover *A. salmonicida* from the mucus samples in which mixed bacterial population usually occur, the use of the selective medium Coomassie Brilliant Blue (CBB) agar is recommended (Cipriano et al., 1992).

A. salmonicida subsp. *salmonicida* can be defined as biochemically, antigenically and genetically homogeneous with no biotypes, serotypes or genotypes being detected (Toranzo et al., 1991a,b; Austin and Austin, 1999; Hiney and Oliver, 1999), which simplify the identification of the typical pigmented strains. Using sensitive DNA-based fingerprinting methodologies such as RAPD analysis, certain genetic heterogeneity can be determined, but no correlation between the generated profiles and the country of origin or host species could be established (Osorio and Toranzo, 2002). All typical *A. salmonicida* strains possess a consistent and distinctive pattern of three or four cryptic plasmid bands (Toranzo et al., 1983; Bast et al., 1988), which has been employed for confirmative identification of this pathogen as well as to design gene-probes or PCR-based methods for rapid diagnosis of furunculosis.

For several years, it has been considered that a correlation exists between virulence and the possession of a cell-surface protein array, the A-layer, this

was further questioned by the isolation of virulent strains lacking this A-layer as well as avirulent strains which retain the A-layer (Toranzo and Barja, 1993). It is now widely accepted that, although cell-surface characteristics can play a role in the pathogenesis of furunculosis, they are not the sole virulent determinants of *A. salmonicida*. In fact, some of the typical signs of the disease are due to the combined effect of two enzymes, a serine protease and a phospholipase (glycerophospholipid cholesterol acyltransferase, GCAT) complexed with LPS (GCAT/LPS) (Lee and Ellis, 1990, 1991).

The slow growth characteristics of this bacterium, the existence of a viable, but not culturable state, as well as the high incidence of covert infections of this pathogen (Austin and Adams, 1996; Enger, 1997), support the need of culture-independent, molecular diagnosis protocols. Many DNA probes and PCR primers have been designed for a rapid and specific detection of *A. salmonicida* subsp. *salmonicida* in pure cultures and in fish tissues. Most of these molecular protocols are based on the use of plasmid sequences, A-layer or 16S rDNA as target genes (Gustafson et al., 1992; Høie et al., 1997; Hiney and Oliver, 1999; Osorio and Toranzo, 2002). Although the highest specificity in the detection of *A. salmonicida* is obtained when the PCR assay is directed to the amplification of the surface A-layer gene (Gustafson et al., 1992), some cross reactivity was observed with *A. hydrophila* strains. Recent studies allowed the design of new primer sets targeted to the gene *fstA* (coding for an outer membrane siderophore-receptor), which showed a total specificity for *A. salmonicida* isolates (Beaz et al., 2003).

Although many furunculosis bacterins have been developed and commercialized from 1980, to be used by injection, immersion or oral route (Newman, 1993; Midtlyng, 1997), their efficacy has been questioned because of the lack of repetitive results and/or the short protection period. The best results in terms of protection have been reported in salmonids with the mineral oil-adjuvanted vaccines. However, these bacterins possess several adverse side-effects such as the induced formation of granulomatous lesions adherent to the viscera and reduction in weight gain (Ellis, 1997; Midtlyng and Lillehaug, 1998). To avoid these drawbacks, new non-mineral oil-adjuvanted vaccines have been recently developed and are

now in the market. Polyvalent vaccines for salmonids including different *Vibrio* species and *A. salmonicida* as antigens are also available which seems to be more effective than monovalent furunculosis bacterins. In addition, the furunculosis vaccines devised for salmonids confer also cross-protection against atypical *A. salmonicida* strains in marine fish such as halibut (*Hippoglossus hippoglossus*) (Gudmundsdóttir et al., 2003). However, in the case of turbot, the protection covered by the typical furunculosis vaccines is very short (about 3 months) even by the i.p. route. Currently, new vaccines and/or immunization strategies are being investigated to achieve a long-term protection of turbot against furunculosis.

Different approaches have been used in the development of live attenuated vaccines against furunculosis. Although A-layer and O-antigen deficient *A. salmonicida* vaccines were effective in providing high levels of fish protection (Thornton et al., 1991, 1994; Munn, 1994), concern exists about a possible reversion to virulence of these incompletely attenuated vaccine strains. However, recombinant DNA technology allowed the construction of highly attenuated and stable *aroA* auxotrophic mutant strains, using an allelic replacement technique, which were employed experimentally as safe live vaccines with high success (Vaughan et al., 1993; Munn, 1994) although approval has not been given for field use.

6. Marine flexibacteriosis

Tenacibaculum maritimum (formerly, *Cytophaga marina*, *Flexibacter marinus* and *F. maritimus*) is the causative agent of flexibacteriosis in marine fish (Wakabayashi et al., 1986; Bernardet and Grimont, 1989; Sukui et al., 2001). Several other names as “gliding bacterial diseases of sea fish”, “eroded mouth syndrome” and “black patch necrosis” were used to designate the disease caused by this bacterium.

Marine flexibacteriosis is widely distributed in cultured and wild fish in Europe, Japan, North America and Australia (McVicar and White, 1979, 1982; Wakabayashi et al., 1986; Pazos et al., 1993; Chen et al., 1995; Devesa et al., 1989; Handlinger et al., 1997; Ostland et al., 1999; Santos et al., 1999; Avendaño-Herrera et al., 2004a). Among the cultured fish, the disease has been reported in turbot, sole,

gilthead seabream, seabass, red seabream, black seabream (*Acanthopagrus schlegeli*), flounder and salmonids. Although both adults and juveniles may be affected by marine flexibacteriosis, younger fish suffer a more severe form of the disease. An increased prevalence and severity of the disease has been reported at higher temperatures (above 15 °C). In addition to water temperature, the disease is influenced by a multiplicity of environmental (stress) and host-related factors (skin surface condition) (Magariños et al., 1995). In general, the affected fish have eroded and haemorrhagic mouth, ulcerative skin lesions, frayed fins and tail rot. A systemic disease can be also established involving different internal organs. The loss of epithelial fish surface, typical of this disease, is also a portal of entry for other bacterial or parasitic pathogens.

The clinical signs, along with the microscopical observation of accumulations of long rods in wet mounts or Gram-stained preparations obtained from gills or lesions, can be used as a initial step for the presumptive diagnosis of marine flexibacteriosis. This preliminary diagnosis must be supported by isolation of the pathogen in the appropriate medium or by the use of specific molecular DNA-based methods applied directly to fish tissues. This bacterium only grow in specific media since it needs an absolute requirement of seawater as well as low concentration of nutrients. Although several media (i.e., Anacker and Ordal, Marine Agar, *Flexibacter maritimus* medium, FMM) have been devised to isolate *F. maritimus*, FMM proved to be the most effective for the recovery of this pathogen from fish tissues (Pazos et al., 1996). Typical colonies of *F. maritimus* are pale-yellow, flat with uneven edges. Although the bacterium is biochemically homogeneous, at least two major O-serogroups can be detected which seem to be related to the host species (Avendaño-Herrera et al., 2004a). Thus, the majority of sole and gilthead seabream isolates constitute a serotype different from those strains isolated from turbot. However, this antigenic heterogeneity would warrant further investigation to clarify the value of serotyping as epidemiological marker in this fish pathogen. Intraspecific genetic variability within *T. maritimum* using RAPD-PCR methodology has been demonstrated regardless of the oligonucleotide primer employed. These strains can be separated in two main clusters strongly

associated with the host and/or serogroups described (Avendaño-Herrera et al., 2004b).

One of the major problems in the study of this bacterium is the difficulty of distinguishing it from other phylogenetically and phenotypically similar species, particularly those of the genera *Flavobacterium* and *Cytophaga*. Therefore, the application of the PCR methodology is important for accurate identification of the pathogen. Although different PCR protocols have been published using the 16S rRNA gene as target (Toyama et al., 1996; Bader and Shotts, 1998), a comparative evaluation of the specificity and sensitivity of both methods (Avendaño-Herrera et al., 2004c) demonstrated that the Toyama PCR protocol was the most adequate for the accurate detection of *T. maritimum* in diagnostic pathology as well as in epidemiological studies of marine flexibacteriosis.

Until recently, no vaccines were available to prevent the disease (Bernadet, 1997), but a flexibacteriosis vaccine (FM 95) has been patented by the University of Santiago (Spain) and is the only bacterin currently in the market to prevent mortalities caused by *F. maritimus* in turbot (Santos et al., 1999). Because this disease affects juvenile and adult turbot, the vaccine is applied by bath when the fish are 1–2 g and later by injection when the fish attain 20–30 g. The percentage of protection by bathing is about 50%, but when the vaccine is administered by i.p. injection the protection increases to more than 85%. Polyvalent formulations to prevent flexibacteriosis and vibriosis or flexibacteriosis and streptococcosis in turbot are also available.

The serological diversity cited above indicates that the vaccine developed for turbot would not be effective in preventing flexibacteriosis in other marine fish. Therefore, a new flexibacteriosis bacterin specific for cultured sole is currently being developed by our research group, and this has conferred RPS values higher than 90% in laboratory trials performed by i.p. injection (Romalde et al., 2005).

7. *Pseudomonadiosis*

Among the *Pseudomonas* species recovered from diseased fish (*P. chlororaphis*, *P. anguilliseptica*, *P. fluorescens*, *P. putida*, *P. plecoglossicida*), *Pseudomonas anguilliseptica* is considered the most signifi-

cant pathogen for cultured fish (Toranzo and Barja, 1993; Austin and Austin, 1999).

P. anguilliseptica was originally described in 1972 as the aetiological agent of “Sekiten-bio” or “red spot disease”, which caused massive mortalities in pond-cultured Japanese eel in Japan (Wakabayashi and Egusa, 1972). Since then, this bacterium has been recorded in European eel reared in Taiwan, Scotland and Denmark (Kuo and Kou, 1978; Stewart et al., 1983). The pathogen was subsequently isolated from other fish species such as black seabream and ayu in Japan (Nakai et al., 1985), salmonids in Finland (Wiklund and Bylund, 1990), wild herring (*Clupea harengus membras*) in the Baltic sea (Lönnström et al., 1994), and from 1995 was considered as responsible agent of the “winter disease syndrome” characteristic of gilthead seabream cultured in the Mediterranean area (Berthe et al., 1995; Doménech et al., 1997). Recently, *P. anguilliseptica* was also recovered as an emerging pathogen of turbot and black spot seabream (*Pagellus bogaraveo*) cultured in Spain (López-Romalde et al., 2003a,c).

The disease occurs at low temperatures (below 16 °C) during the winter months. The main clinical signs of the fish affected by this septicaemia are abdominal distension and haemorrhagic petechia in the skin and internal organs, but the lesions in eels are always more severe than those observed in gilthead seabream.

P. anguilliseptica grows very slowly and weakly on conventional media, but shows enhanced growth on blood agar. *P. anguilliseptica* seems to be a biochemically homogeneous pathogen regardless of the source of isolation (Berthe et al., 1995; Doménech et al., 1997; López-Romalde et al., 2003a). However, the study of the serological characteristics indicate the existence of two major O serotypes related to the fish host, one characteristic of the eel isolates and another typical of the gilthead seabream, turbot and black spot seabream isolates (López-Romalde et al., 2003b,c). In addition, genetic characterization studies employing RAPD techniques revealed the presence of two genetic groups, which were coincident with the two serological groups (López-Romalde et al., 2003a). In addition, Japanese workers described, in the case of eel, that virulence of the strains was related to the presence of a capsular (K) antigen, which confers resistance to the bactericidal action of fish serum

(Nakai, 1985). This information is useful when developing an adequate vaccine against this disease.

Two PCR protocols, based on the amplification of the 16S rRNA gene, have been recently described for a rapid identification of *P. anguilliseptica* (Blanco et al., 2002; Romalde et al., 2004). However, only one (Romalde et al., 2004) showed sufficient sensitivity for the direct detection of the pathogen in the fish tissues, and hence becoming a powerful tool for the diagnosis of fish pseudomoniasis under field conditions.

Recent research efforts by our group led to the development of aqueous and non-mineral oil-adjuvanted bacterins (including the both major serotypes detected), which proved to be effective in experimental trials in gilthead seabream and turbot (Romalde et al., 2005).

8. Streptococcosis

Streptococcal infection of fish is considered a reemerging disease affecting a variety of wild and cultured fish throughout the world (Kitao, 1993; Bercovier et al., 1997; Romalde and Toranzo, 1999, 2002). Classification of Gram positive cocci based on DNA–DNA hybridization coupled with 16S sequencing has shown that at least five different species are considered of significance as fish pathogens: *Lactococcus garvieae* (syn. *Enterococcus seriolicida*), *Lactococcus piscium*, *Streptococcus iniae* (syn. *S. shiloi*), *Streptococcus agalactiae* (syn. *S. difficile*), *Streptococcus parauberis* and *Vagococcus salmoninarum*. Therefore, streptococcosis of fish should be regarded as a complex of similar diseases caused by different genera and species capable of inducing a central nervous damage characterised by suppurative exophthalmia and meningoencephalitis. Whereas “warm water” streptococcosis (causing mortalities at temperatures above 15 °C) typically involves *L. garvieae*, *S. iniae*, *S. agalactiae* and *S. parauberis*, “cold water” streptococcosis (occurring at temperatures below 15 °C) is caused by *Lactococcus piscium* and *V. salmoninarum*. It is important to report that the aetiological agents of “warm water” streptococcosis are considered also as potential zoonotic agents capable to cause disease in humans.

