Furunculosis And Other Diseases Caused By *Aeromonas salmonicida*

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HOST AND GEOGRAPHIC RANGE

After Emmerich and Weibel (1890) first described *Aeromonas salmonicida* (syn. *Bacillus salmonicida, Bacterium salmonicida, Bacterium trutta*) among trout in German hatcheries, it was initially believed that *A. salmonicida* was a pathogen exclusive for instensively cultured salmonid fishes. Current knowledge, however, indicates that relatively few cultured or feral fish in freshwater, brackish or even marine environments are immune to the deleterious effects of this pathogen. Let it suffice to say that all species of salmon, trout, charr and grayling are susceptible to infection. Furthermore, the number of non-salmonid hosts that have become infected with this bacterium has steadily multipled commensurate with the increased conduct of epidemiological investigations. Present indications suggest that there are at least four subspecies of *A. salmonicida*; - *salmonicida, achromogenes, masoucida* and *smithia* (Holt et al. 1994). Strains of *A. salmonicida* subsp. *salmonicida* induce typical furunculosis and cause severe septicemia with resultant mortality especially within coldwater fishes. The other subspecies produce atypical forms of disease that are often characterized by dermal ulcerations and external pathology with or without subsequent septicemia. The number, diversity and distribution of fish species that are susceptible to *A. salmonicida* enhances this bacterium's distribution worldwide. However, both typical and atypical subspecies may not be common to all geographic areas. For example, *A. salmonicda* was not reported within New Zealand or Australia until 1974, when atypical variants of *A. salmonicida* were introduced into Australia by an importation of diseased ornamental goldfish (Trust et al. 1980; Humphrey and Ashburner 1993). Since that introduction, atypical isolates of *A. salmoncida* have become endemic even among feral populations of goldfish (Whittington et al. 1987), but A. *salmonicida* subsp. *salmonicida* has not yet been reported within Australian fishes. Similarly, only *A. salmonicida* subsp. *salmonicida* has reported from salmonids in Nova Scotia, Canada (Hammel 1995). It may be argued, however, that such distinctions are somewhat controversial because atypical isolates of *A. salmonicida* obtained from goldfish can be virulent in Atlantic salmon (*Salmo salar*) and induce pathology indicative of classical furunculosis (Carson et al. 1988).

PATHOLOGICAL CONDITIONS

Salmonid Furunculosis

Aeromonas salmonicida subsp. *salmonicida* causes severe septicemia and acute mortality in susceptible salmonid hosts. The mode of infection, nature of pathology, and the degree of mortality, however, is interrelated with the quality of environmental parameters and furthermore affected by the age and innate resistance of the host. Peracute infections most often occur in fingerling fish, which may darken in color and die without showing marked clinical indications of disease. Only a slight exophthalmia may be evident. Acute infections often occur in juvenile and adult fish that darken in color and hemorrhage at the base of fins and oral cavity. Internal hemorrhages may be evident in the abdominal walls, viscera, and heart of affected fish. The spleen is enlarged, and the liver can have subcapsular hemorrhages, or focal necrosis of parenchymatous tissue. Affected fish may display erratic swimming behavior, become sluggish, and stop feeding. Consequently, the stomach and intestine are usually devoid of food, and the lumen may contain sloughed epithelial cells, mucus, and blood. The reproductive organs are commonly hemorrhaged and the intestine is often severely congested (Scott 1968). The chronic form of furunculosis usually occurs in older fish that have become more refractive to the disease or among species that have greater innate resistance to infection by *A. salmonicida*. One or more furuncle-like lesions may be present on the dermis and ulcers may extend deep into the musculature. Internally, chronically infected salmonids show a general visceral congestion and peritonitis. Hemorrhages may occur over the pyloric area and liver, and kidneys are soft or friable (McCarthy and Roberts 1980).

