

REVIEW

Occurrence and significance of atypical *Aeromonas salmonicida* in non-salmonid and salmonid fish species: a review

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ABSTRACT: Bacterial strains of *Aeromonas salmonicida* included in the recognized subsp. *achromogenes*, subsp. *masoucida*, and subsp. *smithia* in addition to the large number of strains not included in any of the described subspecies are referred to as atypical *A. salmonicida*. The atypical strains form a very heterogeneous group with respect to biochemical characteristics, growth conditions, and production of extracellular proteases. Consequently, the present taxonomy of the species *A. salmonicida* is rather ambiguous. Atypical *A. salmonicida* has been isolated from a wide range of cultivated and wild fish species, non-salmonids as well as salmonids, inhabiting fresh water, brackish water and marine environments in northern and central Europe, South Africa, North America, Japan and Australia. In non-salmonid fish species, infections with atypical strains often manifest themselves as superficial skin ulcerations. The best known diseases associated with atypical *A. salmonicida* are carp *Cyprinus carpio* erythrodermatitis, goldfish *Carassius auratus* ulcer disease, and ulcer disease of flounder *Platichthys flesus*, but atypical strains are apparently involved in more disease outbreaks than previously suspected. Macroscopical and microscopical studies of ulcerated fish indicate internal organs are infrequently invaded by atypical *A. salmonicida*. This view is supported by the fact that atypical strains are irregularly isolated from visceral organs of ulcerated fish. High mortality caused by atypical *A. salmonicida* has been observed in populations of wild non-salmonids and farmed salmonids, although the association between the mortality in the wild fish stocks and atypical *A. salmonicida* has not always been properly assessed. In injection experiments the pathogenicity of the atypical strains examined showed large variation. An extracellular A-layer has been detected in different atypical strains, but virulence mechanisms different from those described for (typical) *A. salmonicida* subsp. *salmonicida*, for example an extracellular metallo-protease and a different iron utilization mechanism, have been described. Limited information is available about the ecology, spread and survival of atypical strains in water. The commonly used therapeutic methods for the control of diseases in farmed fish caused by atypical *A. salmonicida* are generally effective against the atypical strains. Resistance to different antibiotics and transferable plasmids encoding multiple drug resistance have been observed in atypical *A. salmonicida*. Studies aimed at producing a vaccine against atypical strains are in progress.

KEY WORDS: *Aeromonas salmonicida* · Skin ulcers · Fish pathogens · Fish

INTRODUCTION

The expression 'atypical *Aeromonas salmonicida*' was initially used for bacterial strains belonging to the spe-

cies *A. salmonicida* but showing biochemical characteristics, mainly slow growth and late pigment production, different from those described for *A. salmonicida* subsp. *salmonicida*. One of the first papers using the expression 'atypical' or 'aberrant' strain of *A. salmonicida* was published in 1971 (Evelyn 1971), although reports on strains not included in subsp. *salmonicida* had been published earlier (Smith 1963, Kimura 1969a, b).

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Although several subspecies of *Aeromonas salmonicida* have been described, i.e. subsp. *achromogenes*, subsp. *masoucida*, subsp. *salmonicida* and subsp. *smithia* (Holt et al. 1994), a large number of reported strains have not been assigned to any of these subspecies. Presently, strains included in the subsp. *achromogenes*, subsp. *masoucida*, and subsp. *smithia* in addition to the strains not included in any of the described subspecies of *A. salmonicida* are referred to as atypical strains. Strains of subsp. *salmonicida* are consequently referred to as 'typical' *A. salmonicida*. Although strains showing characteristics that are different from those described for typical *A. salmonicida* are considered to be atypical isolates, several papers on typical strains have recently been published showing atypical or aberrant biochemical characteristics, such as non- or late-pigmentation (Wiklund et al. 1993), negative cytochrome oxidase reaction (Chapman et al. 1991), acid production from sucrose (Wiklund et al. 1992), no degradation of aesculin (Austin et al. 1989), production of hydrogen sulfide (Austin et al. 1989) and growth at 37°C (McIntosh & Austin 1991).

The number of published reports of disease outbreaks associated with atypical strains has increased significantly during the last decade, and these isolates have been reported from an increasing number of fish species and geographical areas. Most of the isolated strains have been identified as atypical *Aeromonas salmonicida* and very few of them are included in any of the described subspecies (subsp. *achromogenes*, subsp. *masoucida*, and subsp. *smithia*) (Holt et al. 1994). In fact, new isolates of *A. salmonicida* subsp. *masoucida* and subsp. *smithia* have not been reported since their initial isolation and description (Kimura 1969a, b, Austin et al. 1989).

Diseases caused by atypical strains have been referred to as ulcer disease or 'atypical furunculosis' (Snieszko et al. 1950, Hubbert & Williams 1980, Groman et al. 1992, Wiklund 1994b, Gudmundsdóttir & Magnadóttir 1997) in contrast to furunculosis or classical furunculosis caused by typical *Aeromonas salmonicida*. Unfortunately, clinical infections in fish with atypical *A. salmonicida* have sometimes in the literature been referred to as furunculosis (McCarthy 1975, Bucke 1980, Boomker et al. 1984), which in the opinion of the present authors should be avoided.

Atypical bacterial strains of the species *Aeromonas salmonicida* have been poorly defined, and this group consists of isolates showing a large variety of biochemical, molecular and virulence characteristics. Presently, an atypical strain can be defined only as a strain that does not fit into the existing classification of *A. salmonicida* subsp. *salmonicida*. This situation certainly needs to be adequately solved in future detailed taxonomic studies.

This paper presents the current state of knowledge about the biology and the taxonomy of atypical *Aeromonas salmonicida* strains and disease processes generated by these bacteria.

TAXONOMY

The taxonomic position of *Aeromonas salmonicida* has been reviewed by McCarthy & Roberts (1980) and Austin & Austin (1993). In Bergey's Manual of Systematic Bacteriology (Popoff 1984), the genus *Aeromonas* was included in the family *Vibrionaceae*. The proposal to place this genus in the new family *Aeromonadaceae* (Colwell et al. 1986), which was based on molecular genetic data, has recently been validated (Anonymous 1992). The recognized species in the genus *Aeromonas* represent 14 clearly different DNA homology groups (Esteve et al. 1995) with *A. salmonicida* subsp. *salmonicida* belonging to DNA group 3.

According to the latest edition of Bergey's Manual of Systematic Bacteriology (Popoff 1984), *Aeromonas salmonicida* is divided into 3 subspecies: *A. salmonicida* subsp. *salmonicida* (Griffin et al. 1953a), *A. salmonicida* subsp. *achromogenes* (Smith 1963) and *A. salmonicida* subsp. *masoucida* (Kimura 1969a, b). This differentiation was proposed by Schubert (1967, 1969). These 3 subspecies can be distinguished by a limited number of biochemical characteristics (Popoff 1984).

Since the first description by Smith (1963) of strains different from *Aeromonas salmonicida* subsp. *salmonicida*, a large number of atypical strains of *A. salmonicida* have been isolated (e.g. Wiklund 1990, Austin & Austin 1993, Nakatsugawa 1994, Wiklund & Dalsgaard 1995). McCarthy & Roberts (1980) have proposed another and different division into 3 subspecies, based on epizootiological criteria. The 3 subspecies are subsp. *salmonicida*, subsp. *achromogenes* (incorporating subsp. *masoucida*, including only strains isolated from salmonids), and subsp. *nova* (isolated from non-salmonid fish). This classification has not been approved in Bergey's Manual of Systematic Bacteriology (Popoff 1984). *A. salmonicida* subsp. *salmonicida* and *A. salmonicida* subsp. *masoucida* were found to be a genetically homogenous group in DNA homology studies (MacInnes et al. 1979). Based on numerical taxonomy (McCarthy 1978), sequence homology, and guanine-plus-cytosine content (%G+C) (Belland & Trust 1988), the subspecies *achromogenes* should be restructured to include the present subspecies *masoucida*. The close genetic relatedness of these subspecies was also shown by Martinez-Murcia et al. (1992), in contrast to the findings of Austin et al. (1989), which supported the separation of the 2 subspecies. However, in Bergey's Manual of Determinative Bac-

teriology (Holt et al. 1994) the 3 subspecies from the approved list (Skerman et al. 1980) are included together with a new subgroup, subsp. *smithia*, which was created by Austin et al. (1989).

In the description of *Aeromonadaceae* (Colwell et al. 1986) the %G+C content of the DNA ranged from 40 to 63 mol%. The %G+C contents of the DNA of subspecies of *Aeromonas salmonicida* in different studies ranged from 52 to 67 mol% (Table 1). The %G+C values given in Table 1 do not allow for distinction of the different subspecies.

Although several studies have described the genetic relationships between the species within the genus *Aeromonas*, there is still a lack of reliable traits for subspecies discrimination, especially for the slow-growing fastidious strains which have been isolated during the past few years (Pedersen et al. 1994, Wiklund et al. 1994). Further studies have to be based on large numbers of strains and use more modern techniques such as poly-nucleotide sequencing and DNA-DNA or RNA-DNA hybridization.

IDENTIFICATION AND CHARACTERIZATION

The genus *Aeromonas* (Kluyver & Van Niel 1936) consists of a collection of oxidase- and catalase-positive, glucose-fermenting, facultatively anaerobic, Gram-negative, rod-shaped bacteria (Popoff 1984) that are resistant to vibriostatic agent O/129 (10 µg) and novo-

biocin (5 µg) (Magariños et al. 1992). With respect to motility, the genus consists of 2 well-separated groups with the species and subspecies of *Aeromonas salmonicida* belonging to the non-motile group. The optimal growth temperature for *A. salmonicida* is 20 to 22°C (Paterson et al. 1980a). Growth may be accompanied by the release of a brown pigment which is a property used in the classification of *A. salmonicida* subspecies (Griffin et al. 1953b, Donlon et al. 1983, Altmann et al. 1992). The existence of achromogenic or slowly pigmenting strains of *A. salmonicida* has been described, with the consequence that different strains have not been properly identified (Bulkley 1969, Mawdesley-Thomas 1969, LeTendre et al. 1972, Michel 1981).