Among these fish streptococci, *L. garvieae*, *S. iniae* and *S. parauberis* can be regarded as the main aetiological agents causing diseases in marine aquaculture.

L. garvieae infects saltwater fish species such as yellowtail in Japan and fresh water species like rainbow trout mainly in Italy, Spain, France and, to a lesser extent, in UK and Australia (Kusuda et al., 1991; Eldar et al., 1996, 1999a; Bercovier et al., 1997; Eldar and Ghittino, 1999; Bark and McGregor, 2001; Romalde and Toranzo, 2002). Recently, a case of *L. garvieae* infection was also reported in wild red sea wrasse (*Coris aygula*) (Colomi et al., 2003).

S. iniae is the main aetiological agent of streptococcosis in tilapia (*Oreochromis* spp.) hybrids in USA and Israel, and rainbow trout in Israel. However, it was isolated from marine fish including yellowtail and flounder in Japan, European seabass and red drum (*Sciaenops ocellatus*) in Israel, and barramundi (*Latex calcarifer*) in Australia (Perera et al., 1994; Eldar et al., 1995, 1999b; Eldar and Ghittino, 1999; Nguyen and Kanai, 1999; Bromage et al., 1999). This species was also isolated from wild fish from the Gulf of Eilat (Colomi et al., 2002).

S. parauberis seems to be endemic of cultured turbot (Toranzo et al., 1994, 1995a; Doménech et al., 1996).

Gram positive cocci can be isolated on general purpose media but growth is enhanced on blood agar. Biochemical characterization can be accomplished by traditional tube and plate procedures as well as using commercial miniaturized systems (Eldar et al., 1999a; Vela et al., 2000; Ravelo et al., 2001). The API-32 Strep proved to be useful for a fast presumptive identification of some of the aetiological agents of streptococcosis; however, misidentification of *L. garvieae* with *L. lactis* subsp. *lactis* or *S. iniae* with *S. uberis* can occur (Weinstein et al., 1997; Ravelo et al., 2001). Besides this, the identification of some species remains difficult, based only on phenotypic traits. Therefore, serological confirmation must be performed by a slide agglutination test or fluorescent antibody procedures using the appropriate specific antisera. Whereas in *L. garvieae* the existence of two serogroups associated with the presence (serotype KG⁻) or absence (KG⁺) of a capsule was demonstrated (Yoshida et al., 1996); in the case of *S. iniae*, two serotypes (I and II) with different capsule

composition were described (Bachrach et al., 2001). By contrast, no serogroups were detected among the *S. parauberis* strains.

Recently, molecular techniques such as ribotyping, RAPD and PFGE, have been usefully applied in epidemiological studies to study the heterogeneity within some of the aetiological agents of fish streptococcosis. With regard to *S. iniae*, the ribotyping allowed to differentiate the American and Israeli fish strains regardless of the host, demonstrating a lack of epidemiological links between infections in the two countries (Eldar et al., 1997a). In the case of *L. garvieae*, the RAPD and PFGE methods were able to differentiate distinct genogroups closely related with the host of origin (rainbow trout, yellowtail and catfish) and, in addition, within the rainbow trout strains, there was evidence of the existence of three genetically distinct clones associated within the geographical origin of the isolates (Ravelo et al., 2003; Vela et al., 2000). The strains of *S. parauberis* isolated from turbot in Spain exhibited the same ribopattern; however, the RAPD analysis showed a higher discrimination power allowing differentiation of the isolates on the basis of their farm of origin (Romalde et al., 1999c).

Molecular techniques to diagnose fish streptococcosis have been applied for two aetiological agents, *L. garvieae* and *S. iniae* (Romalde and Toranzo, 2002), and specific PCR-based protocols have been published for each of these species. Among them, the techniques based on amplification of 16S rDNA (Zlotkin et al., 1998a,b) seem to be of choice as a standard method for diagnosis of these Gram positive cocci. In the case of *S. parauberis*, detection can be performed using the procedures described for mammals by Lämmle et al. (1998), which combines PCR amplification and endonuclease restriction.

Several attempts have been made to develop appropriate vaccination programmes for fish streptococcosis. However, considerable variability in the protection was observed depending on the fish and bacterial species as well as the route of administration. All the streptococcosis vaccines rendered good levels of protection only when they were administered by intraperitoneal injection. However, the *L. garvieae* and *S. iniae* experimental vaccines conferred high protection in rainbow trout for only 3–6 months (Bercovier et al., 1997; Eldar et al., 1997b; Romalde

et al., 2005), but the *L. garvieae* and *S. parauberis* bacterins displayed high levels of long-term protection in yellowtail and turbot, respectively (Toranzo et al., 1995b; Romalde et al., 1999d; Ooyama et al., 1999).

Recent evidence has identified several failures in both licenced and autogenous rainbow trout lactococcosis vaccines (which caused heavy losses in the farms). The antigenic composition of these bacterins corresponded to avirulent non-capsulated strains of *L. garvieae*, which gives little protection against a natural infection with virulent capsulated strains. In the case of *S. iniae* vaccines, they must include both serotypes detected in the pathogen since it was demonstrated that vaccines formulated only with serotype I do not protect fish against infection caused by serotype II (Bachrach et al., 2001).

9. Bacterial kidney disease

Bacterial kidney disease (BKD), caused by the Gram positive diplobacillus, *R. salmoninarum*, is a chronic systemic disease of salmonids, which causes mortality in cultured fish in fresh and marine environments (Sanders and Fryer, 1980; Evsenden et al., 1993; Evelyn, 1993; Fryer and Lannan, 1993; Toranzo and Barja, 1993; Kaattari and Piganelli, 1997; Wiens and Kaattary, 1999; OIE, 2000). The pathogen has also been found in wild fish populations. The disease has been reported to occur in North America, Japan, Western Europe and Chile. It is of economic importance specially with regard to Pacific salmon, because of its widespread distribution in fresh and saline waters, its chronic nature which allows the disease to develop before clinical signs, its vertical transmission through sexual products and the inefficiency of the main therapeutic measures used in treating fish. In fact, the intracellular occurrence of the pathogen inside phagocytic fish cells could contribute to the chronic nature of the disease by protecting it from circulating antibodies and chemotherapeutic agents (Bruno and Munro, 1986a; Bandín et al., 1993, 1995).

Overt disease only appears in advanced cases of infection, when the fish have completed their first year of life. The gross external signs are exophthalmia, abdominal distension and petechial haemorrhage.

The infection is characterised by a systemic infiltration of the viscera by the bacterium causing granulomatous lesions specially in the kidney. Greyish abscesses tend to multiply resulting in enlargement and necrosis of the whole kidney, which appears swollen with irregular greyish areas (Bruno, 1986). Clinical observations only provide a suspicion of BKD because other Gram positive bacteria, namely lactic bacteria, have been demonstrated to produce similar infections in salmonids.

R. salmoninarum isolates are biochemically and antigenically homogeneous (Bruno and Munro, 1986b; Kaattari and Piganelli, 1997), which favours the use of specific antisera in identification procedures. The main common antigen is the heat-stable p57 protein which is present in the cell surface and is also released to fish sera and tissues during the infection (Wiens and Kaattari, 1999). The detection of this 57 kDa major soluble antigen was the basis to the development of serological and genetic methods for disease diagnosis.

R. salmoninarum has been also described as a highly conserved genospecies (Starliper, 1996; Grayson et al., 1999), which makes difficult the differentiation of the isolates from distinct geographic areas or biological sources. The DNA fingerprinting technique RAPD applied to strains from USA, Canada and different countries of Europe, detected a weak correlation of the RAPD profiles obtained with the geographic origin of the isolates (Grayson et al., 2000). Therefore, the epidemiology of BKD remains unclear.

Although isolation of *R. salmoninarum* from fish tissues, followed by serological identification by slide agglutination or immunofluorescence, is considered a definitive diagnosis (Romalde et al., 1995), the bacterium is a fastidious growing organism that requires prolonged incubation (from 2–3 weeks to more 2 months in subclinical cases) at 15 °C to produce colonies (Benediktsdóttir et al., 1991). In addition, serum or serum substitutes such as charcoal and specially L-cysteine are requisite growth factors (Daly and Stevenson, 1993; Bandín et al., 1996a), and different media (i.e., KDM-2, KDM-C, SKDM) have been proposed to improve its growth or reduce the development of associated fast growing microorganisms (Evelyn, 1977; Austin and Austin, 1999). Primary isolation can be enhanced by a heavy

inoculum of a “nurse culture” in the centre of a Petri dish or the addition of sterile spent media to the culture plates (Evelyn et al., 1990).

Since culture of *R. salmoninarum* is difficult and time-consuming, several immunodiagnostic assays are currently used for the detection of the agent in infected tissues. The most widely used serological assays are the direct or indirect immunofluorescence antibody tests and ELISA using polyclonal antisera or monoclonal antibodies (MAbs) directed against different epitopes on p57 antigen (Hsu et al., 1991; Olea et al., 1993; Pascho et al., 1987, 1991; Pascho and Mulcahy, 1987). However, to obviate the risk of cross-reaction with other bacteria (Bandín et al., 1993; Brown et al., 1995), the use of MAbs is recommended. Different commercial ELISA kits such as Aquarapid-Rs (Bionor A/S, Norway) and K-Dtect or Kwik-Dtect (Diagxotic, Inc., USA) are available for the specific detection of the microorganism in fish tissues. However, the detection limit of these kits is about 10^6 bacteria/g of tissue, which indicates that their sensitivity may not detect carrier fish (Bandín et al., 1996b).

PCR or nested reverse transcriptase PCR (RT-PCR) based methods using either primers to the 16S ribosomal RNA or the p57 genes proved to be the most sensitive approaches to detect *R. salmoninarum* in kidney tissue, ovarian fluids and salmonid eggs as well as in fish lymphocytes (Brown et al., 1994; Magnússon et al., 1994; McIntosh et al., 1996; Miriam et al., 1997; Chase and Pascho, 1998; Cook and Lynch, 1999; Osorio and Toranzo, 2002). Since it was demonstrated that kidney tissue could produce some inhibitory effect reducing the sensitivity of the assay, it is suggested the use of lymphocyte lysates rather than crude tissues in the PCR technique (McIntosh et al., 1996). In addition, the nested RT-PCR assays (Magnússon et al., 1994; Cook and Lynch, 1999) means an important advancement in *R. salmoninarum* detection protocols since this molecular approach allows the detection of viable *R. salmoninarum*.

Although vaccination trials using classical bacterins, recombinant vaccines or attenuated live vaccines have been reported, and there is evidence that under some conditions *Renibacterium* elicits an immune response in fish (Newman, 1993; Kaattari and Piganelli, 1997; Griffiths et al., 1998; Daly et al.,

2001), the protective ability of a vaccine in field conditions is questionable because the intracellular nature and vertical transmission of the pathogen as well as by the possible immunosuppressive role of the protein p57 (Wood and Kaattari, 1996). Although a whole cell *R. salmoninarum* bacterin in which the p57 protein was eliminated (p57⁻ vaccine) failed to reliably protect salmonids by the i.p. route, promising results were obtained when this vaccine was administered by the oral route (Piganelli et al., 1999).

Recently, a commercial aqueous live vaccine has been licenced under the name of “Renogen” (Salonius et al., 2003). This vaccine is constituted by live cells of *Arthrobacter davidanieli* (proposed nomenclature), a non-pathogenic environmental bacterium which express an extracellular polysaccharide with antigenic homology to that of *R. salmoninarum*. In field trials, the “Renogen” conferred a significant long-term protection of Atlantic salmon against BKD, with RPS values higher than 70% 24 months after vaccination.

10. Mycobacteriosis (fish tuberculosis)

Mycobacteriosis in fish (or fish tuberculosis) is a subacute to chronic wasting disease known to affect near 200 freshwater and saltwater species. Although *Mycobacterium marinum* is considered the primary causative agent of fish mycobacteriosis, a great number of *Mycobacterium* species associated with tubercle granulomas in cultured, aquarium and wild fish populations have been described: *M. marinum*, *M. fortuitum*, *M. chelonae*, *M. smegmatis*, *M. abscessus*, *M. neonarum*, *M. simiae*, *M. scrofulaceum*, *M. poriferae* and *M. triplex*-like (Hedrick et al., 1987; Bragg et al., 1990; Colorni, 1992; Colorni et al., 1996; Lansdell et al., 1993; Bruno et al., 1998b; Talaat et al., 1999; Chinabut, 1999; Diamant et al., 2000; Rhodes et al., 2001; Herbst et al., 2001; dos Santos et al., 2002). All these species can also cause disease in humans (Falkinham, 1996; Decostere et al., 2004).