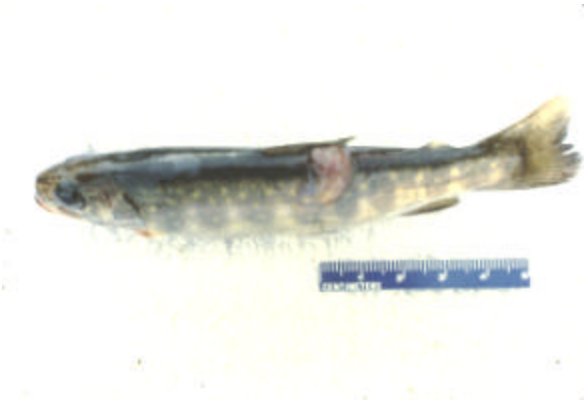

Figure 1: Brook trout (*Salvelinus fontinalis*) showing a furuncle like lesion near its dorsal fin caused by infection with *Aeromonas salmonicida* subsp. *salmonicida*. Photograph by R. Cipriano.

The development of the characteristic "furuncle-like" lesion is not a consistent finding, but is most often associated with chronic infections. When these lesions are present, they consist of tissue fluid exudate, necrotic tissue, and some macrophages. Thus, the furunculosis lesion differs from the true furuncle associated with homeothermic vertebrates, which is characterized by a necrotic mass of polymorphonuclear leukocytes. Degeneration of myofibrils, fragmentation of muscle fibers, and hemorrhage of the entire muscular tissue is evident within the swelling lesion and leads to a colliquative necrosis of the musculature in the most serious lesions (Sakai 1978). Bacteria may also colonize the gill epithelium on or between the secondary lamellae (Bruno 1986) where they may be enclosed within a membrane that is continuous with the basement membrane of the lamellar epithelium (McArdle et al. 1986). Bacterial embolisms may develop in gill lamellae causing a further proliferation of branchial epithelial cells and a subsequent fusion of gill lamellae that impairs circulation (Miyazaki and Kubota 1975a).

Figure 2: Ulceration deep into the musculature of a brook trout (*Salvelinus fontinalis*) caused by *Aeromonas salmonicida* subsp. *salmonicida*. Photograph by R. Cipriano.

Trout Ulcer Disease

Fish (1934) described an Ulcer Disease in Trout among salmonids from a hatchery in Cortland (New York), which he believed was similar to but distinct from the clinical picture observed in typical salmonid furunculosis. He also noted that similar pathology was previously reported among trout at two other New York hatcheries on Long Island (Calkins 1889) and Cold Spring Harbor (Marsh 1905). In each of these cases, disease manifested itself as an external bacterial infection without involvement of the blood, liver, spleen, and kidneys, but the posterior intestine was often congested. Lesions were infrequently observed in the kidneys or livers of affected fish and, if present, they were consistently sterile by culture on nutrient agar. Snieszko (1952) did imply that an internal pathology similar to that described for typical salmonid furunculosis might develop in the most acute cases. Because specific pathogen free fish were not readily available for his experimental purposes, it is difficult to determine if the internal pathology alluded to by Snieszko was actually a clinical indication of ulcer disease or the expression of an underlying *A. salmonicida* subsp. *salmonicida* infection.

The external clinical pathology that was observed in cases of Ulcer Disease, however, was quite dissimilar to typical salmonid furunculosis. Patches of the epidermis initially thickened and then enlarged to form white epidermal tufts from 0.5 to 1.0 mm in diameter. The development of these tufts progressed until the underlying dermis was eroded and a finely delineated dark red or gray ulcer became apparent. Bacteria multiplied within the necrotic debris of the exposed subepithelial connective tissue but did not necrotize or penetrate deeply into the musculature. The lesions, therefore, remained shallow and developed from the outside in. By contrast, the furuncles of typical salmonid furunculosis developed from the inside out and were evident deep into the musculature. Other ulcers were also present on the fins, jaw, or in the oral cavity where the soft tissue covering the roof of the mouth was eroded (Wolf 1938).

Figure 3: Shallow ulceration on the dermis of a brook trout (Salvelinus fontinalis) that is characteristic of the lesions observed in Trout Ulcer Disease caused by *Aeromonas salmonicida* subps. *achromogenes*. The etiology of this condition was formerly ascribed to *Hemophilus piscium*. Photograph courtesy of S. F. Snieszko personal collection.