According to Schubert (1967), *Aeromonas salmonicida* subsp. *achromogenes* differs only in a few properties from subsp. *salmonicida*. Identification of atypical *A. salmonicida* strains to the genus level is easily achieved with sufficient experience. However, reports published on the isolation of cytochrome oxidase negative atypical as well as typical strains (Traxler & Bell 1988, Chapman et al. 1991, Olivier 1992, Pedersen et al. 1994, Wiklund et al. 1994) have complicated identification. In addition, the fermentative reaction of glucose in Hugh & Leifson's medium sometimes requires prolonged incubation, especially for the identification of the slow-growing fastidious strains isolated from flatfish (Pedersen et al. 1994, Wiklund et al. 1994).

Identification to the species level can be further confirmed by demonstrating the antigenic similarity between the typical and atypical strains with respect to the thermostable O-antigen, by macroscopic slide agglutination using antiserum prepared from an isolate of *Aeromonas salmonicida* subsp. *salmonicida* (McCarthy 1977, Trust et al. 1980c, Pedersen et al. 1994) or a hybridoma producing a highly specific monoclonal antibody against *A. salmonicida* (Yoshimizu et al. 1993).

Biochemical characteristics useful for distinguishing the different atypical subspecies of *Aeromonas salmonicida* from the homogenous and well-described *A. salmonicida* subsp. *salmonicida* are summarized in Table 2. The identification of atypical strains and the separation of these from subsp. *salmonicida* has been complicated because strains of subsp. *salmonicida* have been found to differ in the key characteristics: pigment production (Wiklund et al. 1993), cytochrome oxidase activity (Chapman et al. 1991), and acid from sucrose (Wiklund et al. 1992). An accurate biochemical identification of atypical *A. salmonicida* based on a comparison of inter-laboratory evaluations has proven difficult; likewise data presented in the literature on various strains of *A. salmonicida* are not readily comparable (Dalsgaard et al. 1998).

Table 1. *Aeromonas salmonicida* subspecies. Base composition of the DNA. G+C: guanine-plus-cytosine; nd: no data available

Subspecies or group	G+C contents mol%	Source
<i>A. salmonicida</i>	55.1–57.5	McCarthy (1978)
	62.7	MacInnes et al. (1979)
	57–59	Popoff (1984)
	52.6–57.8	Belland & Trust (1988)
<i>A. achromogenes</i>	52.9	McCarthy (1975)
	59.7	Belland & Trust (1988)
<i>A. masoucida</i>	60.0	Belland & Trust (1988)
<i>A. nova</i>	56.1–57.6	McCarthy (1978)
<i>A. smithia</i>	55.9 ± 0.5	Austin et al. (1989)
Phenon 13	nd	Austin et al. (1989)
Slow-growing fastidious (flatfish)	54.0–66.9	Wiklund et al. (1994)
Atypical strains	56.5–58.6	Bootsma et al. (1977)
	55.4–57.1	McCarthy (1978)
	57.9	Paterson et al. (1980b)
	57.0	Shotts et al. (1980)
	54.4–56.1	Trust et al. (1980b)
	57.3	Kitao et al. (1984)
	59.0–59.3	Ohtsuka et al. (1984)
	52.4–57.8	Belland & Trust (1988)

Table 2. *Aeromonas salmonicida*. Characteristics for differentiation between subspecies and atypical isolates. +: positive reaction; -: negative reaction; (): reaction of a few strains; S: sensitive; R: resistant; nd: no data available

Subspecies or group	Pigment production	Oxidase reaction	Gas from glucose	Acid from sucrose	Indole production	Degradation of aesculin	Sensitivity to ampicillin	Sensitivity to cephalothin
<i>A. salmonicida</i> ^a	+(-)	+(-)	+	-(+)	-	+	S	S
<i>A. achromogenes</i> ^a	-(+)	+	-	+	+	-	R	R
<i>A. masoucida</i> ^a	-	+	+	+	+	+	nd	nd
<i>A. nova</i> ^b	-/+	+	-	+/-	+(-)	-/+	R/S	R/S
<i>A. smithia</i> ^c	-(+)	+	nd	+(-)	-	-	S(R)	nd
Phenon 13 ^c	+(-)	+	nd	+	-	-	R	nd
Slow-growing ^d	-	-/+	-	+/-	+(-)	+(-)	S	S(R)
Atypical isolates ^e	+/-	+	-	+	-/+	-(+)	R(S)	R(S)

^aPopoff (1984), Chapman et al. (1991), Wiklund et al. (1992, 1993)
^bMcCarthy (1978), Böhm et al. (1986), Olivier (1992)
^cAustin et al. (1989)
^dPedersen et al. (1994), Wiklund et al. (1994), Wiklund & Dalsgaard (1995)
^eDalsgaard & Paulsen (1986), Hirvelä-Koski et al. (1994), Nakatsugawa (1994)

McCarthy (1978), Böhm et al. (1986) and Olivier (1992) classified the examined atypical strains as subspecies *achromogenes* and *nova*. Some important characteristics of these isolates were the same for all strains (Table 2), but subspecies *nova* seemed to be more fastidious and growth could require up to 5 d incubation at 22°C.

Austin et al. (1989) used numerical taxonomy and DNA:DNA hybridization techniques to group *Aeromonas salmonicida* into 5 phena, which were equated with the subspecies *salmonicida*, *masoucida*, *achromogenes* and *smithia* and a fifth group (phenon 13) comprising unrecognized subspecies. Further strains which were able to grow at 37°C were included in the fifth group (Austin 1993).

The plasmid profiles reported from atypical strains of *Aeromonas salmonicida* were different from the plasmids carried by typical strains (Bast et al. 1988, Belland & Trust 1989, Pedersen et al. 1996), and Belland & Trust (1989) suggested that plasmid content may be a useful epizootiological marker for atypical *A. salmonicida*. The results presented by Pedersen et al. (1996) indicated that plasmid profiles of atypical strains may change considerably *in vivo* and several epizootiologically related strains showed different profiles. Similarly, the variability observed in plasmids in different strains isolated from flounder *Platichthys flesus* seemed to be of no value for epizootiological work because variability occurred within the isolates from the same geographical area (Dalsgaard 1994a). In contrast to plasmid profiles, ribotypes never showed variation upon repeated testing (Pedersen et al. 1996). Most epizootiologically unrelated strains had different ribotypes, whereas isolates from the same outbreak were identical. The data obtained so far suggest that ribotyping can permit the identification of subgroups, which may be useful in epizootiological studies (McCormick et al. 1990, Dalsgaard 1994a, Whittington et al. 1995, Pedersen et al. 1996).

HOST RANGE

Atypical *Aeromonas salmonicida* strains have been isolated from a wide range of fish species belonging to different families and orders (Table 3). More than 20 farmed and 30 wild fish species have been reported to harbor atypical *A. salmonicida* strains. Initially, atypical *A. salmonicida* strains were predominantly recovered from fresh water or anadromous fish species. However, more recently atypical strains have repeatedly been isolated from marine fish species, like flatfish (order Pleuronectiformes) and codfish (order Gadiformes), as well as catadromous fish species, like eels (family Anguillidae) (Table 3).

Non-salmonids

With regard to non-salmonid wild fish, atypical *Aeromonas salmonicida* has been isolated from occasional cases of ulcerated or otherwise diseased fish from nature or from wild fish kept in aquaria or tanks (Evelyn 1971, Cornick et al. 1984, Dalsgaard & Paulsen 1986, Wiklund 1990, Wilson & Holliman 1994). In a few cases, atypical *A. salmonicida* has been associated with epizootics in wild fish populations (Bulkley 1969, McCarthy 1975, Håstein et al. 1978, Michel 1981).

In several studies atypical *Aeromonas salmonicida* has been isolated from fish which after capture were transferred to tanks, aquaria or net pens. This was the case, for example, for sablefish *Anoplopoma fimbria* (Evelyn 1971), Atlantic cod *Gadus morhua* (Cornick et al. 1984) and Pacific herring *Clupea harengus pallasi* in Canada (Traxler & Bell 1988), sand-eels *Hyperoplus lanceolatus* and *Ammodytes lancea* in Denmark (Dalsgaard & Paulsen 1986), and greenback flounder *Rhombosolea tapirina* in Tasmania (Whittington et al. 1995).