Although in cultured fish, mycobacteriosis was documented in Pacific and Atlantic salmon, pejerrey (*Odonthestes bonariensis*), snakehead fish (*Chana striatus*), turbot, tilapia, European seabass and red drum, since 1990 mycobacteriosis caused by *M.*

marinum represents a significant threat specially for seabass cultured on the Mediterranean and the Red Sea coast of Israel (Colorni, 1992; Colorni et al., 1993, 1996; Diamant et al., 2000). Recently, this disease is considered a matter of concern in the turbot culture in Europe (dos Santos et al., 2002).

Among the wild marine fish that have been reported to suffer mycobacteriosis are cod, halibut, striped bass and Atlantic mackerel (*Scomber scomber*) (Chinabut, 1999).

As mycobacteriosis is a chronic disease, it seems likely that the fish maintained in aquaria will show a higher incidence of this disease than cultured or wild species, because aquarium fish are often kept for long periods of time compared with fish raised for commercial purposes.

Internal signs of mycobacteriosis vary according to the fish species but typically include greyish-white nodules (granulomas) in the spleen, kidney and liver. External manifestations include scale loss accompanied by haemorrhagic lesions penetrating the musculature in advanced cases.

Diagnosis of the disease depends on clinical and histological signs and identification of the bacterial pathogen. Smears from spleen and kidney tissues should be made and stained with Ziehl-Neelsen based stains in order to visualize the acid-fast short bacilli characteristic of *Mycobacterium* species. An immunocytochemical method using the avidin–biotin complex was recommended to demonstrate the presence of small number of mycobacteria in affected tissues (Gómez et al., 1993). However, for a precise diagnosis of mycobacterial infection at species level, the isolation and identification of the microorganisms using specific media and phenotypical tests devised for clinical *Mycobacterium*, including fatty acid and mycolic acid analysis, are required. In addition, identification should be confirmed by 16S ribosomal gene DNA sequencing (Bruno et al., 1998b; Knibb et al., 1993; Herbst et al., 2001).

Mycobacteriosis remains asymptomatic for long period, stunts fish growth, is virtually impossible to eradicate with chemotherapeutic agents and renders the affected fish unmarketable. The slow and poor growth exhibited by the majority of the *Mycobacterium* species requires a reliable DNA-based method for fast identification of the main pathogenic *Mycobacterium* species in fish tissues, specially in the case

of latent infections. PCR approaches using the 16S rDNA as target gene, coupled with restriction enzyme analysis of the amplified fragment, were already reported and proved to be highly specific and sensitive for the detection of mycobacteria not only in fish tissues but also in the blood (Colorni et al., 1993; Knibb et al., 1993; Talaat et al., 1997). Therefore, this methodology can constitute an useful non-destructive method to screen broodstock.

11. Piscirickettsiosis

Piscirickettsiosis is a septicemic condition of salmonids (Fryer and Lannan, 1996; Almendras and Fuentealba, 1997; Larenas et al., 1999; Lannan et al., 1999; OIE, 2000). The causative agent of the disease is *Piscirickettsia salmonis* (Fryer et al., 1992), a non-motile Gram negative, obligately intracellular bacterium. The disease was described for the first time in 1989 affecting to coho salmon cultured in Chile (Bravo and Campos, 1989; Branson and Nieto, 1991; Cvitanich et al., 1991) where mortalities between 30% and 90% were reported. From 1992, the disease was also described in Ireland, Norway, Scotland, and both the west and east coasts of Canada (Rodger and Drinan, 1993; Grant et al., 1996; Palmer et al., 1997; Olsen et al., 1997; Jones et al., 1998; OIE, 2000; Birrell et al., 2003). Although *P. salmonis* has been detected in different species of Pacific salmon, Atlantic salmon and rainbow trout, the most susceptible species seems to be coho salmon. Natural outbreaks of piscirickettsiosis typically occur a few weeks after smolts are transferred to the sea (Fryer et al., 1990; Branson and Nieto, 1991; Cvitanich et al., 1991). However, the disease has also been observed in fresh water facilities (Bravo, 1994; Gaggero et al., 1995).

Although horizontal transmission is one of the main routes of infection, in certain cases, the existence of vertical transmission of *P. salmonis* has been demonstrated (Larenas et al., 2003). Therefore, to avoid the possible risk of congenital transmission of the pathogen, the Chilean salmon farming industry has implemented the elimination of carrier broodstock. Intermediate vectors such as external hemaphagous isopods may also play a role in the natural transmission of piscirickettsiosis.

Reported clinical signs of affected fish by piscirickettsiosis are lethargy, anorexia, darkening of the skin, respiratory distress and surface swimming. The first physical evidence of the disease may be the appearance of small white lesions or shallow haemorrhagic ulcers on the skin. However, often the fish die without any visible clinical signs. The most characteristic gross internal lesions are off-white to yellow subcapsular nodules, measuring up to 2 cm in diameter, scattered throughout the liver (Almendras and Fuentealba, 1997; Lannan et al., 1999).

P. salmonis can only be isolated in fish cell lines, without antibiotics added, commonly employed in virology (CHSE-214 or EPC) where it produces a cytopathic effect. Therefore, a preliminary diagnosis of the disease is normally made by examination of Gram, Giemsa or acridine orange-stained kidney or liver imprints, with confirmation by serological methods such as immunofluorescence or immunohistochemistry employing specific antiserum (Lannan et al., 1991; Alday-Sanz et al., 1994). Although an ELISA assay is commercially available (Microteck International Ltd., Canada or DiagXotics, Inc., USA), there are scarce information of its efficacy in field samples. In addition, the identity of the aetiological agent of piscirickettsiosis can be confirmed by PCR-assays. Until present, two different PCR-based protocols have been published for the fast diagnosis of the disease in infected tissues. Whereas one of them is based in a nested PCR assay employing the 16S DNA as the target gene (Mauel et al., 1996), in the other protocol part of the internal transcribed spacer (ITS) of the ribosomal RNA operon is amplified (Marschall et al., 1998). The latter PCR assay was further employed in phylogenetic studies of strains of *P. salmonis* (Mauel et al., 1999; Heath et al., 2000). Both serological and molecular methods must be also utilized to confirm the isolation of *P. salmonis* in fish cell-lines.

It is noteworthy that although kidney and liver tissues are the recommended sources for the isolation of *P. salmonis* (OIE, 2000), it was recently reported that the brain might represent an important residence site of the pathogen, being its bacterial load approximately 100 times higher than the loads observed in liver and kidney (Skarmeta et al., 2000; Heath et al., 2000).

Commercial vaccines against *P. salmonis* are available in Chile, but the efficacy of these bacterins is questioned because the lack of enough protection data from experimental and field trials (Smith et al., 1997; Larenas et al., 1999). Recently, a monovalent recombinant subunit vaccine for *P. salmonis* has been constructed which elicited a high protection in coho salmon in laboratory trials (Kuzyk et al., 2001). In addition, the live vaccine “Renogen” developed to prevent bacterial kidney disease was also effective in reducing mortality from *P. salmonis* in Pacific salmon with significant long-term protection in both laboratory and field conditions (Salonius et al., 2003).

Salmonids have not been the only target fish of *Rickettsia*-like organisms (RLOs), and several reports have been published describing rickettsial infections as the responsible of epizootic outbreaks in non-salmonid fresh water and marine fish such as species of tilapia in Taiwan, imported blue-eyed plecostomus (*Panaque suttoni*) in USA and juvenile seabass in Europe (Comps et al., 1996; Lannan et al., 1999; Steiropoulos et al., 2002; Mauel et al., 2003). In the majority of the cases, no comparison between these *Rickettsia*-like organisms and the *P. salmonis* isolates have been conducted, but recent immunohistochemistry studies (Steiropoulos et al., 2002) demonstrate antigenic similarities between the RLOs from European seabass and *P. salmonis*.

12. Emerging pathologies

Two emerging diseases are affecting cultured Atlantic salmon, pasteurellosis caused by *Pasteurella skyensis* and streptococcosis by *Streptococcus phocae*.

Different outbreaks of pasteurellosis caused by the new *Pasteurella* species, *P. skyensis*, were reported in farmed Atlantic salmon in Scotland from 1995 to 1998 (Jones and Cox, 1999; Birkbeck et al., 2002). The disease occurred between April to October and cumulative mortalities were around 6% of the affected population. Diseased fish show significant cataracts and loss of weight. Internal examination of moribund/dead fish revealed no feed in the stomach and a variable pathology, which appeared to progress over time. This initially consisted of petechia on caecal fat and peritoneal surfaces, and discrete white focal

lesions through the kidney, spleen and heart. In later samples, pericarditis, generalized peritonitis with granulomata formation and the presence of false membranes in the peritoneal organs and swimbladder, became predominant pathological lesions (Jones and Cox, 1999).

The aetiological agent, *P. skyensis*, is an halophilic bacterium which shows an strictly requirement for blood. Therefore, primary isolation of the micro-organism must be done on tripticase soy agar supplemented with 1.5% salt and 5% defibrinated blood. In this medium, convex, smooth and grey colonies appears after 48 h incubation at 22 °C. Growth occurs from 14 to 32 °C, which can explain the stationarity of the disease. *P. skyensis* differs from most other members of *Pasteurellaceae* in lacking catalase and nitrate-reducing activities (Birkbeck et al., 2002).

Since 1999 to date, streptococcosis outbreaks occurred repeatedly during the summer months in Atlantic salmon farmed in Chile affecting both smolts and adult fish. The cumulative mortality can reach the 20% of the affected population. Diseased fish show exophthalmia with accumulation of purulent and haemorrhagic fluid around eyes, and ventral petechial haemorrhage. At necropsy, haemorrhage in the abdominal fat, pericarditis, and enlarged liver (showing a yellowish colour), spleen, and kidney are common pathological changes. Recent molecular studies performed by our research group demonstrated that the causative agent of this streptococcosis belongs to the species *S. phocae* (unpublished results).

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References

- Actis, L.A., Tolmasky, M.E., Crosa, J.H., 1999. Vibriosis. In: Woo, P.T.K., Bruno, D.W. (Eds.), Fish Diseases and Disorders, vol. 3. CAB Intern., Publ., United Kingdom, pp. 523–558.
- Alday-Sanz, V., Rodger, H., Turnbull, T., Adams, A., Richards, R.H., 1994. An immunohistochemical diagnostic test for rickettsial disease. J. Fish Dis. 17, 189–191.