Snieszko and Friddle (1948) originally isolated and Snieszko et al. (1950) then described *Hemophilus piscium* as the etiologic agent of Ulcer Disease in Trout. Paterson et al. *(1980a)* later suggested that this bacterium was actually an atypical, achromogenic strain of *A. salmonicida* by showing that DNA:DNA hybridization results for *H. piscium* were similar to those of *A. salmonicida*, but beyond acceptable limits for the genus *Hemophilus*. These workers further

showed that strains of *H. piscium were* (1) sensitive to *A. slmonicida* bacteriophage; (2) serologically identical to *A. salmonicida;* and (3) phenotypically analogous to achromogenic strains of *A. salmonicida.* Unfotunately, the original isolates described by Snieszko and Friddle (1948) and Snieszko et al. (1950) were not available for comparison. Thornton et al. *(1999)* also showed the SSU rRNA gene sequence identity between *H. piscium* and *A. salmonicida* subp. *salmonicida* is 99.6%. This information strongly argues that the pathology observed in Trout Ulcer Disease is caused by atypical, achromogenic strain of *A. salmonicida*.

Goldfish Ulcer Disease

Goldfish ulcer disease was first described by Mawdesley-Thomas (1969), who termed the condition furunculosis of goldfish because *A. salmonicida* was isolated from skin lesions. However, the organism in those studies was actually a typical biotype of the bacterium, *A. salmonicida* subsp. *salmonicida* and, therefore, the clinical pathology described by Mawdsley-Thomas is quite different than that which is observed in the majority of reports concerning Goldfish Ulcer Disease. True Goldfish Ulcer Disease, however, was actually introduced into goldfish farms in the United States during the 1970's. Although other investigators attributed the etiology of this disease to a fungus (oomycete), a yellow pibmented bacterium *(Flavobacterium columnarae),* or a protozoan *(Epistylis longicorpia),* Elliott and Shotts (1980a, b) established the causal agent to be another atypical, achromogenic variant of *A. salmonicida.*

Figure 4: A dermal ulceration on a goldfish (*Carrasius auratus*) indicative of Goldfish Ulcer Disease caused by an atypical variant of *Aeromonas. salmonicida*. Photograph by R. Cipriano.

The role of ectoparasites in creating a portal of entry for *A. salmonicida* remains unclear, but *A. salmoncida* preferentially adhered to mucus and dead or damaged cells in the dermis. Early infections appeared as white proliferations on the epithelium of fishes. These areas developed peripheral hemorrhages beneath the scales and. as the lesion develops, scales in the affected area were sloughed, the dermis became necrotic, and there was significant degeneration of the musculature. There was also a marked leukocyte infiltration at the site of trauma within 24 – 48 h. Unlike typical salmonid furunculosis, the infiltration persisted throughout a 21-day observation period and consisted of acidophilic, heterophylic, and basophilic granulocytes, lymphocytes, and macrophages. There was no evidence, however, that *A. salmonicida* was phagocytized by the leukocytes. Septecemia caused by *A. salmonicida* was most likely to be found if fish had well-developed dermal lesions. The causative bacterium was most readily isolated just below the dermis at the peripheral margin of the lesion.

Carp Erythrodermatitis

Carp erythrodermatitis is a subacute to chronic skin disease that occurs at temperatures from 4 to 30°C (Pol et al. 1980). This disease was historically associated with Carp Dropsy Syndrome until Fijan (1972) showed that Carp Dropsy actually involved two infections: Spring Viremia of Carp caused by *Rhabdovirus carpio* and Carp Erythrodermatitis. Consequently, the etiologic agent of Carp Erythrodermatitis was isolated and described as an atypical, achromogenic variant of *A. salmonicida* (Bootsma et al. 1977; Bootsma and Blommaert 1978). Although *A. salmonicida* is now recognized to cause Carp Erythrodermatitis, it is important to note that other species of bacteria may also induce external lesions in carp that are macroscopically similar to those that are caused by *A. salminicida* (Schultz 1980).

Figure 5: A dermal ulceration in the mirror carp (*Cyprinus carpio*) indicative of the pathology observed in Carp Erythrodermatitis, as caused by an atypical *Aeromonas salmonicida*. Photograph from NFHRL collection courtesy of R. Bootsma.