Table 3. *Aeromonas salmonicida*. Isolates of atypical *A. salmonicida* from farmed and wild fish species in different countries

Fish species	Scientific name	Country	Source
Order Anguilliformes			
FARMED FISH			
American eel	<i>Anguilla rostrata</i>	USA	Noga & Berkhoff (1990)
European eel	<i>Anguilla anguilla</i>	Denmark ^a	Dalsgaard (1994b)
Japanese eel	<i>Anguilla japonica</i>	Japan	Ohtsuka et al. (1984)
WILD FISH			
American eel	<i>Anguilla rostrata</i>	Canada, USA	Olivier (1992), Noga & Berkhoff (1990)
European eel	<i>Anguilla anguilla</i>	Denmark	Dalsgaard (1994b)
Order Clupeiformes			
WILD FISH			
Pacific herring	<i>Clupea harengus pallasii</i>	Canada ^d	Traxler & Bell (1988)
Order Cypriniformes			
FARMED FISH			
Carp	<i>Cyprinus carpio</i>	Australia Denmark ^b Germany Hungary Netherlands ^b UK ^b Yugoslavia	Humphrey & Ashburner (1993) Belland & Trust (1988) Böhm et al. (1986), Mirlé et al. (1986) Csaba et al. (1980a) Ishiguro et al. (1986) Austin (1993) Bootsma et al. (1977)
Goldfish	<i>Carassius auratus</i>	Australia Denmark Germany Italy ^b Japan Netherlands ^b Singapore UK USA Yugoslavia ^b Germany	Trust et al. (1980c), Humphrey & Ashburner (1993) Dalsgaard (unpubl. data) Böhm et al. (1986) Whittington et al. (1987) Elliott & Shotts (1980a) Evenberg et al. (1982) Whittington et al. (1995) Elliott & Shotts (1980a) Elliott & Shotts (1980a) Evenberg et al. (1982) Böhm et al. (1986)
'Shubunkin'	<i>Carassius</i> sp.	Germany	Böhm et al. (1986)
WILD FISH			
Bream	<i>Abramis brama</i>	Finland	Wiklund (unpubl. data)
Chub	<i>Leuciscus cephalus</i>	UK	Wilson & Holliman (1994)
Crucian carp	<i>Carassius carassius</i>	Hungary	Csaba et al. (1980a)
Dace	<i>Leuciscus leuciscus</i>	Finland	Hirvelä-Koski et al. (1994)
Goldfish	<i>Carassius auratus</i>	Australia Canada	Whittington et al. (1987) Munkittrick & Leatherland (1984)
Minnow	<i>Phoxinus phoxinus</i>	Norway	Håstein et al. (1978)
Roach	<i>Rutilus rutilus</i>	Australia Finland Sweden UK	Humphrey & Ashburner (1993) Hirvelä-Koski et al. (1994) Wichardt et al. (1989) McCarthy (1975)
Rudd	<i>Scardinius erythrophthalmus</i>	UK ^b	Gudmundsdóttir et al. (1990)
Silver bream	<i>Blicca bjoerkna</i>	UK	McCarthy (1975)
Order Gadiformes			
WILD FISH			
Cod	<i>Gadus morhua</i>	Canada ^d Iceland ^a	Cornick et al. (1984) Guðmundsdóttir et al. (1995)
Four bearded rockling	<i>Enchelyopus cimbrius</i>	Denmark	Dalsgaard (1994b)
Haddock	<i>Melanogrammus aeglefinus</i>	Canada ^e Iceland ^b	Olivier (1992) Gudmundsdóttir (1996)
Tomcod	<i>Gadus microgadus</i>	Canada	Olivier (1992)
Viviparous blenny	<i>Zoarces viviparus</i>	Baltic Sea Denmark	Wiklund (unpubl. data) Dalsgaard (1994b)
Whiting	<i>Merlangius merlangus</i>	Iceland	Guðmundsdóttir et al. (1996)
Order Perciformes			
FARMED FISH			
Common wolfish	<i>Anarhichas lupus</i>	Norway ^a	Hellberg et al. (1996)
Silver perch	<i>Bidyanus bidyanus</i>	Australia	Humphrey & Ashburner (1993)
Spotted wolfish	<i>Anarhichas minor</i>	Norway	M. Valheim (pers. comm.)
Wrasse	<i>Labrus berggylta</i>	Norway	A. B. Olsen (pers. comm.)
WILD FISH			
Goldsinny wrasse	<i>Ctenolabrus rupestris</i>	Ireland Norway ^a	Frerichs et al. (1992) Gravningen et al. (1996)
Perch	<i>Perca fluviatilis</i>	France ^d Sweden UK	Michel (1981) Wichardt et al. (1989) Bucke et al. (1979)
Sand-eels	<i>Ammodytes lancea</i> <i>Hyperoplus lanceolatus</i>	Denmark ^o	Dalsgaard & Paulsen (1986)
Smallmouth bass ^c	<i>Micropterus dolomieu</i>	USA	LeTendre et al. (1972)
Yellow bass ^c	<i>Morone mississippiensis</i>	USA	Bulkley (1969)

(Table continued on next page)

Table 3 (continued)

Fish species	Scientific name	Country	Source
Order Pleuronectiformes			
FARMED FISH			
Shotted halibut	<i>Eopsetta grigorjewi</i>	Japan	Nakatsugawa (1994)
Turbot	<i>Scophthalmus maximus</i>	Denmark Norway	Pedersen et al. (1994) Gravningen & Dydland (1991)
WILD FISH			
American plaice	<i>Hippoglossoides platessoides</i>	Canada ^a	Olivier (1992)
Dab	<i>Limanda limanda</i>	Baltic Sea Denmark	Wiklund (unpubl. data) Wiklund & Dalsgaard (1995)
Flounder	<i>Platichthys flesus</i>	Denmark Finland Southern Baltic Sea	Wiklund & Dalsgaard (1995) Wiklund & Bylund (1991) Wiklund & Dalsgaard (1995)
Greenback flounder	<i>Rhombosolea tapirina</i>	Australia ^a	Whittington et al. (1995)
Halibut	<i>Hippoglossus hippoglossus</i>	Canada ^a Norway ^b	Cornick et al. (1984) Gudmundsdóttir (1996)
Plaice	<i>Pleuronectes platessa</i>	Denmark	Wiklund & Dalsgaard (1995)
Turbot	<i>Scophthalmus maximus</i>	Baltic Sea	Wiklund (unpubl. data)
Order Salmoniformes			
FARMED FISH			
Arctic char	<i>Salvelinus alpinus</i>	Canada Finland Iceland ^b Norway Sweden	Olivier (1992) Hirvelä-Koski et al. (1994) Gudmundsdóttir (1996) M. Valheim (pers. comm.) Ljungberg & Johansson (1977)
Atlantic salmon	<i>Salmo salar</i>	Canada Faeroe Islands Finland Iceland ^b Norway Sweden	Paterson et al. (1980b) Dalsgaard & Mortensen (1988) Rintamäki & Valtonen (1991) Gudmundsdóttir et al. (1990) Olsen et al. (1989) Ljungberg & Johansson (1977)
Brook trout	<i>Salvelinus fontinalis</i>	Canada Sweden USA	Bullock et al. (1971) Ljungberg & Johansson (1977) Snieszko et al. (1950)
Brown trout	<i>Salmo trutta m. fario</i>	Finland Iceland ^b Sweden	Hirvelä-Koski et al. (1994) Gudmundsdóttir (1996) Ljungberg & Johansson (1977)
Chum salmon	<i>Oncorhynchus keta</i>	Canada	Evelyn (1971)
Grayling	<i>Thymallus thymallus</i>	Finland Sweden	Rintamäki & Valtonen (1991) Ljungberg & Johansson (1977)
Lake trout	<i>Salmo trutta m. lacustris</i>	Finland Sweden	Ojala (1966) Ljungberg & Johansson (1977)
Masu salmon	<i>Oncorhynchus masou</i>	Japan	Kimura (1969a)
Pink salmon	<i>Oncorhynchus gorbuscha</i>	Japan	Kimura (1969a)
Rainbow trout	<i>Oncorhynchus mykiss</i>	Canada Finland South Africa Sweden	Olivier (1992) Hirvelä-Koski et al. (1994) Boomker et al. (1984) Ljungberg & Johansson (1977)
Sea trout	<i>Salmo trutta m. trutta</i>	Faeroe Islands ^b Finland Norway ^b Sweden	Gudmundsdóttir (1990) Rintamäki & Valtonen (1991) Pedersen et al. (1996) Wichardt (1983a)
Whitefish	<i>Coregonus sp.</i>	Finland	Hirvelä-Koski et al. (1994)
WILD FISH			
Atlantic salmon	<i>Salmo salar</i>	Canada	Olivier (1992)
Grayling	<i>Thymallus thymallus</i>	Finland	Hirvelä-Koski et al. (1994)
Pike	<i>Esox lucius</i>	Finland Sweden UK ^b	Wiklund (1990) Wichardt et al. (1989) Austin et al. (1989)
Sea trout	<i>Salmo trutta m. trutta</i>	Scotland	Smith (1963)
Sockeye salmon	<i>Oncorhynchus nerka</i>	Canada ^d	Evelyn (1971)
Order Scorpaeniformes			
WILD FISH			
Sablefish	<i>Anoplopoma fimbria</i>	Canada ^d	Evelyn (1971)

^aWild fish kept in tanks, aquaria or net pens

^bNot reported whether isolated from wild or farmed fish

^cPresent identification of isolates as atypical *Aeromonas salmonicida* is based on slow pigment production

^dIsolated strains subsequently identified as likely atypical *Aeromonas salmonicida* (C. Michel pers. comm.)

In most studies the fish showed signs of disease shortly after transfer to the tanks or aquaria. However, the source of the infection usually remained unknown, and it was not definitely established whether the fish infected with atypical *A. salmonicida* harbored the pathogen at the time of capture or whether the fish were infected during transport or from the water or equipment in the tanks or aquaria (Evelyn 1971, Cornick et al. 1984, Dalsgaard & Paulsen 1986, Whittington et al. 1995). In other studies atypical strains have been isolated from the skin (Hubbert & Williams 1980), gills (Benediktsdóttir & Helgason 1990), and kidney (Frerichs et al. 1992) of clinically healthy fish without any signs of disease, indicating that atypical *A. salmonicida* might be present in or on the fish and that stressful conditions (e.g. capture and transfer to tanks) may cause subsequent outbreaks of disease.

Salmonids

Fish belonging to different species of the family Salmonidae have frequently been reported to be infected with atypical *Aeromonas salmonicida* (see Table 3); increased reporting is probably due to the intensive farming of, and economical interest in, these species. More than 10 different salmonid species have been found to be infected with atypical *A. salmonicida*. There are, however, comparably few reports on the isolation of atypical strains from wild fish belonging to family Salmonidae (Evelyn 1971, Olivier 1992, Hirvelä-Koski et al. 1994) (Table 3).