- Almendras, F., Fuentealba, C., 1997. Salmonid rickettsial septicemia caused by *Piscirickettsia salmonis*: a review. *Dis. Aquat. Org.* 29, 137–144.
- Amaro, C., Biosca, E.G., 1996. *Vibrio vulnificus* biotype 2, pathogenic for eels, is also an opportunistic pathogen for humans. *Appl. Environ. Microbiol.* 62, 1454–1457.
- Amaro, C., Biosca, E.G., Esteve, C., Fouz, B., Toranzo, A.E., 1992. Comparative study of phenotypic and virulence properties in *Vibrio vulnificus* biotype 1 and 2 obtained from a European eel farm experiencing mortalities. *Dis. Aquat. Org.* 13, 29–35.
- Arias, C.R., Garay, E., Aznar, R., 1995. Nested PCR method for rapid and sensitive detection of *Vibrio vulnificus* in fish, sediments, and water. *Appl. Environ. Microbiol.* 61, 3476–3478.
- Arias, C.R., Verdonck, L., Swings, J., Garay, E., Aznar, R., 1997. Intraspecific differentiation of *Vibrio vulnificus* biotypes by amplified fragment length polymorphism and ribotyping. *Appl. Environ. Microbiol.* 63, 2600–2666.
- Austin, B., Adams, C., 1996. Fish pathogens. In: Austin, B., Altwegg, A., Gosling, P.J., Joseph, S. (Eds.), *The Genus Aeromonas*. John Wiley & Sons, Chichester, UK, pp. 197–244.
- Austin, B., Austin, D.A., 1999. *Bacterial Fish Pathogens. Diseases of Farmed and Wild Fish*. Springer-Praxis Publishing, Ltd., United Kingdom.
- Avendaño-Herrera, R., Magariños, B., López-Romalde, S., Romalde, J.L., Toranzo, A.E., 2004a. Phenotypic characterization and description of two major O-serotypes in *Tenacibaculum maritimum* strains from marine fishes. *Dis. Aquat. Org.* 58, 1–8.
- Avendaño-Herrera, R., Rodríguez, J., Magariños, B., Romalde, J.L., Toranzo, A.E., 2004b. Intraspecific diversity of the marine fish pathogen *Tenacibaculum maritimum* as determined by randomly amplified polymorphic DNA-PCR. *J. Appl. Microbiol.* 96, 871–877.
- Avendaño-Herrera, R., Magariños, B., Toranzo, A.E., Beaz, R., Romalde, J.L., 2004c. Comparative evaluation of species-specific polymerase chain reaction primer sets for the routine identification of *Tenacibaculum maritimum*. *Dis. Aquat. Org.* 62, 75–83.
- Aznar, R., Ludwig, W., Schleifer, K.H., 1993. Ribotyping and randomly amplified polymorphic DNA analysis of *Vibrio vulnificus* biotypes. *Syst. Appl. Microbiol.* 16, 303–309.
- Bachrach, G., Zlotkin, A., Hurvitz, A., Evans, D.L., Eldar, A., 2001. Recovery of *Streptococcus iniae* from diseased fish previously vaccinated with a *Streptococcus* vaccine. *Appl. Environ. Microbiol.* 67, 3756–3758.
- Bader, J.A., Shotts, E.B., 1998. Identification of *Flavobacterium* and *Flexibacter* species by species-species polymerase chain reaction primers to the 16S ribosomal RNA gene. *J. Aquat. Anim. Health* 10, 311–319.
- Bakopoulos, V., Volpatti, D., Papapanagiotou, E., Richards, R., Galleotti, M., Adams, A., 1997. Development of an ELISA to detect *Pasteurella piscicida* in cultured and 'spiked' fish tissue. *Aquaculture* 156, 359–366.
- Bandín, I., Santos, Y., Barja, J.L., Toranzo, A.E., 1993. Detection of a common antigen among *Renibacterium salmoninarum*, *Corynebacterium aquaticum*, and *Carnobacterium piscicola* by the Western blot technique. *J. Aquat. Anim. Health* 5, 172–176.
- Bandín, I., Rivas, C., Santos, Y., Secombes, J.C., Barja, J.L., 1995. Effect of serum factors on the survival of *Renibacterium salmoninarum* within rainbow trout macrophages. *Dis. Aquat. Org.* 23, 221–227.
- Bandín, I., Santos, Y., Barja, J.L., Toranzo, A.E., 1996a. Growth of the fish pathogen *Renibacterium salmoninarum* on different media. *Microbiología (SEM)* 12, 439–442.
- Bandín, I., Heinen, P., Brown, L.L., Toranzo, A.E., 1996b. Comparison of different ELISA Kits for detecting *Renibacterium salmoninarum*. *Bull. Eur. Assoc. Fish Pathol.* 16, 19–22.
- Barja, J.L., Dopazo, C.P., 2003. Posibilidades prácticas y limitaciones del control sanitario en el salmón Atlántico. Actuciones desde la Consellería de Medio Ambiente. In: Lamuela, M., Alvarez, J. (Eds.), *IV Jornadas del Salmón Atlántico en la Península Ibérica*. Departamento de Medio Ambiente, Gobierno de Navarra, pp. 145–154.
- Bark, S., McGregor, D., 2001. The first occurrence of lactococcosis in farmed trout in England. *Trout News* 31, 9–11.
- Bast, L., Daly, J.G., De Grandis, S.A., Stevenson, R.M.W., 1988. Evaluation of profiles of *Aeromonas salmonicida* as epidemiological markers of furunculosis infections in fish. *J. Fish Dis.* 11, 133–145.
- Beaz, R., Romalde, J.L., Ravelo, C., Barja, J.L., 2003. Nuevos cebadores del gen *fstA*, receptor del complejo hierro-sideróforo para detectar *Aeromonas salmonicida* por PCR. Abstract XIX Congreso Nacional de Microbiología. Santiago de Compostela, Spain, p. 16.
- Benediktsdóttir, E., Helgason, S., Gudmundsdóttir, S., 1991. Incubation time for the cultivation of *Renibacterium salmoninarum* from Atlantic salmon, *Salmo salar* L., broodfish. *J. Fish Dis.* 14, 97–102.
- Benediktsdóttir, E., Helgason, S., Sigurjónsdóttir, H., 1998. *Vibrio* spp. isolated from salmonids with shallow skin lesions and reared at low temperature. *J. Fish Dis.* 21, 19–28.
- Benediktsdóttir, E., Verdonck, L., Spröer, C., Helgason, S., Swings, J., 2000. Characterization of *Vibrio viscosus* and *Vibrio wodanis* isolated at different geographical locations: a proposal for reclassification of *Vibrio viscosus* as *Moritella viscosa* comb. nov. *Int. J. Syst. Evol. Microbiol.* 50, 479–488.
- Bercovier, H., Ghittino, C., Eldar, A., 1997. Immunization with bacterial antigens: infections with streptococci and related organisms. In: Gudding, R., Lillehaug, A., Midtlyng, P.J., Brown, F. (Eds.), *Fish Vaccinology*. Karger, Basel, Switzerland, pp. 153–160.
- Bernadet, J.F., 1997. Immunization with bacterial antigens: *Flavobacterium* and *Flexibacter* infections. In: Gudding, R., Lillehaug, A., Midtlyng, P.J., Brown, F. (Eds.), *Fish Vaccinology*. Karger, Basel, Switzerland, pp. 179–188.
- Bernadet, J.F., Grimont, P.A.D., 1989. Deoxyribonucleic acid relatedness and phenotypic characteristics of *Flexibacter columnaris* sp. nov. nom. rev. *Flexibacter psychrophilus* sp. nov. nom. rev. and *Flexibacter maritimus* Wakabayashi, Hikida and Masamura, 1986. *Int. J. Syst. Bacteriol.* 39, 346–354.
- Bernoeth, E.M., 1997. Furunculosis: the history of the disease and of disease research. In: Bernoeth, E.M., Ellis, A.E., Midtlyng, P.J., Smith, P. (Eds.), *Furunculosis. Multidisciplinary Fish Disease Research*. Academic Press, United Kingdom, pp. 1–20.

- Berthe, F.C.J., Michel, C., Bernardet, J.-F., 1995. Identification of *Pseudomonas anguilliseptica* isolated from several fish species in France. *Dis. Aquat. Org.* 21, 151–155.
- Biosca, E.G., Amaro, C., Esteve, C., Alcaide, E., Garay, E., 1991. First record of *Vibrio vulnificus* biotype 2 from diseased European eel, *Anguilla anguilla*, L. *J. Fish Dis.* 14, 103–109.
- Biosca, E.G., Amaro, C., Larsen, J.L., Pedersen, K., 1997. Phenotypic and genotypic characterization of *Vibrio vulnificus*: proposal for the substitution of the subspecific taxon for serovar. *Appl. Environ. Microbiol.* 63, 1460–1466.
- Birkbeck, T.H., Laidler, L.A., Grant, A.N., Cox, D.I., 2002. *Pasteurella skyensis* sp. nov., isolated from Atlantic salmon (*Salmo salar* L.). *Int. J. Syst. Evol. Microbiol.* 52, 699–704.
- Birrell, J., Mitchell, S., Bruno, D.W., 2003. *Piscirickettsia salmonis* in farmed Atlantic salmon, *Salmo salar* in Scotland. *Bull. Eur. Assoc. Fish Pathol.* 23, 213–217.
- Blanco, M.M., Gibello, A., Vela, A.I., Moreno, M.A., Domínguez, L., Fernández-Garayzábal, J.F., 2002. PCR detection and PFGE DNA macrorestriction analysis of clinical isolates of *Pseudomonas anguilliseptica* from winter disease outbreaks in sea bream *Sparus aurata*. *Dis. Aquat. Org.* 50, 19–27.
- Bragg, R.R., Huchzermeyer, H.F., Hanisch, M.A., 1990. *Mycobacterium fortuitum* isolated from three species of fish in South Africa. *Onderstepoort J. Vet. Res.* 57, 101–102.
- Branson, E.J., Nieto, D., 1991. Description of a new disease condition occurring in farmed coho salmon, *Oncorhynchus kisutch* (Walbaum), in South America. *J. Fish Dis.* 14, 147–156.
- Bravo, S., 1994. Piscirickettsiosis in fresh water. *Bull. Eur. Assoc. Fish Pathol.* 14, 137–138.
- Bravo, S., Campos, M., 1989. Coho salmon syndrome in Chile. *FHS/AFS Newsl.* 17, 3.
- Bricknell, I.R., Bowden, T.J., Bruno, D.W., MacLachlan, P., Johnstone, R., Ellis, A.E., 1999. Susceptibility of halibut, *Hippoglossus hippoglossus* (L.), to infection with *Aeromonas salmonicida*. *Aquaculture* 175, 1–13.
- Bromage, E.S., Thomas, A., Owens, L., 1999. *Streptococcus iniae*, a bacterial infection in barramundi *Lates calcarifer*. *Dis. Aquat. Org.* 36, 177–181.
- Brown, L.L., Evelyn, T.P.T., Iwama, G.K., Nelson, W.S., Levine, R.P., 1994. Use of polymerase chain reaction (PCR) to detect DNA from *Renibacterium salmoninarum* within individual salmonid eggs. *Dis. Aquat. Org.* 18, 165–171.
- Brown, L.L., Iwama, G.K., Evelyn, T.P.T., Nelson, W.S., Levine, R.P., 1995. Bacterial species other than *Renibacterium salmoninarum* cross-react with antisera against *R. salmoninarum* but are negative for the p57 gene of *Renibacterium salmoninarum* as detected by the polymerase chain reaction (PCR). *Dis. Aquat. Org.* 21, 227–231.
- Bruno, D.W., 1986. Histopathology of bacterial kidney disease in laboratory infected rainbow trout, *Salmo gairdneri* (Richardson), and Atlantic salmon, *Salmo salar* L., with reference to naturally infected fish. *J. Fish Dis.* 9, 523–537.
- Bruno, D.W., Munro, A.L.S., 1986a. Observations on *Renibacterium salmoninarum* and the salmonid egg. *Dis. Aquat. Org.* 1, 83–87.
- Bruno, D.W., Munro, A.L.S., 1986b. Uniformity in the biochemical properties of *Renibacterium salmoninarum* isolates obtained from several sources. *FEMS Microbiol. Lett.* 33, 247–250.
- Bruno, D.W., Hastings, T.S., Ellis, A.E., 1986. Histopathology, bacteriology and experimental transmission of a cold water vibriosis in Atlantic salmon *Salmo salar*. *Dis. Aquat. Org.* 1, 163–168.
- Bruno, D.W., Griffiths, J., Petrie, J., Hastings, T.S., 1998a. *Vibrio viscosus* in farmed Atlantic salmon *Salmo salar* in Scotland: field and experimental observations. *Dis. Aquat. Org.* 34, 161–166.
- Bruno, D.W., Griffiths, J., Mitchell, C.C., Wood, B.P., Fletcher, Z.J., Brobniewski, F.A., Hastings, T.S., 1998b. Pathology attributed to *Mycobacterium chelonae* infection among farmed and laboratory-infected Atlantic salmon *Salmo salar*. *Dis. Aquat. Org.* 33, 101–109.
- Chase, D.M., Pascho, R.J., 1998. Development of a nested polymerase chain reaction for amplification of a sequence of the p57 gene of *Renibacterium salmoninarum* that provides a highly sensitive method for detection of the bacterium in salmonid kidney. *Dis. Aquat. Org.* 34, 223–229.
- Chen, M.F., Henry-Ford, D., Groff, J.M., 1995. Isolation of *Flexibacter maritimus* from California. *FHS/AFS Newsl.* 22, 7–11.
- Chinabut, S., 1999. Mycobacteriosis and Nocardiosis. In: Woo, P.T.K., Bruno, D.W. (Eds.), *Fish Diseases and Disorders*, vol. 3. CAB Intern. Publ., United Kingdom, pp. 319–340.
- Cipriano, R.C., Ford, L.A., Teska, J.D., Hale, L.E., 1992. Detection of *Aeromonas salmonicida* in the mucus of salmonid fish. *J. Aquat. Anim. Health* 4, 114–118.
- Cipriano, R.C., Ford, L.A., Shachte, J.H., Petrie, C., 1994. Evaluation of mucus as a valid site to isolate *Aeromonas salmonicida* among asymptomatic populations of lake trout (*Salvelinus mayaycush*). *Biomed. Lett.* 49, 229–233.
- Cipriano, R.C., Ford, L.A., Teska, J.D., Shachte, J.H., Petrie, C., Novak, B.M., Flint, D.E., 1996. Use of non-lethal procedures to detect and monitor *Aeromonas salmonicida* in potentially endangered or threatened populations of migrating and post-spawning salmon. *Dis. Aquat. Org.* 27, 233–236.
- Coleman, S.S., Oliver, J.D., 1996. Optimization of conditions for the polymerase chain reaction amplification of DNA from culturable and nonculturable cells of *Vibrio vulnificus*. *FEMS Microbiol. Ecol.* 19, 127–132.
- Colorni, A., 1992. A systemic mycobacteriosis in the European seabass *Dicentrarchus labrax* cultured in Eilat (Red Sea). *Isr. J. Aquac.-Bamidgah* 44, 75–81.
- Colorni, A., Ankaoua, M., Diamant, A., Knibb, W., 1993. Detection of mycobacteriosis in fish using the polymerase chain reaction technique. *Bull. Eur. Assoc. Fish Pathol.* 13, 195–198.
- Colorni, A., Ucko, M., Knibb, W., 1996. Epizootiology of *Mycobacterium* spp. in seabass, seabream and other commercial fish. *Seabass and Seabream Culture: Problems and Prospects*. Eur. Aquacult. Soc. Spec. Publ., Verona, Italy, pp. 259–261.
- Colorni, A., Diamant, A., Eldar, A., Kvitt, H., Zlotkin, A., 2002. *Streptococcus iniae* infections in red seacage-cultured and wild fishes. *Dis. Aquat. Org.* 49, 165–170.
- Colorni, A., Ravelo, C., Romalde, J.L., Toranzo, A.E., Diamant, A., 2003. *Lactococcus garvieae* in wild red sea wrasse *Coris aygula* (Labridae). *Dis. Aquat. Org.* 56, 275–278.