Clinical indicatications of Carp Ertythrodermatitis become evident as small inflamed hemorrhagic areas on the skin that develop into white erosions surrounded by a narrow red zone and darkened pigmentation. Extensive hemorrhage in the dermis is followed by a necrosis of the epidermis with strong infiltration by polymorphonuclear leukocytes. Edema in the dermis displaces "knots" in the epidermis while hemorrhage occurs around the veins inducing a persistent inflammatory infiltration that extends into the stratum compactum, subdermis and underlying musculature. Irregular, hemorrhagic, ulcers proliferate in the central necrotic areas (Gayer et al. 1980).

Common carp with extensive lesions may also exhibit exophthalmia, hemorrhage in the gills, abdominal distention and anemia. In more advanced cases, a transudate may be present within the abdominal cavity, and the internal organs may become edematous (Fijan 1972). The causative bacterium is present exclusively in lesions between the dermis and epidermis (Bootsma et al. 1977), but is not cultured from the kidneys of infected carp.

TAXONOMY AND CLASSIFICATION

In the ninth edition of "Bergey's Manual of Determinative Bacteriology," Holt et al. (1994) recognize four subspecies of *A. salmonicida*; - namely, *salmonicida, masoucida, achromogenes* and *smithia* (Table 1). Although phenotypic (Griffin 1954; Popoff 1969; Dalsgaard et al. 1994;

Hanninen et al. 1995) and genotypic (Belland and Trust 1988; Umelo and Trust 1998; Boyd et al. 1994; Nielsen et al. 1994; Garcia et al. 2000; O'Hici et al. 2000) characterizations have consistently indicated that strains of *A. salmonicida* subsp. *salmoncida* form a homogeneous clonal group, more variation exists among "atypical" isolates (Powell et al. 1998; Hoie et al. 1999; Yamada et al. 2000). In fact, molecular and genotypic analyses have not necessarily correlated with phenotypic subspeciation (Austin et al. 1998) and the degree of heterogeneity is sufficiently greater among atypical isolates than that which is apparent within the three subspecies *masoucida, achromogenes* and *smithia* (O'Hici et al. 2000). In fact, Austin et al. (1989) have shown that numerical taxonomy and DNA: DNA hybridization techniques divided *A. salmonicida* into five phena. In their studies, subspecies masoucida, *salmonicida, achromogenes,* and *smithia* grouped within phena 10, 11, 12, and 14, respectively. Phenon 13 contained an additional group of atypical isolates that did not group within any of the recognized subspecies. Isolates from this phenon have been obtained from both salmonid and non-salmonid sources, can induce ulcers and septicemia in rainbow trout and goldfish, and produce brown pigment. Unlike all previously reported isolates of *A. salmonicida*, most (87%) of the isolates in phenon 13 grow at 37°C (Austin 1993).

Pavan et al. (2000) have also proposed a new subspecies, *"pectinolytica,"* based upon phenotypic and DNA: DNA hybridization studies of isolates obtained from polluted waters of the Mantanza River, near Buenos Aires, Brazil. Their isolates were unusually dissimilar to hitherto reported isolates of *A. salmonicida* in their ability to ferment sorbitol and degrade polypectate. Analysis of 16S rDNA sequences indicated that these isolates constituted a tightly knit genomic group that was most closely related to *A. salmonicida* subspp. *salmonicida, masoucida* and *achromogenes*.

Table 1: Phenotypic Differentiation Of Different Subspecies Of *Aeromonas salmonicida* described in "Bergey's Manual od Determinative Bacteriology", 9th ed. (Holt et al 1994)

Characteristic	*Aeromonas salmonicida* subp.			
	salmonicida	***achromogenes***	***masoucida***	***smithia***
Indole production	-	+	+	-
Methyl Red	+	+	+	-
Vogues Prokauer	-	-	+	-
H_2S production	-	-	+	+
Lysine decarboxylase	d	d	d	-
Arginine dihydrolase	+	+	+	[-]
D-Glucose acid	+	+	+	[+]
D-Glucose gas	+	-	+	[+]
L-Arabinose acid	+	-	+	
D-Galactose acid	+	+	+	-
Glycerol acid	d	d	d	[-]
Maltose acid	+	+	+	-
D-Mannitol acid	+	-	+	-
Sucrose acid	-	+	+	d
Trehalose acid	+	+	+	-
Esculine hydrolysis	+	-	+	-
Lipase (corn oil)	+	+	+	-
ONPG	d	d	d	+
Brown pigment	+	-	-	-

+ = 90% or more strains are positive; - = 90% or more strains are negative; [-] = 0-10% are positive; d = 11-75% of strains are positive; [+] = 76-89% of strains are positive.