GEOGRAPHICAL DISTRIBUTION

The geographical distribution of reported isolations of atypical *Aeromonas salmonicida* is indicated in Table 3. Available reports suggest that atypical *A. salmonicida* strains infect fish mainly in the temperate regions of the northern hemisphere, that is, Canada, the USA, Japan, and central and northern Europe including the Nordic countries. In addition, atypical strains have been isolated from fish in southern Australia including Tasmania (Whittington et al. 1987, Whittington et al. 1995), South Africa (Boomker et al. 1984) and from fish exported from Singapore (Whittington et al. 1995). There are also a few reports of atypical strains isolated from fish in the Mediterranean area: from carp *Cyprinus carpio* and goldfish *Carassius auratus* in former Yugoslavia (Bootsma et al. 1977, Evenberg et al. 1982) and from goldfish in Italy (Whittington et al. 1987). To date, there is no information available about atypical *A. salmonicida* infecting fish in South America, Russia or northern Asia.

Atypical *Aeromonas salmonicida* strains have been isolated from fish in fresh water (Bulkley 1969, Håstein et al. 1978, Michel 1981, Whittington et al. 1987, Noga & Berkhoff 1990, Wilson & Holliman 1994), brackish water (Wiklund 1990, Wiklund & Bylund 1991, Wiklund et al. 1994), as well as salt water habitats (Boomker et al. 1984, Cornick et al. 1984, Dalsgaard & Paulsen 1986, Pedersen et al. 1994, Whittington et al. 1995, Wiklund & Dalsgaard 1995, Hellberg et al. 1996).

PATHOLOGY

Non-salmonids

Atypical *Aeromonas salmonicida* infection in non-salmonids often manifests itself predominantly as a skin infection (skin ulceration) without subsequent general septicaemia. Skin ulcerations and skin lesions on wild and farmed non-salmonid fish have been observed in connection with most disease outbreaks associated with atypical *A. salmonicida*. The best described diseases showing these symptoms are carp erythrodermatitis (CE; Fijan 1972, Bootsma et al. 1977, Csaba et al. 1980a, b, Sövényi et al. 1984, Böhm et al. 1986, Mirle et al. 1986), goldfish ulcer disease (GUD; Mawdesley-Thomas 1969, Elliott & Shotts 1980a, Shotts et al. 1980, Trust et al. 1980c, Böhm et al. 1986, Whittington et al. 1987, Humphrey & Ashburner 1993, Whittington et al. 1995), and ulcer disease of flounder (UDF) in the Baltic and the North Sea, including adjacent rivers (Nounou et al. 1981, Möller 1990, Wiklund & Bylund 1991, Ullrich 1992, Vethaak 1992, Wiklund & Bylund 1993, Wiklund 1994a, b, Wiklund et al. 1994, Wiklund & Dalsgaard 1995). In addition, many other fish species have been reported to be affected by ulcerations associated with atypical *A. salmonicida*, and the pathogen is apparently involved in more disease outbreaks than previously suspected (McCarthy 1975, Håstein et al. 1978, Ohtsuka et al. 1984, Dalsgaard & Paulsen 1986, Noga & Berkhoff 1990, Wiklund 1990, Pedersen et al. 1994, Wilson & Holliman 1994, Larsen & Pedersen 1996).

In carp affected by CE, the ulcers can be found all over the body surface except on the head (Jeney & Jeney 1995). The scales of the fish have been reported to be initially surrounded by inflammation with infiltration of inflammatory cells. The scales are subsequently shed. The epithelium and corium become necrotized with marked hyperaemia surrounding the resulting ulcer. The inflammatory cells subsequently spread into the muscle (Gayer et al. 1980, Jeney & Jeney 1995).

Mawdesley-Thomas (1969) reported that the general health of goldfish infected with *Aeromonas salmonicida* was variable. Affected fish developed skin ulcers of

varying size and depth, which could appear on any part of the body. In some specimens even the face, operculum and the eyes were affected. A marked increase in pigmentation in and around the lesions and inflammatory cells penetrating far into the surrounding musculature were observed. Some of the ulcers were secondarily infected with *Saprolegnia* sp. Some fish died within a few days; others showed lethargy, loss of orientation and abnormal swimming movements.

In flounder affected by UDF, the ulcers have been described as open, dark red, rounded, and superficial (Wiklund & Bylund 1993). Ulcer size varied from small lesions (1 to 2 mm) to large eroded areas (10 to 20 mm in diameter). The development of the ulcers was divided into 3 stages: (1) initially weak haemorrhage in the skin which developed into (2) a white lesion surrounded by a red rim of haemorrhagic inflammatory tissue; (3) in the final, true ulcer stage the skin tissue was eroded and the muscle tissue was exposed (Wiklund & Bylund 1993). Unfortunately, there are no reports on histological studies of skin ulcers in flounder.

Although there is accumulating evidence that atypical *Aeromonas salmonicida* is the aetiological agent of CE, GUD and UDF, the subject is a matter of discussion, and under certain circumstances other (opportunistic) pathogens can be isolated. In addition to *A. salmonicida*, the aetiology of CE, GUD and UDF has been associated with *Aeromonas hydrophila*, *Vibrio anguillarum*, *Flexibacter columnaris* or opportunistic pathogens (Takahashi et al. 1975a, b, c, Nounou et al. 1981, Sioutas et al. 1991, Ullrich 1992, Vethaak 1992, reviewed by Wiklund 1994b). However, some of these studies were inadequately reported or performed and, hence, a successful isolation of atypical *A. salmonicida* strains could not be ensured. Sioutas et al. (1991) and Vethaak (1992) did not specify the incubation time when examining ulcerated carp and flounder. Slow-growing strains of atypical *A. salmonicida* could have been overlooked due to a short incubation time. Vethaak (1992) isolated *A. salmonicida* from flounder, but it was not stated whether this was a typical or an atypical strain. Nounou et al. (1981) used selective or non-enriched media in the isolation procedure, thus hampering the probable isolation of fastidious atypical *A. salmonicida* from ulcerated flounder.

In American eel *Anguilla rostrata*, European eel *Anguilla anguilla*, as well as Japanese eel *Anguilla japonica*, atypical *Aeromonas salmonicida* strains have been reported to cause severe necrosis, lesions of the skin, and tissue swelling on the head of affected specimens (Iida et al. 1984, Kitao et al. 1984, 1985, Ohtsuka et al. 1984, Noga & Berkhoff 1990, Dalsgaard 1994b). The disease in eels has thus been referred to as 'head ulcer disease' (Ohtsuka et al. 1984). A mild to severe, primarily mononuclear infiltrate was observed in the

ulcers (Noga & Berkhoff 1990). Many lesions had extensive collagen deposition, contributing to the tissue swelling. Bacterial microcolonies were common on the surface and within ulcerations (Noga & Berkhoff 1990).

In addition to skin ulcerations, atypical *Aeromonas salmonicida* has also been associated with erosions on the mouth, varying from weak haemorrhage on the snout (sand-eels; Dalsgaard & Paulsen 1986), and erosion of the lip (shotted halibut *Eopsetta grigorjewi*; Nakatsugawa 1994) to destruction of the bones in the upper and lower jaws (minnow *Phoxinus phoxinus*; Håstein et al. 1978) of non-salmonid fish. Additionally, haemorrhage and erosion of the fins (McCarthy 1975, Trust et al. 1980c, Wilson & Holliman 1994, Jeney & Jeney 1995), with subsequent necrosis of the tail (Dalsgaard & Paulsen 1986), and haemorrhage or lesions in the eyes (Mawdesley-Thomas 1969, Håstein et al. 1978) have been associated with infections caused by atypical *A. salmonicida*. Hyperplasia or proliferation of the gills sometimes containing bacterial colonies has been reported (Mawdesley-Thomas 1969, Morrison et al. 1984). In histological studies, the ulcers showed pathological changes with oedema, distortion of the scales, hyperaemia, haemorrhage, leucocytic infiltration and the presence of fibroblast-like cells often causing granulomatous tissue in the dermis as well as in the spleen and the kidney (Håstein et al. 1978, Morrison et al. 1984).

In non-salmonids there are few reports on signs of pathological disease in internal organs of fish infected with atypical *Aeromonas salmonicida*. Congested and dark kidneys, spleens and gills were detected in goldfish naturally infected with atypical *A. salmonicida*. Additionally, enlargement of the hepatocytes, degenerative changes in the kidney, congestion and in some cases hyperplasia in the spleen were observed (Mawdesley-Thomas 1969). Pathological signs of disease in the intestine (haemorrhage and hyperaemia) have been observed in captive sand-eels (Dalsgaard & Paulsen 1986) and farmed common wolffish *Anarhichas lupus* (Hellberg et al. 1996). Additionally, haemorrhages in the liver and in the musculature of infected sand-eels have been reported (Dalsgaard & Paulsen 1986). In infection experiments with atypical *A. salmonicida* isolated from cod, degenerative changes, accumulated leucocytes, and cyst formations were seen in the spleen and kidney of infected cod, while in Atlantic salmon *Salmo salar* the injected bacteria caused no cellular host reaction except for the presence of necrotic muscle fibers at the injection site (Morrison et al. 1984). In farmed wolffish, neither a corresponding leucocyte reaction nor fibroblast encystment of atypical *A. salmonicida* was observed (Hellberg et al. 1996). However, Bulkley (1969), McCarthy (1975), Håstein et al. (1978), Michel (1981), Wilson &

Holliman (1994), and Jeney & Jeney (1995) reported that macroscopical signs of disease in internal organs of fish (silver bream *Blicca bjoerkna*, carp, minnow, perch *Perca fluviatilis*, yellow bass *Morone mississippiensis*) infected with atypical *A. salmonicida* were not observed, although Jeney & Jeney (1995) stated that a slight fatty degeneration was sometimes visible in carp affected by carp erythrodermatitis. According to Fijan (1972), the internal organs of carp affected with small ulcers appeared normal, but in the terminal stages fluid accumulation was found in the abdominal cavity and the internal organs were oedematous. In several studies no indication of pathological signs in internal organs of non-salmonids infected with atypical *A. salmonicida* have been reported (Trust et al. 1980c, Whittington et al. 1987, Noga & Berkhoff 1990, Wiklund 1990, Wiklund & Bylund 1993, Pedersen et al. 1994, Wiklund & Dalsgaard 1995).