- Comps, M., Raymond, J.C., Plassiart, G.N., 1996. Rickettsia-like organism infecting juvenile sea-bass *Dicentrarchus labrax*. Bull. Eur. Assoc. Fish Pathol. 16, 30–33.
- Cook, M., Lynch, W.H., 1999. A sensitive nested reverse transcriptase PCR-assay to detect viable cells of the fish pathogen *Renibacterium salmoninarum* in Atlantic salmon (*Salmo salar* L.). Appl. Environ. Microbiol. 65, 3042–3047.
- Cvitanich, J.D., Gárate, O., Smith, C.E., 1991. The isolation of a Rickettsia-like organism causing disease and mortality in Chilean salmonids and its confirmation by Koch's postulate. J. Fish Dis. 14, 121–145.
- Dalsgaard, I., Hoi, L., Siebeling, R.J., Dalsgaard, A., 1998. Indole-positive *Vibrio vulnificus* isolated from outbreaks on a Danish eel farm. Dis. Aquat. Org. 35, 187–194.
- Daly, J.G., Stevenson, R.M.W., 1993. Nutritional requirements of *Renibacterium salmoninarum* on agar and in broth media. Appl. Environ. Microbiol. 59, 2178–2183.
- Daly, J.G., Griffiths, S.G., Kew, A.K., Moore, A.R., Olivier, G., 2001. Characterization of attenuated *Renibacterium salmoninarum* strains and their use as live vaccines. Dis. Aquat. Org. 44, 121–126.
- Decostere, A., Hermans, K., Haesebrouck, F., 2004. Piscine mycobacteriosis: a literature review covering the agent and the disease it causes in fish and humans. Vet. Microbiol. 99, 159–166.
- Devesa, S., Barja, J.L., Toranzo, A.E., 1989. Ulcerative and skin and fin lesions in reared turbot (*Scophthalmus maximus* L.). J. Fish Dis. 12, 323–333.
- Diamant, A., Banet, A., Ucko, M., Colomi, A., Knibb, W., Kvitt, H., 2000. Mycobacteriosis in wild rabbitfish *Siganus rivulatus* associated with cage farming in the Gulf of Eilat, Red Sea. Dis. Aquat. Org. 39, 211–219.
- Doménech, A., Fernández-Garayzábal, J.F., Pascual, C., García, J.A., Cutúli, M.T., Moreno, M.A., Collins, M.D., Domínguez, L., 1996. Streptococcosis in cultured turbot, *Scophthalmus maximus* (L.), associated with *Streptococcus parauberis*. J. Fish Dis. 19, 33–38.
- Doménech, A., Fernández-Garayzábal, J.F., Lawson, P., García, J.A., Cutúli, M.T., Blanco, M., Gibello, A., Moreno, M.A., Collins, M.D., Domínguez, L., 1997. Winter disease outbreak in sea bream (*Sparus aurata*) associated with *Pseudomonas anguilliseptica* infection. Aquaculture 156, 317–326.
- dos Santos, N.M.S., do Vale, A., Sousa, M.J., Silva, M.T., 2002. Mycobacterial infection in farmed turbot *Scophthalmus maximus*. Dis. Aquat. Org. 52, 87–91.
- Egidius, E., Wiik, R., Andersen, K., Holff, K.A., Hjeltness, B., 1986. *Vibrio salmonicida* sp. nov., a new fish pathogen. Int. J. Syst. Bacteriol. 36, 518–520.
- Eldar, A., Ghittino, C., 1999. *Lactococcus garvieae* and *Streptococcus iniae* infections in rainbow trout *Oncorhynchus mykiss*: similar, but different diseases. Dis. Aquat. Org. 36, 227–231.
- Eldar, A., Frelie, P.F., Assenta, L., Varner, P.W., Lawhon, S., Bercovier, H., 1995. *Streptococcus shiloi*, the name for an agent causing septicemic infection in fish, is a junior synonym of *Streptococcus iniae*. Int. J. Syst. Bacteriol. 45, 840–842.
- Eldar, A., Ghittino, C., Asanta, L., Bozzetta, E., Goria, M., Prearo, M., Bercovier, H., 1996. *Enterococcus seriolicida* is a junior synonym of *Lactococcus garvieae*, a causative agent of septicemia and meningoencephalitis in fish. Curr. Microbiol. 32, 85–88.
- Eldar, A., Lawhon, S., Frelie, P.F., Assenta, L., Simpson, B.R., Varner, P.W., Bercovier, H., 1997a. Restriction fragment length polymorphisms of 16S rDNA and of whole rRNA genes (ribotyping) of *Streptococcus iniae* strains from the United States and Israel. FEMS Microbiol. Lett. 151, 155–162.
- Eldar, A., Horovitz, A., Bercovier, H., 1997b. Development and efficacy of a vaccine against *Streptococcus iniae* infection in farmed rainbow trout. Vet. Immunol. Immunopathol. 56, 175–183.
- Eldar, A., Goria, M., Ghittino, C., Zlotkin, A., Bercovier, H., 1999a. Biodiversity of *Lactococcus garvieae* isolated from fish in Europe, Asia, and Australia. Appl. Environ. Microbiol. 65, 1005–1008.
- Eldar, A., Perl, S., Frelie, P.F., Bercovier, H., 1999b. Red drum *Sciaenops ocellatus* mortalities associated with *Streptococcus iniae* infection. Dis. Aquat. Org. 36, 121–127.
- Ellis, A.E., 1997. Immunization with bacterial antigens: furunculosis. In: Gudding, R., Lillehaug, A., Midtlyng, P.J., Brown, P.R. (Eds.), Fish Vaccinology. Karger, Basel, pp. 107–116.
- Enger, Ø., 1997. Survival and inactivation of *Aeromonas salmonicida* outside the host—a most superficial way of life. In: Bernoth, E.M., Ellis, A.E., Midtlyng, P.J., Smith, P. (Eds.), Furunculosis. Multidisciplinary Fish Disease Research. Academic Press, United Kingdom, pp. 159–177.
- Espelid, S., Hjelmeland, K., Jørgensen, T., 1987. The specificity of Atlantic salmon antibodies made against the fish pathogen *Vibrio salmonicida*, establishing the surface protein VS-P1 as the predominant antigen. Dev. Comp. Immunol. 11, 529–537.
- Esteve-Gassent, D., Fouz, B., Barrera, R., Amaro, C., 2004. Efficacy of oral reimmunisation after immersion vaccination against *Vibrio vulnificus* in farmed European eels. Aquaculture 231, 9–22.
- Evelyn, T.P.T., 1977. An improved growth medium for the kidney disease bacterium and some notes using the medium. Bull.-Off. Int. Epizoot. 87, 511–513.
- Evelyn, T.P.T., 1993. Bacterial kidney diseases—BKD. In: Inglis, V., Roberts, R.J., Bromage, N.R. (Eds.), Bacterial Diseases of Fish. Blackwell Sci. Publ., Oxford, pp. 177–195.
- Evelyn, T.P.T., Prosperi-Porta, L., Ketcheson, J.E., 1990. Two new techniques for obtaining consistent results when growing *Renibacterium salmoninarum* on KDM2 culture. Dis. Aquat. Org. 9, 209–212.
- Evsenden, A.E., Grayson, T.H., Gilpin, M.L., Munn, C., 1993. *Renibacterium salmoninarum* and bacterial kidney disease—the unfinished jigsaw. Annu. Rev. Fish Dis. 3, 87–104.
- Falkinham III, J.O., 1996. Epidemiology of infection by non-tuberculosis mycobacteria. Clin. Microbiol. Rev. 9, 177–215.
- FAO, 2003. Overview of Fish Production, Utilization, Consumption and Trade. FAO, Rome, Italy.
- Fouz, B., Amaro, C., 2003. Isolation of a new serovar of *Vibrio vulnificus* pathogenic for eels cultured in freshwater farms. Aquaculture 217, 667–682.
- Fouz, B., Esteve-Gassent, M.D., Barrera, R., Larsen, J.L., Nielsen, M.E., Amaro, C., 2001. Field testing of a vaccine against eel

- diseases caused by *Vibrio vulnificus*. Dis. Aquat. Org. 45, 183–189.
- Fryer, J.L., Lannan, C.N., 1993. The history and current status of *Renibacterium salmoninarum*, the causative agent of bacterial kidney disease in Pacific salmon. Fish. Res. 17, 15–33.
- Fryer, J.L., Lannan, C.N., 1996. Rickettsial infections of fish. Annu. Rev. Fish Dis. 6, 3–13.
- Fryer, J.L., Lannan, C.N., Garcés, L.H., Larenas, J.J., Smith, P.A., 1990. Isolation of a rickettsiales-like organism from diseased coho salmon (*Oncorhynchus kisutch*) in Chile. Fish Pathol. 25, 107–114.
- Fryer, J.L., Lannan, C.N., Giovannoni, S.J., Wood, N.D., 1992. *Piscirickettsia salmonis* gen. nov., sp. nov., the causative agent of an epizootic disease in salmonid fishes. Int. J. Syst. Bacteriol. 42 (20), 120–126.
- Gaggero, A., Castro, H., Sandino, A.M., 1995. First isolation of *Piscirickettsia salmonis* from coho salmon, *Oncorhynchus kisutch* (Walbaum), and rainbow trout, *Oncorhynchus mykiss* (Walbaum), during the freshwater stage of their life cycle. J. Fish Dis. 18, 227–229.
- Gómez, S., Bernabé, A., Gómez, M.A., Navarro, J.A., Sánchez, J., 1993. Fish mycobacteriosis: morphological and immunocytochemical aspects. J. Fish Dis. 16, 137–141.
- Grant, A.N., Brown, A.G., Cox, D.I., Birbeck, T.H., Griffen, A.A., 1996. Rickettsia-like organism in farmed salmon. Vet. Rec. 138, 423–424.
- Grayson, T.H., Cooper, L.F., Atienzar, F.A., Knowles, M.R., Gilpin, M.L., 1999. Molecular differentiation of *Renibacterium salmoninarum* isolates from worldwide locations. Appl. Environ. Microbiol. 65, 961–968.
- Grayson, T.H., Atienzar, F.A., Alexande, S.M., Cooper, L.F., Gilpin, M.L., 2000. Molecular diversity of *Renibacterium salmoninarum* isolates determined by random amplified polymorphic DNA analysis. Appl. Environ. Microbiol. 66, 435–438.
- Greger, E., Goodrich, T., 1999. Vaccine development for winter ulcer disease, *Vibrio viscosus*, in Atlantic salmon, *Salmo salar* L.. J. Fish Dis. 22, 193–199.
- Griffiths, S.G., Melville, K.J., Salenius, K., 1998. Reduction of *Renibacterium salmoninarum* culture activity in Atlantic salmon following vaccination with avirulent strains. Fish Shellfish Immunol. 8, 607–619.
- Gudmundsdóttir, S., Lange, S., Magnadóttir, B., Gudmundsdóttir, B.K., 2003. Protection against atypical furunculosis in Atlantic halibut, *Hippoglossus hippoglossus* (L); comparison of a commercial furunculosis vaccine and an autogenous vaccine. J. Fish Dis. 26, 331–338.
- Gustafson, C.E., Thomas, C.J., Trust, T.J., 1992. Detection of *Aeromonas salmonicida* from fish by using polymerase chain reaction amplification of the virulence surface array protein gene. Appl. Environ. Microbiol. 58, 3816–3825.
- Handlinger, J., Soltani, H., Percival, V., 1997. The pathology of *Flexibacter maritimus* in aquaculture species of Tasmania, Australia. J. Fish Dis. 20, 159–168.
- Hawke, J.P., Thune, R.L., Cooper, R.K., Judice, E., Kelly-Smith, M., 2003. Molecular and phenotypic characterization of *Photobacterium damsela* subsp. *piscicida* isolated from hybrid striped bass cultured in Louisiana, USA. J. Aquat. An. Health 15, 189–201.
- Heath, S., Pak, S., Marschall, S., Prager, E.M., Orrego, C., 2000. Monitoring *Piscirickettsia salmonis* by denaturant gel electrophoresis and competitive PCR. Dis. Aquat. Org. 41, 19–29.
- Hedrick, R.P., McDowell, T., Groff, J., 1987. Mycobacteriosis in cultured striped bass from California. J. Wildl. Res. 23, 391–395.
- Herbst, L.H., Costa, S.F., Weiss, L.M., Johnson, L.K., Bartell, J., Davis, R., Walsh, M., Levi, M., 2001. Granulomatous skin lesions in Moray eels caused by a novel *Mycobacterium* species related to *Mycobacterium triplex*. Infect. Immun. 69, 4639–4646.
- Hiney, M., Oliver, G., 1999. Furunculosis (*Aeromonas salmonicida*) In: Wo, P.T.K., Bruno, D.W. (Eds.), Fish Diseases and Disorders, vol. 3. CAB Intern., Publ., United Kingdom, pp. 341–426.
- Hjelmeland, K., Stensvåg, K., Jørgensen, T., Espelid, S., 1988. Isolation and characterization of a surface layer antigen from *Vibrio salmonicida*. J. Fish Dis. 11, 197–205.
- Høie, S., Heum, M., Thoresen, O.F., 1997. Evaluation of a polymerase chain reaction-based assay for the detection of *Aeromonas salmonicida* subsp. *salmonicida* in Atlantic salmon, *Salmo salar*. Dis. Aquat. Org. 30, 27–35.
- Hsu, H.-M., Bowser, P.R., Chachte, J.H., 1991. Development and evaluation of a monoclonal-antibody-based-enzyme-linked immunosorbent assay for the diagnosis of *Renibacterium salmoninarum* infection. J. Aquat. Anim. Health 3, 168–175.
- Jones, M.W., Cox, D.I., 1999. Clinical disease in sea farmed Atlantic salmon (*Salmo salar*) associated with a member of the family *Pasteurellaceae*. Bull. Eur. Assoc. Fish Pathol. 19, 75–78.
- Jones, S.R.M., Markham, R.J.F., Groman, D.B., Cusack, R.R., 1998. Virulence and antigenic characteristics of a cultured Rickettsiales-like organism isolated from farmed Atlantic salmon *Salmo salar* in eastern Canada. Dis. Aquat. Org. 33, 25–31.
- Kaattari, S.L., Piganelli, J.D., 1997. Immunization with bacterial antigens: bacterial kidney disease. In: Gudding, R., Lillehaug, A., Midtlyng, P.J., Brown, F. (Eds.), Fish Vaccinology. Karger, Basel, Switzerland, pp. 145–152.
- Kitao, T., 1993. Streptococcal infections. In: Inglis, V., Roberts, R.J., Bromage, N.R. (Eds.), Bacterial Diseases of Fish. Blackwell Scientific Publications, Oxford, UK, pp. 196–210.
- Knibb, W., Colomi, A., Ankaoua, M., Lindell, D., Diamant, A., Gordon, H., 1993. Detection and identification of a pathogenic *Mycobacterium* from European seabass *Dicentrarchus labrax* using polymerase chain reaction and direct sequencing of 16S rRNA sequences. Mol. Mar. Biol. Biotechnol. 2, 225–232.
- Kuo, S.-C., Kou, G.-H., 1978. *Pseudomonas anguilliseptica* isolated from red spot disease of pond-cultured eel, *Anguilla japonica*. Rep. Inst. Fish. Biol., Min. Econ. Aff. Nat. Taiwan Univ. 3, 19–23.
- Kusuda, R., Kawai, K., Salati, F., Banner, C.R., Fryer, J.L., 1991. *Enterococcus seriolicida* sp. nov., a fish pathogen. Int. J. Syst. Bacteriol. 41, 406–409.
- Kuzyk, M.A., Burian, J., Machander, D., Dolhaine, D., Cameron, S., Thornton, J.C., Kay, W.W., 2001. A recombinant subunit vaccine against *Piscirickettsia salmonis*. 10th int. Conf. of the