DETECTION AND DIAGNOSIS

Presumptive diagnoses of typical and atypical variants of *A. salmonicida* from clinically diseased or moribund fish are based on clinical indications of disease, the type and number of species infected, and the clinical history of disease within the affected population or facility. Presumptive diagnosis of atypical *A. salmonicida* infections in salmonids or nonsalmonids is more difficult than the diagnosis of typical furunculosis because clinical signs vary and dermal lesions are often contaminated with opportunistic fungi and bacteria. Definitive diagnosis of all *A. salmonicida* infections requires isolation and identification of the causative bacterium. Pigmented typical *A. salmonicida* strains can be readily isolated from kidney tissues of dead or moribund fishes using commercial media such as trypticase soya agar or brain-heart infusion agar. Differentiation of colonial types that grow upon primary isolation can be facilitated by the simple addition of 0.1% (weight: volume) Coomassie Brilliant Blue (CBB) R-250 into either of the aforementioned media. When cultured on CBB agar, the A-layer protein that is present in virulent strains of *A. salmonicida* will absorb the Coomassie Brilliant Blue protein-specific dye. Consequently, virulent *A. salmonicida* develop dark blue to deep violet, friable colonies on CBB agar (Cipriano and Bertolini 1988; Cipriano and Blanch 1989). Although CBB agar facilitates presumptive identification, it is not a selective medium and a definitive diagnosis should be confirmed via additional biochemical tests (Teska and Cipriano 1993).

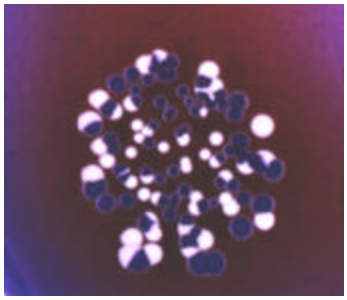

Figure 6: Growth on Coomassie Brilliant Blue (CBB) agar differentiates between A-layer positive colonies (blue) and A-layer negative colonies (white) of *Aeromonas salmonicida*. Most isolates from fish contain the A-layer and the characteristic blue growth of such colonies on CBB agar also facilitates presumptive diagnosis. Photograph by R. Cipriano.

Atypical pigmented or nonpigmented strains of *A. salmonicida* are fastidious on initial isolation from lesion material and the presence of biotic contaminants may interfere with an accurate diagnosis. McCarthy *(1977a)* suggested, therefore, that isolation should be attempted from early and advanced lesions using a minimum of six fish from each epizootic. Elliott and Shotts *(1980a)* found that primary isolation of atypical isolates causing Goldfish Ulcer Disease was facilitated on blood agar. Additionally, Bootsma et al. (1977) suggested that diagnosis of Carp Erythrodermatitis should be enacted first by experimentally infecting healthy common carp by contact with diseased carp; and second, by inoculation of lesion material from experimentally infected carp onto a medium consisting of tryptose blood agar base (DIFCO), 10% blood serum, and filter decontaminated ampicillin and polymyxin B sulfate to final concentrations of 50 µg/mL and 50 IU/mL, respectively. These fastidious atypical isolates have a specific requirement for haem that is essential for the initiation of growth, especially when the bacteria are in low concentrations within the initial inoculum. Incorporation of haematoporphyrin and haemoglobin into the medium may, therefore, provide a suitable for of haem and increase plating efficieny (Ishiguro et al. 1986).