Salmonids

In salmonids different atypical *Aeromonas salmonicida* strains have been associated with different pathological gross signs. Wichardt (1983a) concluded that the signs of disease in sea trout *Salmo trutta* m. *trutta*, which were caused by pigment-producing atypical *A. salmonicida*, more closely resembled those induced by achromogenic variants of atypical *A. salmonicida* than those caused by typical strains. In contrast, Rintamäki & Valtonen (1991) found pigment-producing atypical *A. salmonicida* caused signs of disease in salmonids (mainly brown trout *Salmo trutta* m. *fario* and Atlantic salmon) that were similar to those caused by typical *A. salmonicida* but different from those caused by achromogenic variants of atypical *A. salmonicida*. The achromogenic strains caused mainly skin ulcerations and other external or internal signs of disease were not detectable. According to Groman et al. (1992), both *A. salmonicida* subsp. *nova* and typical *A. salmonicida* produced similar signs of disease in salmon, i.e. lethargy, aimless swimming, respiratory distress, fin erosion and haemorrhagic cutaneous and muscular lesions. In some of the disease outbreaks in salmonids caused by atypical *A. salmonicida* strains, ulcerations in the infected fish were reported (Snieszko et al. 1950, Paterson et al. 1980b, Boomker et al. 1984, Rintamäki & Valtonen 1991). Ulcerations were characteristically observed in disease outbreaks in brook trout *Salvelinus fontinalis* associated with *Hemophilus piscium* (Snieszko et al. 1950, Bullock et al. 1971), now classified as atypical *A. salmonicida* (Paterson et al. 1980a). Histologically, cellular infiltration, degenerative changes in the epithelial and superficial muscle cells and considerable tissue destruction have been observed in

ulcerations of rainbow trout *Oncorhynchus mykiss* caused by atypical *A. salmonicida*. However, tissue reaction or attempts at regeneration were not observed (Boomker et al. 1984).

Haemorrhages in the gills, liver, heart, intestine as well as in the peritoneal cavity of sea trout and lake trout *Salmo trutta* m. *lacustris* have been reported (Ojala 1966, Wichardt 1983a). Boomker et al. (1984) observed the presence of a few gross changes in internal organs, like slight splenomegaly, discoloration of the liver and enlargement of the kidney of rainbow trout infected with atypical *A. salmonicida*. Microscopically, the spleen and the liver were congested and in the kidney slight vasculitis and early degeneration of tubular epithelium were encountered. Bacterial colonies were scarce and present only in sections of the skin.

The few signs of disease reported to be present in internal organs of fish indicate that atypical *Aeromonas salmonicida* seldom colonizes those organs. This conclusion is in accordance with the fact that atypical *A. salmonicida* is only occasionally isolated from the internal organs of fish with an ulcerative infection (see 'Isolation' and Table 5). Why the organism is retained at the site of the local skin lesion is unknown, but it might be a result of the effects of local inflammation and the host's immune response. Certainly there are also differences in the invasive capacity between different atypical isolates. More detailed studies on the infective mechanisms of different atypical strains and corresponding immune response in the host are needed.

MORTALITY

Wild fish

Epizootics, occasionally with high mortality, have been observed in wild fish in connection with the isolation of atypical *Aeromonas salmonicida* (Table 4). However, for practical reasons the mortality rate for epizootics in wild fish has seldom been presented (McCarthy 1975). Additionally, the relationship between the mortality recorded and the isolated atypical *A. salmonicida* strains has not always been properly established (Bucke et al. 1979). High mortality in wild fish, suggested to be caused by atypical strains, has been described among yellow bass (Bulkley 1969), silver bream (McCarthy 1975), minnow (Håstein et al. 1978), and perch (Michel 1981; strains subsequently identified as a likely atypical *A. salmonicida*, C. Michel pers. comm.) (Table 4). In some of these cases, inadequate nutrition (Bulkley 1969), transport of live fish (McCarthy 1975), or low water associated with spawn-

Table 4. Mortality rates in wild fish species and farmed salmonids associated with atypical *Aeromonas salmonicida*

Fish species	Country	Mortality	Year	Source
Wild fish species				
Silver bream	UK	25% morbidity	1976	McCarthy (1975)
Perch	UK	>98%		Bucke et al. (1979)
Yellow bass	USA	Considerable		Bulkley (1969)
Minnow	Norway	Mass mortality		Håstein et al. (1978)
Perch	France	Mass mortality		Michel (1981)
Farmed salmonids				
Sea trout	Sweden	60–65%	1983	Wichardt (1983b)
Atlantic salmon	Canada	~50%	1974	Paterson et al. (1980b)
Rainbow trout	South Africa	30%	1981	Boomker et al. (1984)
Atlantic salmon	Canada	>25%	1991	Groman et al. (1992)
Different species	Iceland	15–25%		Guðmundsdóttir et al. (1995)
Sea trout	Finland	2–20%	1988	Rintamäki & Valtonen (1991)
Salmon	Faroe Islands	7%	1986	Dalsgaard & Mortensen (1988)

ing season (Håstein et al. 1978), which all may cause stressful conditions for the fish, were suggested as the primary cause of the infection with atypical *A. salmonicida* and subsequent heavy mortality observed. In some studies of ulcerated fish, mortality in the affected population has not been reported (Wiklund 1990, Wiklund & Bylund 1993, Wiklund & Dalsgaard 1995). However, low mortality in a fish population, whatever the reason, will most probably remain undetected. Low numbers of weak and moribund fish will be eliminated due to predation by fish, birds and mammals.

Farmed fish

In farmed fish high cumulative mortality has been reported in several studies (Table 4). Wichardt (1983b) reported very high mortality (60 to 65%) in farmed sea trout in one farm in Sweden due to atypical *Aeromonas salmonicida* infections during unfavourable environmental conditions. In farmed Atlantic salmon in Canada, 50% cumulative mortality was observed in 1974 (Paterson et al. 1980b). In 1991 over \$500 000 in potential sales were lost during disease outbreaks caused by atypical *A. salmonicida*, which was considered to be the major economic constraint to the commercial salmonid culture in the area (Groman et al. 1992). In Iceland atypical *A. salmonicida* infections in farmed fish have caused losses equal to 15–25% of the total slaughter production (Guðmundsdóttir et al. 1995). In general, however, the mortality due to atypical strains has generally stayed low although occasional mortality up to 20% (Rintamäki & Valtonen 1991), 30% (Boomker et al. 1984) and 35% (calculated from Pedersen et al. 1994) have been reported (Table 4).

The mortality in carp caused by atypical *Aeromonas salmonicida* has been found to reach 25% in pond culture (Sövényi 1986). However, it has been suggested

that the mortality in carp populations was caused by invasion of opportunistic pathogens, facilitated by the immunosuppressive substances released by atypical *A. salmonicida* (Evenberg et al. 1986, Pourreau et al. 1986, Sövényi et al. 1988).

ISOLATION

While the isolation of typical *Aeromonas salmonicida* is mostly a standard procedure and the cells readily grow on basic isolation agar, the recovery of atypical *A. salmonicida* is more difficult. Fastidious, slow-growing strains of atypical *A. salmonicida* (Bullock et al. 1971, Ishiguro et al. 1986, Wiklund & Bylund 1991, Olivier 1992, Pedersen et al. 1994, Wiklund & Dalsgaard 1995) have been reported to require agar supplemented with blood or serum for isolation and further cultivation (Paterson et al. 1980b, Ishiguro et al. 1986, Olivier 1992, Wiklund et al. 1994, Wiklund & Dalsgaard 1995). McCarthy (1977) reported that tryptone soya agar was superior to blood-containing medium for the isolation of atypical as well as typical *A. salmonicida*. However, this view was not confirmed by any comparative analysis, and it represented merely an opinion based on laboratory experience. Austin (1993) found that atypical strains did grow on blood agar, but not on brain-heart infusion agar or tryptone soya agar at isolation, but subcultures grew well on all 3 media. It has been suggested that the incidence of atypical *A. salmonicida* as a pathogen of different fish species may have been underestimated in studies using agar medium without blood (Paterson et al. 1980b).

Several studies show that atypical *Aeromonas salmonicida* is predominantly isolated from skin ulcers and only occasionally from visceral organs of fish of different species (Table 5). This has been observed in investigations of ulcer disease of brook trout (Snieszko

Table 5. Isolation (% of total number of fish examined) of atypical *Aeromonas salmonicida* from ulcerations and visceral organs of different fish species. nr: atypical *A. salmonicida* isolated but exact number of isolates not reported

Fish species	Country	No. of fish examined	Isolation from ulcerations (%)	Isolation from visceral organs (%)	Source
Flounder	Finland	162	54	2	Wiklund & Bylund (1991)
Chub	UK	14	64	0	Wilson & Holliman (1994)
Cod	Canada	22	77	22	Cornick et al. (1984)
Goldfish	Japan, UK, USA	83	77	26 ^a	Elliott & Shotts (1980a)
Flounder	Denmark & southern Baltic Sea	16	81	0	Wiklund & Dalsgaard (1995)
Roach	UK	75	95	24	Hubbert & Williams (1980)
American eel	USA	20	nr	0	Noga & Berkhoff (1990)

^aNumber of fish examined for infection in visceral organs = 31

et al. 1950), CE (Csaba et al. 1980a, Sövényi et al. 1988, Austin 1993), GUD (Elliott & Shotts 1980a, Whittington et al. 1987, Austin 1993), head ulcer disease of eel (Noga & Berkhoff 1990), ulcerations in perch (Michel 1981), ulcerated cod kept in tanks (Cornick et al. 1984), ulcerations in roach *Rutilus rutilus* (Hubbert & Williams 1980, Austin 1993), and skin ulcerations in different wild flatfish species like flounder, dab *Limanda limanda*, plaice *Pleuronectes platessa* and turbot *Scophthalmus maximus* from the Baltic Sea and the North Sea (Wiklund & Bylund 1991, Wiklund & Dalsgaard 1995) (Table 5). In other studies isolation of atypical *A. salmonicida* has successfully been achieved from internal organs of non-salmonid wild fish kept in tanks or net-pens (Dalsgaard & Paulsen 1986, Traxler & Bell 1988).