- European Association of Fish Pathologists: Diseases of Fish and Shellfish. Dublin, Ireland, pp. O–107.
- Kvitt, H., Ucko, M., Colorni, A., Batargias, C., Zlotkin, A., Knibb, W., 2002. *Photobacterium damsela* ssp. *piscicida*: detection by direct amplification of 16S rRNA gene sequences and genotypic variation as determined by amplified fragment length polymorphism (AFLP). *Dis. Aquat. Org.* 48, 187–195.
- Laidler, L.A., Grant, A.N., Wadsworth, S., 1999. Preliminary investigations into the bacteriology of skin lesions of Atlantic salmon reared in seawater in Scotland. *Fish Vet. J.* 3, 31–37.
- Lämmle, Ch., Abdulmawjood, A., Danic, G., Vaillant, S., Weiß, R., 1998. Differentiation of *Streptococcus uberis* and *Streptococcus parauberis* by restriction fragment length polymorphism analysis of the 16S ribosomal RNA gene and further studies on serological properties. *Med. Sci. Res.* 26, 177–179.
- Lannan, C.N., Ewing, S.A., Fryer, J.L., 1991. A fluorescent antibody test for detection of the rickettsia causing disease in Chilean salmonids. *J. Aquat. Anim. Health* 3, 229–234.
- Lannan, C.N., Bartholomew, J.L., Fryer, J.L., 1999. Rickettsial and chlamydial infections. In: Woo, P.T.K., Bruno, D.W. (Eds.), *Fish diseases and disorders*, vol. 3. CABI Publ., United Kingdom, pp. 245–268.
- Lansdell, W., Dixon, B., Smith, N., Benjamin, L., 1993. Isolation of several *Mycobacterium* species from fish. *J. Aquat. Anim. Health* 5, 73–76.
- Larenas, J.J., Contreras, J., Smith, P., 1999. Estado actual de la Piscirickettsiosis en salmones. *AquaTIC* 1, 1–20 (Electronic Journal URL: <http://www.aquatic.unizar.es/N1/art505/piscirick.htm>).
- Larenas, J.J., Bartholomew, J., Troncoso, O., Fernández, S., Ledezma, H., Sandoval, N., Vera, P., Contreras, J., Smith, P., 2003. Experimental vertical transmission of *Piscirickettsia salmonis* and in vitro study of attachment and mode of entrance into the fish ovum. *Dis. Aquat. Org.* 56, 25–30.
- Larsen, J.L., Pedersen, K., Dalsgaard, I., 1994. *Vibrio anguillarum* serovars associated with vibriosis in fish. *J. Fish Dis.* 17, 259–267.
- Lee, K.K., Ellis, A.E., 1990. Glycerophospholipid: cholesterol acyltransferase complexed with LPS is a major lethal exotoxin and cytolysin of *Aeromonas salmonicida*: LPS stabilises and enhances toxicity of the enzyme. *J. Bacteriol.* 172, 5382–5393.
- Lee, K.K., Ellis, A.E., 1991. The role of extracellular lethal cytolysin of *Aeromonas salmonicida* in the pathology of furunculosis. *J. Fish Dis.* 14, 453–460.
- Lillehaug, A., Sørum, R.H., Ramstad, A., 1990. Cross-protection after immunization of Atlantic salmon *Salmo salar* L., against different strains of *Vibrio salmonicida*. *J. Fish Dis.* 13, 519–523.
- Lönnström, L., Wiklund, T., Bylund, G., 1994. *Pseudomonas anguilliseptica* isolated from Baltic herring *Clupea harengus* membras with eye lesions. *Dis. Aquat. Org.* 18, 143–147.
- López-Romalde, S., Magariños, B., Núñez, S., Toranzo, A.E., Romalde, J.L., 2003a. Phenotypic and genetic characterization of *Pseudomonas anguilliseptica* strains isolated from fish. *J. Aquat. Anim. Health* 15, 39–47.
- López-Romalde, S., Magariños, B., Ravelo, C., Toranzo, A.E., Romalde, J.L., 2003b. Existence of two O-serotypes in the emerging fish pathogen *Pseudomonas anguilliseptica*. *Vet. Microbiol.* 94, 325–333.
- López-Romalde, S., Núñez, S., Toranzo, A.E., Romalde, J.L., 2003c. Black spot seabream (*Pagellus bogaraveo*), a new susceptible host for *Pseudomonas anguilliseptica*. *Bull. Eur. Assoc. Fish Pathol.* 23, 258–264.
- Lunder, T., Evensen, Ø., Holstad, G., Håstein, T., 1995. “Winter ulcer” in the Atlantic salmon *Salmo salar*. Pathological and bacteriological investigations and transmission experiments. *Dis. Aquat. Org.* 23, 39–49.
- Lunder, T., Sørum, H., Holstad, G., Steigerwalt, A.G., Mowinckel, P., Brenner, D.J., 2000. Phenotypic and genotypic characterization of *Vibrio viscosus* sp. nov. and *Vibrio wodanis* sp. nov. isolated from Atlantic salmon (*Salmo salar*) with “winter ulcer”. *Int. J. Syst. Bacteriol.* 50, 427–450.
- Magariños, B., Romalde, J.L., Bandín, I., Fouz, B., Toranzo, A.E., 1992. Phenotypic, antigenic and molecular characterization of *Pasteurella piscicida* isolated from fish. *Appl. Environ. Microbiol.* 58, 3316–3322.
- Magariños, B., Santos, Y., Romalde, J.L., Rivas, C., Barja, J.L., Toranzo, A.E., 1992. Pathogenic activities of the live cells and extracellular products of the fish pathogen *Pasteurella piscicida*. *J. Gen. Microbiol.* 138, 2491–2498.
- Magariños, B., Romalde, J.L., Barja, J.L., Toranzo, A.E., 1994. Evidence of a dormant but infective state of the fish pathogen *Pasteurella piscicida* in sea water and sediment. *Appl. Environ. Microbiol.* 60, 180–186.
- Magariños, B., Pazos, F., Santos, Y., Romalde, J.L., Toranzo, A.E., 1995. Response of *Pasteurella piscicida* and *Flexibacter maritimus* to the skin mucus of marine fish. *Dis. Aquat. Org.* 21, 103–108.
- Magariños, B., Toranzo, A.E., Romalde, J.L., 1996. Phenotypic and pathobiological characteristics of *Pasteurella piscicida*. *Annu. Rev. Fish Dis.* 6, 41–64.
- Magariños, B., Osorio, C.R., Toranzo, A.E., Romalde, J.L., 1997. Applicability of ribotyping for intraspecific classification and epidemiological studies of *Pasteurella piscicida*. *Syst. Appl. Microbiol.* 20, 634–639.
- Magariños, B., Romalde, J.L., Barja, J.L., Núñez, S., Toranzo, A.E., 1999. Protection of gilthead seabream against pasteurellosis at the larval stages. *Bull. Eur. Assoc. Fish Pathol.* 19, 159–161.
- Magariños, B., Toranzo, A.E., Barja, J.L., Romalde, J.L., 2000. Existence of two geographically linked clonal lineages in the bacterial pathogen *Photobacterium damsela* subsp. *piscicida*. *Epidemiol. Infect.* 125, 213–219.
- Magariños, B., Couso, N., Noya, M., Merino, P., Toranzo, A.E., Lamas, J., 2001. Effect of temperature on the development of pasteurellosis in carrier gilthead seabream (*Sparus aurata*). *Aquaculture* 195, 17–21.
- Magariños, B., Romalde, J.L., López-Romalde, S., Moriñigo, M.A., Toranzo, A.E., 2003. Pathobiological characterization of *Photobacterium damsela* subsp. *piscicida* strains isolated from cultured sole (*Solea senegalensis*). *Bull. Eur. Assoc. Fish Pathol.* 23, 183–190.
- Magnússon, H.B., Frigjónsson, O.H., Andrússon, O.S., Benediktsdóttir, E., Guðmundsdóttir, S., Andrúsdóttir, V., 1994. *Renibacterium salmoninarum*, the causative agent of bacterial kidney