In farmed fish, atypical *Aeromonas salmonicida* is often readily isolated from internal organs as well as from ulcers. This has been shown for farmed turbot in Denmark, where atypical *A. salmonicida* was isolated from external ulcers and kidneys of ulcerated specimens (Pedersen et al. 1994). Elliott & Shotts (1980a) reported that atypical *A. salmonicida* was more prevalent in kidneys of goldfish which had ulcerations in the late stage of development than in goldfish with ulcers in the early or intermediate stage of development.

In several studies it was observed that atypical *Aeromonas salmonicida* could not be isolated from the ulcers of all examined ulcerated fish (Table 5). Additionally, atypical *A. salmonicida* was isolated mainly from lesions or ulcers in an early stage of development, and more seldom from chronic, terminal or healing lesions (Elliott & Shotts 1980a, Whittington et al. 1987, Wiklund & Dalsgaard 1995). Generally, in later developmental stages the ulcers had often been invaded by opportunistic pathogens and contaminating water microorganisms such as motile *Aeromonas* sp., *Pseudomonas* sp. and *Saprolegnia* sp. (Bootsma et al. 1977, Elliott & Shotts 1980a, Noga & Berkhoff 1990). The slow growth of atypical *A. salmonicida*, overgrowth by

other bacteria, and/or growth suppression of *A. salmonicida* by contaminants may have contributed to the failure to isolate this pathogen (Bootsma et al. 1977, Elliott & Shotts 1980a, Bullock et al. 1983, Humphrey & Ashburner 1993).

In conclusion it seems essential to perform bacterial isolation on recently developed skin ulcerations as well as on the internal organs of fish, using agar supplemented with blood or serum, in order to make a proper identification of the aetiologic agent of the skin ulcer disease, especially in wild fish.

PATHOGENICITY

The pathogenicity of different atypical *Aeromonas salmonicida* strains seems to be highly variable (Table 6). However, comparison of the virulence of the examined strains is difficult due to the varying methods used in the different studies. The assessment of virulence has been performed on different fish species, and the administration of the bacteria by different routes of challenge (e.g. intraperitoneal, intramuscular, subcutaneous, bath and rubbing into disrupted skin); the amount of administered bacteria has varied greatly, and several studies did not report whether the strain tested was freshly isolated or passed on artificial media (agar) once or several times before the challenge experiment was performed. In some studies the injected amount of bacteria was not reported (Bucke 1980, Michel 1981, Sövényi & Ruttkay 1986, Sövényi et al. 1988). The age and the genetic strain of the fish (e.g. carp) can also significantly affect the resistance against bath challenge with atypical *A. salmonicida* (Wiegertjes et al. 1993). Additionally, the nutritional status of the fish (e.g. Arctic char *Salvelinus alpinus*, carp, grayling *Thymallus thymallus*) and the feeding regime have also been shown to affect the resistance to challenge with atypical *A. salmonicida* (Sövényi & Ruttkay 1986, Pylkkö 1993).

Table 6. *Aeromonas salmonicida*. Virulence of atypical *A. salmonicida* strains isolated from various fish species. i.p.: intraperitoneal; i.m.: intramuscular; s.c.: subcutaneous; MLD: minimum lethal dose

Host fish species	Challenged fish species	Challenge mode	Injected amount of bacteria	Mortality or LD ₅₀ (no. of dead fish/no. of infected)	Source
Silver bream	Rainbow trout	i.p.	1.7 × 10 ⁹ (MLD)	Not reported	McCarthy (1975)
Minnow	Minnow	i.p.	6 × 10 ⁸	5/5	Håstein et al. (1978)
Turbot	Atlantic salmon	i.p.	1.3 × 10 ⁸	0/6	Pedersen et al. (1994)
Wrasse	Atlantic salmon	i.p.	1.4 × 10 ⁶	0/10	Frerichs et al. (1992)
		Bath	4.7 × 10 ⁶	0/10	Frerichs et al. (1992)
Sand eels	Rainbow trout	i.p.	8.0 × 10 ⁶	5/10	Dalsgaard & Paulsen (1986)
Flounder	Flounder	i.p.	3.7 × 10 ⁶ ^a	4/9	Wiklund (1995b)
Flounder	Rainbow trout	i.p.	3.7 × 10 ⁷ ^a	4/12 ^b	Wiklund (1995b)
Flounder	Roach	i.p.	0.8 × 10 ³ ^a	4/5 ^b	Wiklund (1995b)
Flounder	Bleak	i.p.	0.8 × 10 ³ ^a	3/4 ^b	Wiklund (1995b)
Cod	Atlantic salmon	i.m.	2.8 × 10 ⁶	4/4	Cornick et al. (1984)
		i.p.	2.0 × 10 ⁸	5/5	Cornick et al. (1984)
Goldfish	Goldfish	i.p., i.m., s.c.	1 × 10 ⁵	10/10	Elliot & Shotts (1980b)
Goldfish	Goldfish	i.m.	1.0 × 10 ⁴	8/8	Trust et al. (1980c)
Cyprinid	Goldfish	i.m.	10 ³	7/10	Austin (1993)
Cyprinid	Rainbow trout	i.m.	10 ³	6/10	Austin (1993)
Wrasse	Goldsinny wrasse	i.p.	5 × 10 ²	27 and 28/30	Gravningen et al. (1996)
Japanese eel	Japanese eel	i.m.	1.0 × 10 ² ^a	LD ₅₀ < 100 ^c	Ohtsuka et al. (1984)
Pacific herring	Pacific herring	i.p.	1.4 × 10 ⁹	LD ₅₀ = 14 (10/20)	Traxler & Bell (1988)
Goldfish	Atlantic salmon	i.p.	3.0 × 10 ⁹	LD ₅₀ = 3	Carson & Handlinger (1988)
Goldfish	Atlantic salmon	i.p.	<10	LD ₅₀ = 7.4 × 10 ⁻³ ^d	Whittington & Cullis (1988)
Goldfish	Brown trout	i.p.	<10	LD ₅₀ = 3.0 × 10 ⁻² [*]	Whittington & Cullis (1988)
Goldfish	Brook trout	i.p.	<10	LD ₅₀ = 3.7 × 10 ² ^d	Whittington & Cullis (1988)
Goldfish	Rainbow trout	i.p.	37	LD ₅₀ = 6.4 × 10 ³ ^d	Whittington & Cullis (1988)

^aLowest concentration of bacteria giving mortality; ^binjected bacteria not re-isolated; ^cper 100 g fish; ^dcalculated 10 d LD₅₀

In some studies the virulence of isolated strains was tested on several fish species (Bucke 1980, Michel 1981, Whittington & Cullis 1988, Austin 1993, Wiklund 1995b). Some of the examined strains were pathogenic only for the original host and not for other fish species challenged (Michel 1981, Wiklund 1995b), while in other studies the examined strain was pathogenic for several fish species (Bucke 1980, Whittington & Cullis 1988, Austin 1993). Michel (1981) found that an atypical strain isolated from perch was non-pathogenic to carp, possibly pathogenic to rainbow trout and pathogenic to perch when injected intramuscularly. Wiklund (1995b) reported an atypical *A. salmonicida* strain isolated from flounder to be weakly pathogenic for flounder and non-pathogenic for rainbow trout. Additionally, mortality among challenged roach and bleak *Alburnus alburnus* occurred, but the injected pathogen could not be re-isolated; instead other bacteria including *Aeromonas* sp., *Pseudomonas* sp. and Enterobacteria were isolated. It was suggested that the injected atypical *A. salmonicida* strain caused an immunosuppressive effect on the challenged fish, allowing opportunistic pathogens to invade the fish (Wiklund 1995b).

Evenberg et al. (1986) concluded that the pathogenesis of CE caused by atypical *Aeromonas salmonicida* is characterised by a state of immune suppression in the infected fish. This may explain why opportunistic pathogens such as *Aeromonas hydrophila*, *Pseudomonas* sp., *Vibrio* sp. and *Vibrio anguillarum* can be isolated from internal organs of moribund, seriously ulcerated fish or from fish injected with atypical strains (Csaba et al. 1980a, Cornick et al. 1984, Evenberg et al. 1986, Wiklund 1995b, Gravningen et al. 1996). The cause of a possible immune suppression in fish infected with atypical *A. salmonicida* could be strain specific, because in other experimental studies invasion of opportunistic pathogens has not been observed in injected fish (Dalsgaard & Paulsen 1986). In infection experiments on carp, opportunistic pathogens (previously isolated from ulcers) failed to produce ulcers in injected fish (Csaba et al. 1980a, Evenberg et al. 1986). In another study, *A. hydrophila*, isolated from ulcerated carp, produced clinical and pathological disease signs identical to CE, but the amount of bacteria injected or rubbed into scarified skin of carp was rather high (3.4 × 10⁹ bacteria) (Sioutas et al. 1991), making the experimental evidence questionable. Elliott &

Shotts (1980b) reported that an anaerogenic member of the *A. hydrophila* complex, isolated from ulcers of diseased goldfish, did not produce ulcerations or mortality in bath challenged goldfish at a concentration of 7.2×10^6 colony-forming units (CFU) ml⁻¹.