- disease in salmonid fish, detected by nested reverse transcription-PCR of 16S rRNA sequences. *Appl. Environ. Microbiol.* 60, 4580–4583.
- Marco-Noales, E., Milán, M., Fouz, B., Sanjuán, E., Amaro, C., 2001. Transmission to eels, portals of entry, and putative reservoirs of *Vibrio vulnificus* serovar E (biotype 2). *Appl. Environ. Microbiol.* 67, 4717–4725.
- Marschall, S., Heath, S., Henríquez, V., Orrego, C., 1998. Minimally invasive detection of *Piscirickettsia salmonis* in cultivated salmonids via PCR. *Appl. Environ. Microbiol.* 64, 3066–3069.
- Mauel, M.J., Giovannoni, S.J., Fryer, J.L., 1996. Development of polymerase chain reaction assays for detection, identification, and differentiation of *Piscirickettsia salmonis*. *Dis. Aquat. Org.* 26, 189–195.
- Mauel, M.J., Giovannoni, S.J., Fryer, J.L., 1999. Phylogenetic analysis of *Piscirickettsia salmonis* by 16S, internal transcribed spacer (ITS) and 23S ribosomal DNA sequencing. *Dis. Aquat. Org.* 35, 115–123.
- Mauel, M.J., Miller, D.L., Frazier, K., Liggett, A.D., Styer, L., 2003. Characterization of a piscirickettsiosis-like disease in Hawaiian tilapia. *Dis. Aquat. Org.* 53, 249–255.
- McIntosh, D., Meaden, P.G., Austin, B., 1996. A simplified PCR-based method for the detection of *Renibacterium salmoninarum* utilizing preparations of rainbow trout (*Oncorhynchus mykiss*, walbaum) lymphocytes. *Appl. Environ. Microbiol.* 62, 2932–2929.
- McVicar, A.H., White, P.G., 1979. Fin and skin necrosis of Dover sole *Solea solea* (L.). *J. Fish Dis.* 2, 557–562.
- McVicar, A.H., White, P.G., 1982. The prevention and cure of an infectious disease in cultivated juvenile Dover sole *Solea solea* (L.). *Aquaculture* 26, 213–222.
- Midtlyng, P.J., 1997. Vaccination against furunculosis. In: Bernoth, E.M., Ellis, A.E., Midtlyng, P.J., Smith, P. (Eds.), *Furunculosis. Multidisciplinary Fish Disease Research*. Academic Press, United Kingdom, pp. 382–404.
- Midtlyng, P.J., Lillehaug, A., 1998. Growth of Atlantic salmon *Salmo salar* after intraperitoneal administration of vaccines containing adjuvants. *Dis. Aquat. Org.* 32, 91–97.
- Miriam, A., Griffiths, S.G., Lovely, J.E., Lynch, W.H., 1997. PCR and probe-PCR assays to monitor broodstock Atlantic salmon (*Salmo salar*, L.) ovarian fluid and kidney tissue for presence of DNA of the fish pathogen *Renibacterium salmoninarum*. *J. Clin. Microbiol.* 35, 1322–1326.
- Munn, C.B., 1994. The use of recombinant DNA technology in the development of fish vaccines. *Fish Shellfish Immunol.* 4, 459–473.
- Mutharia, L.W., Raymond, B.T., Dekievit, T.R., Stevenson, R.M.W., 1992. Antibody specificities of polyclonal rabbit and rainbow trout antisera against *Vibrio ordalii* and serotype O2 strains of *Vibrio anguillarum*. *Can. J. Microbiol.* 39, 492–499.
- Nakai, T., 1985. Resistance of *Pseudomonas anguilliseptica* to bactericidal action of fish serum. *Bull. Jpn. Soc. Sci. Fish.* 51, 1431–1436.
- Nakai, T., Muroga, K., Wakabayashi, H., 1985. First record of *Pseudomonas anguilliseptica* infection in cultured ayu, *Plecoglossus altivelis*. *Fish Pathol.* 20, 481–484.
- Newman, S.G., 1993. Bacterial vaccines of fish. *Annu. Rev. Fish Dis.* 3, 145–186.
- Nguyen, H.T., Kanai, K., 1999. Selective agars for the isolation of *Streptococcus iniae* from Japanese flounder, *Paralichthys olivaceus*, and its cultural environment. *J. Appl. Microbiol.* 86, 769–776.
- Noya, N., Magariños, B., Lamas, J., 1995. Interactions between peritoneal exudate cells (PECs) of gilthead seabream (*Sparus aurata*) and *Pasteurella piscicida*. A morphological study. *Aquaculture* 131, 11–21.
- Office International des Epizooties (O.I.E.), 2000. *Diagnostic Manual for Aquatic Animal Diseases*, 3rd ed. Off. Int. Epizoot., Paris.
- Olea, I., Bruno, D.W., Hastings, T.S., 1993. Detection of *Renibacterium salmoninarum* in naturally infected Atlantic salmon *Salmo salar* L., and rainbow trout *Oncorhynchus mykiss* Walbaum using an enzyme linked immunosorbent assay. *Aquaculture* 116, 99–110.
- Olsen, J.E., Larsen, J.L., 1993. Ribotypes and plasmid contents of *Vibrio anguillarum* strains in relation to serovars. *Appl. Environ. Microbiol.* 59, 3863–3870.
- Olsen, A.B., Melby, H.P., Speilberg, L., Evensen, O., Hastein, T., 1997. *Piscirickettsia salmonis* infection in Atlantic salmon *Salmo salar* in Norway—epidemiological, pathological and microbiological findings. *Dis. Aquat. Org.* 31, 35–48.
- Ooyama, T., Kera, A., Okada, T., Inglis, V., Yoshida, T., 1999. The protective immune response of yellowtail *Seriola quinqueradiata* to the bacterial fish pathogen *Lactococcus garvieae*. *Dis. Aquat. Org.* 37, 121–126.
- Osorio, C., Toranzo, A.E., 2002. DNA-based diagnostics in sea farming. In: Fingerhman, M., Nagabhushanam, R. (Eds.), *Recent Advances in Marine Biotechnology Series, Seafood Safety and Human Health*, vol. 7. Science Publishers, Inc., Plymouth, UK, pp. 253–310.
- Osorio, C.R., Collins, M.D., Toranzo, A.E., Barja, J.L., Romalde, J.L., 1999. 16S rRNA sequence analysis of *Photobacterium damsela* ssp. *piscicida* and nested PCR method for the rapid detection of the causative agent of fish pasteurellosis. *Appl. Environ. Microbiol.* 65, 2942–2946.
- Osorio, C.R., Toranzo, A.E., Romalde, J.L., Barja, J.L., 2000. Multiplex PCR assay for ureC and 16S rRNA genes clearly discriminates between both subspecies of *Photobacterium damsela*. *Dis. Aquat. Org.* 40, 177–183.
- Ostland, V.E., la Trace, C., Morrison, D., Ferguson, H.W., 1999. *Flexibacter maritimus* associated with a bacterial stomatitis in Atlantic salmon smolts reared in net-pens in British Columbia. *J. Aquat. Anim. Health* 11, 35–44.
- Palmer, R., Rutledge, M., Callanan, K., Drinan, E., 1997. A piscirickettsiosis-like disease on farmed Atlantic salmon in Ireland—isolation of the agent. *Bull. Eur. Assoc. Fish Pathol.* 17, 68–72.
- Pascho, R.J., Mulcahy, D., 1987. Enzyme-linked immunosorbent assay for a soluble antigen of *Renibacterium salmoninarum*, the causative agent of bacterial kidney disease. *Can. J. Fish. Aquat. Sci.* 44, 183–191.
- Pascho, R.J., Elliot, D.G., Mallet, R.W., Mulcahy, D., 1987. Comparison of five techniques for the detection of *Renibacte-*

- rium salmoninarum* in coho salmon. Trans. Am. Fish. Soc. 11, 882–890.
- Pascho, R.J., Elliot, D.G., Treufert, J.M., 1991. Brood stock segregation of spring salmon *Oncorhynchus tshawytscha* by use of the enzyme linked immunosorbent assay (ELISA) and the fluorescent antibody technique (FAT) affects the prevalence and levels of *Renibacterium salmoninarum* infection in progeny. Dis. Aquat. Org. 12, 25–40.
- Pazos, F., Santos, Y., Núñez, S., Toranzo, A.E., 1993. Increasing occurrence of *Flexibacter maritimus* in marine aquaculture of Spain. FHS/AFS Newsl. 21, 1–2.
- Pazos, F., Santos, Y., Macías, A.R., Núñez, S., Toranzo, A.E., 1996. Evaluation of media for the successful culture of *Flexibacter maritimus*. J. Fish Dis. 19, 193–197.
- Pedersen, K., Larsen, J.L., 1993. rRNA gene restriction patterns of *Vibrio anguillarum* serogroup O1. Dis. Aquat. Org. 16, 121–126.
- Pedersen, K., Grisez, L., van Houdt, R., Tiainen, T., Ollevier, F., Larsen, J.L., 1999. Extended serotyping scheme for *Vibrio anguillarum* with the definition of seven provisional O-serogroups. Curr. Microbiol. 38, 183–189.
- Perera, R.P., Johnson, S.K., Collins, M.D., Lewis, D.H., 1994. *Streptococcus iniae* associated with mortality of *Tilapia nilotica* × *T. aurea* hybrids. J. Aquat. Anim. Health 6, 335–340.
- Piganelli, J.D., Wiens, G.D., Zhang, J.A., Christenson, J.M., Kaattari, S.L., 1999. Evaluation of a whole cell, p57-vaccine against the pathogen *Renibacterium salmoninarum*. Dis. Aquat. Org. 39, 37–44.
- Ransom, D.P., Lannan, C.N., Rohovec, J.S., Fryer, J.L., 1984. Comparison of histopathology caused by *Vibrio anguillarum* and *Vibrio ordalii* and three species of Pacific salmon. J. Fish Dis. 7, 107–115.
- Ravelo, C., Magariños, B., Toranzo, A.E., Romalde, J.L., 2001. Conventional versus miniaturized systems for the phenotypic characterization of *Lactococcus garvieae*. Bull. Eur. Assoc. Fish Pathol. 21, 136–144.
- Ravelo, C., Magariños, B., Toranzo, A.E., Romalde, J.L., 2003. Molecular fingerprinting of *Lactococcus garvieae* strains by RAPD analysis. J. Clin. Microbiol. 41, 751–756.
- Rhodes, M.W., Kator, H., Kotob, S., van Berkum, P., Kaattari, I., Vogelbein, W., Floyd, M.M., Butler, W.R., Quinn, F.D., Ottinger, C., Shotts, E., 2001. A unique *Mycobacterium* species isolated from an epizootic of striped bass (*Morone saxatilis*). Emerg. Infect. Dis. 7, 896–899.
- Rodger, H.D., Drinan, E.M., 1993. Observation of a rickettsia-like organism in Atlantic salmon, *Salmo salar* L., in Ireland. J. Fish Dis. 16, 361–369.
- Romalde, J.L., Magariños, B., 1997. Immunization with bacterial antigens: pasteurellosis. In: Gudding, R., Lillehaug, A., Midtlyng, J., Brown, F. (Eds.), Fish Vaccinology. Karger, Basel, Switzerland, pp. 167–177.
- Romalde, J.L., Toranzo, A.E., 1999. Streptococcosis of marine fish. In: Olivier, G. (Ed.), ICES Identification Leaflets for Diseases and Parasites of Fish and Shellfish. No. 56. International Council for the Exploration of the Sea. Copenhagen, Denmark, pp. 1–8.
- Romalde, J.L., Toranzo, A.E., 2002. Molecular approaches for the study and diagnosis of salmonid streptococcosis. In: Cunningham, C.O. (Ed.), Molecular Diagnosis of Salmonid Diseases. Kluwer Academic Publ, Netherlands, pp. 211–223. Chap. 8.
- Romalde, J.L., Magariños, B., Fouz, B., Bandín, I., Núñez, S., Toranzo, A.E., 1995. Evaluation of Bionor mono-kits for rapid detection of bacterial fish pathogens. Dis. Aquat. Org. 21, 25–34.
- Romalde, J.L., Magariños, B., Toranzo, A.E., 1999a. Pasteurellosis: pathological and epizootiological aspects of the *Pasteurella piscicida* infection. In: Olivier, G. (Ed.), ICES Identification Leaflets for Diseases and Parasites of Fish and Shellfish. No. 54. International Council for the Exploration of the Sea. Copenhagen, Denmark, pp. 1–6.
- Romalde, J.L., Magariños, B., Lores, F., Toranzo, A.E., 1999b. Assessment of a magnetic bead-EIA based kit for rapid diagnosis of fish pasteurellosis. J. Microbiol. Methods 38, 147–154.
- Romalde, J.L., Magariños, B., Villar, C., Barja, J.L., Toranzo, A.E., 1999c. Genetic analysis of turbot pathogenic *Streptococcus parauberis* strains by ribotyping and random amplified polymorphic DNA. FEMS Microbiol. Lett. 459, 297–304.
- Romalde, J.L., Magariños, B., Toranzo, A.E., 1999d. Prevention of streptococcosis in turbot by intraperitoneal vaccination: a review. J. Appl. Ichthyol. 15, 153–158.
- Romalde, J.L., López-Romalde, S., Ravelo, C., Magariños, B., Toranzo, A.E., 2004. Development and validation of a PCR-based protocol for the detection of *Pseudomonas anguilliseptica*. Fish Pathol. 39, 33–41.
- Romalde, J.L., Ravelo, C., López-Romalde, S., Avendaño-Herrera, R., Magariños, B., Toranzo, A.E., 2005. Vaccination strategies to prevent important emerging diseases for Spanish aquaculture. In: Midtlyng, P.J. (Ed.), Fish vaccinology. Karger, Switzerland, pp. 85–95.
- Salonius, K., Siderakis, C.V., Griffiths, S.G., 2003. Further characterization of *Arthrobacter davidanieli* and use as a live vaccine to immunize against intracellular pathogens of salmonids. Abstracts 3rd International Symposium on Fish Vaccinology. Bergen, Norway, p. 41.
- Salte, R., Rørvik, K.A., Reed, E., Norberg, K., 1994. Winter ulcers of the skin in Atlantic salmon, *Salmo salar* L., pathogenesis and possible aetiology. J. Fish Dis. 17, 661–665.
- Sanders, J.E., Fryer, J.L., 1980. *Renibacterium salmoninarum* gen. nov., sp. nov., the causative agent of bacterial kidney disease in salmonid fishes. Int. J. Syst. Bacteriol. 30, 496–502.
- Santos, Y., Pazos, F., Bandín, I., Toranzo, A.E., 1995. Analysis of antigens present in the extracellular products and cell surface of *Vibrio anguillarum* O1, O2 and O3. Appl. Environ. Microbiol. 61, 2493–2498.
- Santos, Y., Pazos, F., Barja, J.L., 1999. *Flexibacter maritimus*, causal agent of flexibacteriosis in marine fish. In: Olivier, G. (Ed.), ICES Identification Leaflets for Diseases and Parasites of Fish and Shellfish. No. 55. International Council for the Exploration of the Sea. Copenhagen, Denmark, pp. 1–6.
- Schieve, M.H., Crosa, J.H., 1981. Molecular characterization of *Vibrio anguillarum* biotype 2. Can. J. Microbiol. 27, 1011–1018.
- Skarmeta, A.M., Bandín, I., Santos, Y., Toranzo, A.E., 1995. In vitro killing of *Pasteurella piscicida* by fish macrophages. Dis. Aquat. Org. 23, 51–57.
- Skarmeta, A.M., Henríquez, V., Zahr, M., Orrego, C., Marshall, S.H., 2000. Isolation of a virulent *Piscirickettsia salmonis* from