In infection experiments Bucke (1980) found that a weakly pigment-producing strain of *Aeromonas salmonicida* was more virulent for several fish species (brown trout, carp, goldfish, roach, rudd *Scardinius erythrophthalmus* and perch) than a non-pigmented strain of *A. salmonicida*. Unfortunately, the amount of bacteria injected was not reported. The different numbers of cells of the 2 strains injected into the fish could explain the difference in the virulence observed. Additionally, it was reported that a weakly pigmented variant of *A. salmonicida* could be experimentally transferred by co-habitation from infected brown trout to several fish species such as brown trout, carp, goldfish and roach (Bucke 1980).

Some strains of atypical *Aeromonas salmonicida* isolated from non-salmonids were reported as extremely virulent (Ohtsuka et al. 1984, Carson & Handlinger 1988, Traxler & Bell 1988, Whittington & Cullis 1988), with calculated 10 d LD₅₀ values as low as 7.4×10^{-3} CFU by intraperitoneal injection (i.p.) (Whittington & Cullis 1988). The LD₅₀ value in this study was estimated, by regression analysis, to be the dose for which the median lifetime of challenged fish was 10 d. As the atypical strain used in the experiments autoagglutinated in broth, 1 CFU consisted of a clump of bacteria, which accounts for the LD₅₀ values that were less than 1 unit (Whittington & Cullis 1988). Other examined strains seemed to be totally non-virulent as determined in injection experiments (Pedersen et al. 1994, McCarthy 1975) (Table 6).

In many studies the virulence of atypical strains isolated from non-salmonid fish was assessed on fish used for farming, mainly rainbow trout or Atlantic salmon, while the virulence for the host fish species was not determined (Table 6). The explanation for this could be the need to gain knowledge about the possible spread of the disease from wild fish to the more economically important farmed fish. Tested atypical *Aeromonas salmonicida* strains isolated from non-salmonids seem to be non-virulent or low-virulent for salmonids (Table 6), except for strains isolated from goldfish in Australia, which have proved to be extremely virulent by i.p. injection of different salmonids (Carson & Handlinger 1988, Whittington & Cullis 1988). However, in experiments performed simultaneously with i.p. injection, the atypical strain was not transmitted via water from the injected fish to Atlantic salmon and rainbow trout kept in the same tanks as the infected fish (within-tank control or co-habitation) but was transmitted to a low number of brown trout and brook trout. This indicates

that the invading capacity of the highly pathogenic (at i.p. injection) strain tested through the water, into salmonids, was rather limited, although Whittington & Cullis (1988) concluded that the degree of bacterial contamination of atypical *A. salmonicida* of the tank environment was probably low and that the low concentrations were infective. Unfortunately, no studies on the concentration of atypical *A. salmonicida* in the water were done, so the above conclusion is based more on assumption than on fact. Additionally, in bath experiments, injection (bacterial concentrations: 2.0×10^5 to 2.0×10^6 CFU ml⁻¹) with the same highly pathogenic atypical strain as used for i.p. resulted in low mortality with challenged Atlantic salmon and rainbow trout, but high mortality with brown trout and brook trout. The co-habitation and bath experiments probably give a better estimate of the virulence and invasive capacity of the tested pathogen than the injection experiments. In a similar experiment done with another atypical strain isolated from ulcerated goldfish high mortality was obtained with i.p. injection (LD₅₀ = 3 CFU) as well as bath challenge (8.0×10^5 CFU ml⁻¹) of Atlantic salmon (Carson & Handlinger 1988). Whittington & Cullis (1988) found that an atypical strain isolated from goldfish could establish a carrier state in i.p.-injected Atlantic salmon. These authors concluded that outbreaks of furunculosis in salmonid fish will occur if the tested strain of atypical *A. salmonicida* is transmitted from goldfish to salmonids.

Although atypical *Aeromonas salmonicida* has been isolated from several salmonid fish species (Table 3), there are surprisingly few reports on the virulence of the isolated strains. Olivier (1992) reported that strains (subsp. *nova*) isolated from salmonids in Newfoundland, Canada, were highly pathogenic (LD₅₀ < 100 bacteria) for juvenile Atlantic salmon and brook trout.

Unfortunately, several virulence and transmission studies of atypical *Aeromonas salmonicida* strains were done by injecting the pathogen into the fish (Table 6), which does not give a good picture of the infection capacities of the examined bacteria. The technique using application of bacteria into scarified skin has been applied mainly in the examination of CE (Bootsma et al. 1977, Csaba et al. 1980a, Gayer et al. 1980, Mirle et al. 1986). However, the amount of bacteria applied to the scarified skin is difficult to control using this method, and observed differences in virulence (40 to 100%) of examined strains (Bootsma et al. 1977) could be explained by the use of different concentrations of the organisms tested. In the analysis of GUD and UDF, abrasion of the skin and bathing in a bacterial suspension of *A. salmonicida* has been performed in different studies. The concentration of bacteria in the suspension has often been rather high (2×10^5 to 4.3×10^7 CFU ml⁻¹) (Elliott & Shotts 1980b,

Carson & Handlinger 1988, Whittington & Cullis 1988, Wiklund 1995b). A method using micro-injection, with Hamilton syringe, of a small amount of bacteria has been used as a compromise between scarification technique and immersion route (Evenberg et al. 1986, Evenberg et al. 1988). However, in order to obtain an appropriate description of the virulence of atypical strains, future investigations on this issue need to use other methods like co-habitation, which so far has been employed only in a few studies on the pathogenicity of atypical *A. salmonicida* (Bucke 1980, Whittington & Cullis 1988).

VIRULENCE FACTORS

Present knowledge about virulence factors in atypical strains is rather limited compared to knowledge about typical strains of *Aeromonas salmonicida*. The presence of extracellular products (ECPs) including proteases, A-layer of the cell wall, and iron-uptake mechanisms of atypical strains have been described in several studies. ECPs from atypical strains have been examined by Pol et al. (1980), Hastings & Ellis (1985), Evenberg et al. (1988), Gudmundsdottir et al. 1990, and Gudmundsdóttir (1996). Pol et al. (1980) reported that crude ECP of an atypical *A. salmonicida* strain was lethal for carp, but the nature of the toxin(s) was not determined. They concluded that ulcers occurring in connection with CE are partly or exclusively caused by a bacterial toxin produced by atypical *A. salmonicida*. Evenberg et al. (1988) found that the lethality of cell-free culture supernatants of atypical *A. salmonicida* for carp varied significantly depending on the composition of the growth medium and incubation time. The mortality of the challenged fish ranged from zero (over 10 d) to death within 48 h. Hastings & Ellis (1985) examined an atypical strain from Iceland which did not produce any detectable haemolysin or gelatinase and, in contrast to typical strains, had caseinase properties comparable to those of a metallo-protease. Subsequently, Gudmundsdottir et al. (1990) reported that a metallo-protease with a molecular weight of approximately 20 kDa was the major extracellular lethal toxin of atypical strains isolated from salmonids in Iceland. The 24 h LD₅₀ value of the protease was 0.03 µg protein g⁻¹ fish. This protease was different from the 2 extracellular proteases (P1 and P2) described from *A. salmonicida* subsp. *salmonicida*. In contrast, strains isolated from cyprinids (goldfish, carp, and rudd) did not produce this metallo-protease (Gudmundsdottir et al. 1990). In a recent study, 25 atypical *A. salmonicida* strains showed significant differences in the production of extracellular proteases (Gudmundsdóttir 1996). Five different protease groups among the examined

atypical strains were detected, while the examined typical strains formed a homogenous, single protease group. The results clearly indicate that with respect to extracellular proteases the atypical strains also form a very heterogeneous group. Gudmundsdóttir (1996) concluded that exoprotease production appeared to be strongly associated with geographical location of the host rather than the host fish species itself.

A relationship between the presence of an extracellular A-layer, external to the cell wall of atypical and typical *Aeromonas salmonicida*, and virulence has been suggested (Kay et al. 1981, Evenberg et al. 1988, Austin & Austin 1993). However, the close association between virulence and the presence of A-layer in typical strains is still being debated, and there are reports of non-virulent isolates possessing an A-layer and virulent isolates with no detectable A-layer (Ellis et al. 1988, Olivier 1990, Austin & Austin 1993). A-layer-possessing as well as A-layer-deficient atypical *A. salmonicida* strains have been isolated from different fish species (Trust et al. 1980a, Kay et al. 1981, Evenberg & Lugtenberg 1982, Evenberg et al. 1982, Chart et al. 1984, Kay et al. 1984, Pedersen et al. 1994, Wiklund et al. 1994). Detailed investigations on the virulence of A-layer-possessing and -deficient atypical strains have not been performed, but as previously indicated the virulence of different atypical strains can be host-specific and the virulence of a certain strain has to be assessed on the same fish species from which the examined strain was originally isolated (Table 6).

Bacteria have an absolute requirement for iron, and a bacterial pathogen must possess high-affinity iron-uptake mechanisms which can compete with the iron-binding proteins (such as transferrin) in the serum and extracellular fluids of the host. Such mechanisms include the production of siderophores and the induction of transferrin-binding proteins under conditions of iron restriction. Siderophore production has not been observed in atypical strains (Chart & Trust 1983, Hirst et al. 1991), and iron-uptake has been suggested to be performed by a probable proteolytic degradation of transferrin bound iron by an extracellular metallo-protease (Hirst & Ellis 1996). A probable correlation between established protease groups (Gudmundsdóttir 1996) and uptake of iron might explain the large variations in virulence observed in different atypical strains (Table 6). However, future research will hopefully resolve this issue. The utilisation of haem-bound sources of iron have been reported to be A-layer dependent both in atypical and typical *Aeromonas salmonicida* (Hirst et al. 1994). Also, the utilisation of haem-bound iron by atypical *A. salmonicida* indicated that the mechanism of haem utilisation was not siderophore-mediated, due to the lack of siderophores in atypical strains (Hirst et al. 1991).

ECOLOGY

Compared to typical *Aeromonas salmonicida* (Michel & Dubois-Darnaudpeys 1980, Allen-Austin et al. 1984, Rose et al. 1990, Morgan et al. 1991, Effendi & Austin 1994, Husevåg 1994, Ferguson et al. 1995), there is limited information available about the ecology, spread and survival of atypical strains in water (Evelyn 1971, Wiklund 1995a). Evelyn (1971) found that an atypical strain isolated from sablefish survived better in sea water than in fresh water (distilled or tap water). The addition of peptone to the sea water extended the survival time significantly. Wiklund (1995a) examined the survival of atypical *A. salmonicida* (isolated from ulcerated flounder from brackish water) under laboratory conditions in glass bottles (microcosms) containing sterilised water and sediment. In sterilised water the examined strains showed better survival at 4°C than at 15°C. In contrast, in water and sediment slightly increased survival was observed at 15°C compared to 4°C. The strains tested showed the highest survival in brackish water ($S = 6.4 \pm 0.5\%$) compared to fresh water or salt water ($S = 30\%$). Wiklund (1995a) concluded that bacteria shed from ulcers of flounder may survive in the bottom sediment of brackish water environments in excess of 60 d. The sediment can thus act as a reservoir for the pathogen, facilitating the spread of the infection, although the question of whether or not the virulence of the pathogen was preserved was not examined (Wiklund 1995a). Although the atypical strains isolated from ulcerated flounder have been reported to be fastidious, requiring serum or blood when cultivated on artificial agar plates (Wiklund & Dalsgaard 1995), their survival in nutrient-limited conditions (sediment and brackish water) was clearly extended. However, these survival studies were done in sterilised water in microcosms, without any exchange of nutrients and waste products or interaction between the bacteria tested and other bacteria or protozoans. Therefore, caution must be used in the interpretation of the results, and the information obtained does not necessarily reflect the behaviour of the pathogen in the natural aquatic environment (Austin & Austin 1993).

The few data presently available about the survival of atypical strains are based only on laboratory results. For a proper evaluation of the possible spread of atypical strains from infected populations to uninfected ones more information is needed, especially on survival of different atypical strains in natural habitats. Additionally, more knowledge is needed on the effect of prolonged survival and starvation in water habitats on the maintenance of the virulence of the surviving cells.

CONTROL

The prophylactic and therapeutic methods available for the control of diseases caused by typical *Aeromonas salmonicida* have been reviewed by Austin & Austin (1993). For diseases caused by atypical *A. salmonicida* less information about control is available. It appears that potentiated sulphonamide, chloramphenicol, neomycin, nitrofurantoin and oxytetracycline are generally effective against these pathogens (Bootsma et al. 1977, Csaba et al. 1980a, Gayer et al. 1980, Böhm et al. 1986, Groman et al. 1992, Pedersen et al. 1994, Jeney & Jeney 1995).

The development of resistance to antibiotics among atypical strains has been observed by Hirvelä-Koski et al. (1994), who found resistance to sulfonamides. Strains isolated from turbot (Pedersen et al. 1994) were resistant to trimethoprim but sensitive to potentiated sulphonamide. Transferable drug resistance has been known to occur in typical *Aeromonas salmonicida* since 1959, when Aoki et al. (1971) demonstrated the presence of R-factors. Sandaa & Enger (1996) demonstrated transfer of a naturally occurring plasmid (pRAS1), encoding multiple drug resistance, from atypical *A. salmonicida* to a variety of marine bacteria.

Little information is available about immunological protection of fish against infection with atypical *Aeromonas salmonicida*. Evenberg et al. (1986) and Pourreau et al. (1986) showed that carp infected with atypical *A. salmonicida* had a suppressed humoral immune response, while an experimental vaccine consisting of the same strain, demonstrated that ECP is important for protection (Evenberg et al. 1988). Daly et al. (1994) found that carp have memory formation in response to previous infection with atypical *A. salmonicida* and that the protection lasted for at least 5 mo. Vaccination against atypical 'furunculosis' (caused by atypical *A. salmonicida*) in reared Atlantic salmon in Newfoundland has not successfully eliminated carriers or alleviated disease in fresh or sea water (Groman et al. 1992). Hirst & Ellis (1994) found that iron-regulated outer membrane proteins (IROMPs) of *A. salmonicida* represent protective antigens, which have an important role in the formulation of a successful vaccine against both typical and atypical strains of *A. salmonicida*.

Farmed salmonids have been vaccinated with good results against atypical 'furunculosis' in Iceland with an autogenous bacterin since 1992. Field studies indicated some cross-protection of the autogenous bacterin of *Aeromonas salmonicida* subsp. *achromogenes* against classical furunculosis (Guðmundsdóttir et al. 1996). However, in a recent study protection against classical furunculosis was not observed in fish vaccinated with an *A. salmonicida* subsp. *achromogenes*

bacterin. The fish were kept in tanks under laboratory conditions. The authors suggested that a constant exposure to the bacterium under field conditions boosts and improves the immune response (Gudmundsdóttir & Gudmundsdóttir 1997). Gudmundsdóttir & Magnadóttir (1997) have recently assessed the protective role of cell-associated and extracellular antigens of *A. salmonicida* subsp. *achromogenes*. The results indicate that a specific humoral immune response evoked in Atlantic salmon by a 20 kDa extracellular metallo-caseinase, AsaP1, is important in protection against experimental infection of *A. salmonicida* subsp. *achromogenes*. Jones et al. (1996) observed that vaccination against typical furunculosis protects against atypical *A. salmonicida* in salmonids and cyprinid fish. These authors suggested that antigens capable of eliciting protective immunity are conserved among biochemically diverse strains of *A. salmonicida*.

CONCLUSIONS

This review on atypical *Aeromonas salmonicida* demonstrates that, despite the large amount of information available about this important fish pathogen, substantial gaps remain in our knowledge not only about the non-specific factors influencing host specificity, virulence determinants, and survival of the pathogen in the environment but also about specific factors related to the atypical strains, including the relevance of these strains to farmed fish, their taxonomy, and the question of whether individual strains spread and, if so, how far.

Atypical *Aeromonas salmonicida* seems to infect a large number of fish species worldwide. The disease problems have shown themselves to be more conspicuous in farmed fish than in wild fish, where generally only small numbers of fish are diagnosed with ulcerations associated with atypical *A. salmonicida*. The infection studies carried out so far show that the virulence of atypical *A. salmonicida* varies according to the host species. These observations indicate, therefore, that there might be host species variation in susceptibility of different strains of atypical *A. salmonicida*. Some of the atypical strains isolated from wild fish adversely affect salmonids and therefore may represent a significant risk to farmed fish, both salmonids and non-salmonids. At present, there is a trend on introducing new non-salmonid fish species for farming purpose and the susceptibility of these fish species to atypical *A. salmonicida* has to be examined. Additionally, we need more information about the possible change of host specificity of atypical strains.

Studies on the role of the environment as a reservoir of atypical *Aeromonas salmonicida* would seem to be of particular importance. More work on the survival of

the pathogen in water and sediment is needed, and it will be necessary to assess the capacity of the bacteria to survive in a non-culturable state and, in particular, to determine whether the virulence capability of the starving cells changes in aquatic environments. The bacteria have been isolated from clinically healthy fish, and it seems most likely that the fish have to be subjected to stressful conditions before the disease breaks out. At present, it is unknown whether the bacteria constitute a part of the normal flora of the fish, whether the pathogen is transmitted for long distances through the water, or whether transfer occurs from fish to fish, during spawning for example.

The biochemical identification of *Aeromonas salmonicida* to species level can be done, remembering that several of the atypical strains are fastidious and they need special growth media. In addition, strains of *A. salmonicida* with divergent characters are isolated both within the typical and atypical groups. Until the last decade the subspecies proposed by Schubert (1967) were easily distinguished by a limited number of biochemical characters. This classification was accepted by Bergey's Manual of Systematic Bacteriology (Popoff 1984). The proposal of McCarthy & Roberts (1980) that *A. salmonicida* should be divided into different subspecies based on epizootiological criteria, with most of the atypical strains included into the subspecies *nova*, in hindsight seems to be too simple a solution. However, the present situation with 2 completely different systematic groups of subspecies is impractical and taxonomically inappropriate. There is an urgent need to clarify the taxonomic position of atypical strains, and identification of the different subspecies or groups of atypical *A. salmonicida* using molecular techniques will be a priority for future work.

With respect to fish health, the atypical strains are becoming more and more important, resulting in an increase in the demand for a valid definition of these pathogens and also for improved prevention and control procedures. Fish populations infected with atypical *Aeromonas salmonicida* have been transferred to geographical areas (countries) that are considered free of (classical) furunculosis (Whittington & Cullis 1988), where spread of infection occurred because of unsuccessful attempts at treating the disease and absence of legislation. Should we accept the trading of fish infected with certain biotypes of atypical *A. salmonicida*, while infection with others is prohibited? Additionally, can a geographical area or country be considered free of *A. salmonicida* if atypical strains have been isolated from fish in that area. Certainly, more information, research and discussion are needed on these issues!

This review will hopefully provide a basis of information that can be useful in future studies on the species *Aeromonas salmonicida*.

Acknowledgements. The authors thank their colleagues for the information and unpublished results reported in this paper.

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Editorial responsibility: David Bruno,
Aberdeen, Scotland, UK

Submitted: April 4, 1997; Accepted: November 14, 1997
Proofs received from author(s): February 10, 1998