- the brain of naturally infected coho salmon. *Bull. Eur. Assoc. Fish Pathol.* 20, 261–264.
- Skov, M.N., Pedersen, K., Larsen, J.L., 1995. Comparison of pulse-field gel electrophoresis, ribotyping and plasmid profiling for typing of *Vibrio anguillarum* serovar O1. *Appl. Environ. Microbiol.* 61, 1540–1545.
- Smith, P.A., Contreras, J.R., Larenas, J.J., Aguillón, J.C., Garcés, L.H., Pérez, B., Fryer, J.L., 1997. Immunization with bacterial antigens: *Piscirickettsiosis*. In: Gudding, R., Lillehaug, A., Midtlyng, P.J.F., Brown, B. (Eds.), *Fish Vaccinology*. Karger, Basel, Switzerland, pp. 161–166.
- Sørensen, U.B.S., Larsen, J.L., 1986. Serotyping of *Vibrio anguillarum*. *Appl. Environ. Microbiol.* 51, 593–597.
- Sorum, H., Hvaal, A.B., Heum, M., Daae, F.L., Wiik, R., 1990. Plasmid profiling of *Vibrio salmonicida* for epidemiological studies of cold-water vibriosis in Atlantic salmon (*Salmo salar*) and cod (*Gadus morhua*). *Appl. Environ. Microbiol.* 56, 1033–1037.
- Starliper, C.E., 1996. Genetic diversity of North American isolates of *Renibacterium salmoninarum*. *Dis. Aquat. Org.* 27, 207–213.
- Steiropoulos, N.A., Yuksel, S.A., Thompson, K.-D., Adams, A., Ferguson, H.W., 2002. Detection of *Rickettsia*-like organisms (RLOs) in European seabass (*Dicentrarchus labrax*, L.). *Bull. Eur. Assoc. Fish Pathol.* 22, 338–342.
- Stewart, D.J., Woldemariam, K., Dear, G., Mochaba, F.M., 1983. An outbreak of “sekiten-byo” among cultured European eels, *Anguilla anguilla* L., in Scotland. *J. Fish Dis.* 6, 75–76.
- Sukui, M., Nakagawa, Y., Harayama, S., Yamamoto, S., 2001. Phylogenetic analysis and taxonomic study of marine *Cytophaga*-like bacteria: proposal for *Tenacibaculum* gen. nov. with *Tenacibaculum maritimum* comb. nov. and *Tenacibaculum ovolyticum* comb. nov., and description of *Tenacibaculum mesophilum* sp. nov. and *Tenacibaculum amyolyticum* sp. nov. *Int. J. Syst. Evol. Microbiol.* 51, 1639–1652.
- Tajima, K., Ezura, Y., Kimura, T., 1985. Studies on the taxonomy and serology of causative organisms of fish vibriosis. *Fish Pathol.* 20, 131–142.
- Talaat, A.M., Reimschuessel, R., Trucksis, M., 1997. Identification of mycobacteria infecting fish to the species level using polymerase chain reaction and restriction enzyme analysis. *Vet. Microbiol.* 58, 229–237.
- Talaat, A.M., Trucksis, M., Kane, A.S., Reimschuessel, R., 1999. Pathogenicity of *Mycobacterium fortuitum* and *Mycobacterium smegmatis* to gold fish, *Carassius auratus*. *Vet. Microbiol.* 66, 151–164.
- Thornton, J.C., Garduño, R.A., Newman, S.G., Kay, W.W., 1991. Surface disorganized, attenuated mutants of *Aeromonas salmonicida* as furunculosis vaccines. *Microb. Pathog.* 11, 85–89.
- Thornton, J.C., Garduño, R.A., Kay, W.W., 1994. The development of live vaccines for furunculosis lacking the A-layer and O-antigen of *Aeromonas salmonicida*. *J. Fish Dis.* 17, 195–204.
- Thune, R.L., Fernández, D.H., Hawke, J.P., Miller, R., 2003. Construction of a safe, stable, efficacious vaccine against *Photobacterium damsela* sp. *piscicida*. *Dis. Aquat. Org.* 57, 51–58.
- Thyssen, A., van Eygen, S., Hauben, L., Goris, J., Swings, J., Ollivier, F., 2000. Application of AFLP for taxonomic and epidemiological studies of *Photobacterium damsela* ssp. *piscicida*. *Int. J. Syst. Evol. Microbiol.* 50, 1013–1019.
- Tiainen, R., Pedersen, K., Larsen, J.L., 1995. Ribotyping and plasmid profiling of *Vibrio anguillarum* serovar O2 and *Vibrio ordalii*. *J. Appl. Bacteriol.* 79, 384–392.
- Tison, D.L., Nishibuchi, M., Greenwood, J.D., Seidler, R.J., 1982. *Vibrio vulnificus* biotype 2: new biogroup pathogenic for eels. *Appl. Environ. Microbiol.* 44, 640–646.
- Toranzo, A.E., Barja, J.L., 1990. A review of the taxonomy and seroepizootiology of *Vibrio anguillarum*, with special reference to aquaculture in the northwest of Spain. *Dis. Aquat. Org.* 9, 73–82.
- Toranzo, A.E., Barja, J.L., 1992. First report of furunculosis in turbot reared in floating cages in northwest of Spain. *Bull. Eur. Assoc. Fish Pathol.* 12, 147–149.
- Toranzo, A.E., Barja, J.L., 1993. Virulence factors of bacteria pathogenic for cold water fish. *Annu. Rev. Fish Dis.* 3, 5–36.
- Toranzo, A.E., Barja, J.L., Colwell, R.R., Hetrick, F.M., 1983. Characterization of plasmids in bacterial fish pathogens. *Infect. Immun.* 39, 184–192.
- Toranzo, A.E., Baya, A., Roberson, B.S., Barja, J.L., Grimes, D.J., Hetrick, F.M., 1987. Specificity of slide agglutination test for detecting bacterial fish pathogens. *Aquaculture* 61, 81–97.
- Toranzo, A.E., Barreiro, S., Casal, J.F., Figueras, A., Magariños, B., Barja, J.L., 1991a. Pasteurellosis in cultured gilthead seabream (*Sparus aurata*): first report in Spain. *Aquaculture* 99, 1–15.
- Toranzo, A.E., Santos, Y., Núñez, S., Barja, J.L., 1991b. Biochemical and serological characteristics, drug resistance, and plasmid profiles of Spanish isolates of *Aeromonas salmonicida*. *Fish Pathol.* 26, 55–60.
- Toranzo, A.E., Devesa, S., Heinen, P., Riaz, A., Núñez, S., Barja, J.L., 1994. Streptococcosis in cultured turbot caused by an *Enterococcus*-like bacterium. *Bull. Eur. Assoc. Fish Pathol.* 14, 19–23.
- Toranzo, A.E., Cutrín, J.M., Núñez, S., Romalde, J.L., Barja, J.L., 1995a. Antigenic characterization of *Enterococcus* strains pathogenic for turbot and their relationship with other gram positive bacteria. *Dis. Aquat. Org.* 21, 187–191.
- Toranzo, A.E., Devesa, S., Romalde, J.L., Lamas, J., Riaz, A., Leiro, J., Barja, J.L., 1995b. Efficacy of intraperitoneal and immersion vaccination against *Enterococcus* sp. infection in turbot. *Aquaculture* 134, 17–27.
- Toranzo, A.E., Santos, Y., Barja, J.L., 1997. Immunization with bacterial antigens: *Vibrio* infections. In: Gudding, R., Lillehaug, A., Midtlyng, P.J., Brown, F. (Eds.), *Fish Vaccinology*. Karger, Basel, Switzerland, pp. 93–105.
- Toyama, T., Tsukamoto, K.K., Wakabayashi, H., 1996. Identification of *Flexibacter maritimus*, *Flavobacterium branchiophilum* and *Cytophaga columnaris* by PCR targeted 16S ribosomal DNA. *Fish Pathol.* 31, 25–31.
- Vaughan, L.M., Smith, P.R., Foster, T.J., 1993. An aromatic-dependent mutant of the fish pathogen *Aeromonas salmonicida*, is attenuated and its effective as a live vaccine against the salmonid disease, furunculosis. *Infect. Immun.* 61, 2172–2181.
- Vela, A.I., Vázquez, J., Gibello, A., Blanco, M.M., Moreno, M.A., Liebana, P., Albendea, C., Alcalá, B., Méndez, A.,

- Willey, B.M., Fernández-Garayzabal, J.F., 2000. Phenotypic and genetic characterization of *Lactococcus garvieae* isolated in Spain from lactococcosis outbreaks in comparison with isolates of other countries and sources. J. Clin. Microbiol. 38, 3791–3795.
- Wakabayashi, H., Egusa, S., 1972. Characteristics of a *Pseudomonas* sp. from an epizootic of pond-cultured eels (*Anguilla japonica*). Bull. Jpn. Soc. Sci. Fish. 38, 577–587.
- Wakabayashi, H., Hikida, H., Masumura, K., 1986. *Flexibacter maritimus* sp. nov., a pathogen of marine fishes. Int. J. Syst. Bacteriol. 36, 396–398.
- Weinstein, M.R., Litt, M., Kertesz, D.A., Wyper, P., Rose, D., Coulter, M., McGeer, A., Facklam, R.R., Ostach, C., Willey, B.M., Borczyk, A., Low, D.E., 1997. Invasive infections due to a fish pathogen, *Streptococcus iniae*. N. Engl. J. Med. 337, 589–594.
- Wiens, G.D., Kaattary, S.L., 1999. Bacterial kidney disease (*Renibacterium salmoninarum*). In: Woo, P.T.K., Bruno, D.W. (Eds.), Fish Diseases and Disorders, vol. 3. CAB Intern. Intern. Publ., United Kingdom, pp. 269–302.
- Wiklund, T., Bylund, G., 1990. *Pseudomonas anguilliseptica* as a pathogen of salmonid fish in Finland. Dis. Aquat. Org. 8, 13–19.
- Wood, P.A., Kaattari, S.L., 1996. Enhanced immunogenicity of *Renibacterium salmoninarum* in chinook salmon after removal of the bacterial cell surface-associated 57 kDa protein. Dis. Aquat. Org. 25, 71–79.
- Yoshida, T., Eshima, T., Wada, Y., Yamada, Y., Kakizaki, E., Sakai, M., Kitao, T., Inglis, V., 1996. Phenotypic variation associated with an antiphagocytic factor in the bacterial fish pathogen *Enterococcus seriolicida*. Dis. Aquat. Org. 25, 81–86.
- Zlotkin, A., Hershko, H., Eldar, A., 1998a. Possible transmission of *Streptococcus iniae* from wild fish to cultured marine fish. Appl. Environ. Microbiol. 64, 4065–4067.
- Zlotkin, A., Eldar, A., Ghittino, C., Bercovier, H., 1998b. Identification of *Lactococcus garvieae* by PCR. J. Clin. Microbiol. 36, 983–985.
- Zorrilla, I., Balebona, M.C., Moriñigo, M.A., Sarasquete, C., Borrego, J.J., 1999. Isolation and characterization of the causative agent of pasteurellosis, *Photobacterium damsela* ssp. *piscicida*, from sole *Solea senegalensis* (Kaup). J. Fish Dis. 22, 167–171.