Following primary isolation, purified clones of typical and atypical strains of *A. salmonicida* are readily confirmed if resultant bacteria are gram-negative nonmotile rods that are cytochrome oxidase positive, ferment glucose, and differentiate according to the biochemical criteria listed in Table 1. Dalsgaard et al. (1998) noted that the identification of atypical isolates is particulary troublesome unless biochemical tests are interpreted within a standardized time frame. It is also important to note, that some strains of *A. salmonicida* may be cytochrome oxidase negative; - a result that is inconsistent for this species (Chapman et al. 1991; Pedersen et al. 1994; Wiklund et al. 1994). A number of polymerase chain reaction (PCR) assays have also been developed that can be used either to achieve an initial diagnosis of furunculosis in clinically infected samples (Gustafson et al. 1992; Calabrez et al 1993; Mooney et al. 1995; Høie et al. 1997) or to confirm the identity of isolates that have been cultured subsequent to primary isolation (Hiney et al. 1992; Miyata 1996; Oakey et al. 1998). Because PCR-assays may often detect concentrations of bacteria below those obtained by culture, predictive validation and interpretation of results in the absence of definitive culture-positive results is often problematic (Hiney and Smith 1998).

The production of a brown water soluble pigment remains a principal phenotypic characteristic in the presumptive identification of most *A. salmonicida* subsp. *salmonicida*. Griffin et al. (1953) noted that the incorporation of L-phenylalanine and L-tyrosine in media (pH = 6.5 to6.8) enhanced pigmentation. However, the presence of fermentable sugars (e.g. – maltose), 10% carbon dioxide, simply tightening the caps of test tubes, or incubation of cultures above 22^{o}C delayed pigmentation (Griffin 1953). The incorporation of 0.1% glucose in bacteriological media may not only inhibit pigmentation but also cause an overall reduction in the size of the bacterial colonies (Altman et al. 1992). Although the presence of brown pigment may facilitate diagnosis, similar pigments are also produced by other aquatic aeromonads (Ross 1962) and pseudomonads (Hamilton-Miller 1975; Altman et al. 1992).

A number of different serological techniques may be used for identification (Rabb et al. 1964; McCarthy and Rawle 1975; McCarthy 1975b; McCarthy and Whitehead 1977; Eurell et al. 1979). Sakai et al. (1986) noted that enzyme immunoassays (ELISA) amplified with secondary and tertiary antibodies may detect as little as 10^0 colony forming units (cfu) of *A. salmonicida* per mL. They also found that normal ELISA and immunofluorescence assays detected a minimum of 10^2 and 10^3 cfu of the pathogen, respectively; but it required as many as 10^7 cfu for detection using either the latex agglutination or coagglutination procedures. Adams and Thompson (1990) also demonstrated that the threshold limit for detection of *A. salmonicida* by a normal ELISA test was approximately 10^3 cfu/mL.

Fish may be covert carriers of *A. salmonicida* even when culture or other methods of diagnosis are negative (Hiney et al. 1997). In such cases, the stress induced furunculosis test as described by Bullock and Stuckey (1975) and modified by McCarthy (1977b) has proven to be the most reliable procedure for detection of *A. salmonicida* among carrier fish (Hiney et al. 1994; Cipriano et al. 1997). In this procedure, fish are injected with 20 mg of prednisolone acetate per kilogram of fish and maintained for 14 d in water at 18^{o}C. All mortality is cultured to confirm the presence or absence of *A. salmonicida*. If no fish die, the observation period may be extended or all fish may be sacrificed and cultures are then prepared from their kidneys. Stress inducible furunculosis may be seasonal or transient in nature and can be enhanced by certain physiological (Scallan and Smith 1993) and environmental stressors (Scallan et al. 1994). Filtration (Maheshkumar et al. 1990; Ford 1994) or PCR (O'Brien et al. 1994) analyses may also be adapted to detect and monitor concentrations of *A. salmonicida* in culture water or facility effluents.

BACTERIAL ECOLOGY

Rose et al. (1990a) have indicated that within most microcosms, viability of *A. salmonicida* (as measured by cultivation on bacteriological media) was generally less than 10 days, which is consistent with the obligate nature of this pathogen. Using a streptomycin-resistant mutant of *A. salmonicida*, McCarthy (1977b) determined that the bacterium survived 24 days in natural brackish water, 17 days in nonsterile freshwater, and 8 days in seawater. He also found that *A. salmonicida* survived on wet or dry nets for 6 days and in tissues or carcasses of fish that died from furunculosis for 32 days. On fish husbandry equipment, the bacterium may adhere more preferentially to plastics than it does to stainless steel (Carballo and Seoane 2000). Sakai (1986) has shown that virulent strains may survive for more than 15 weeks in the presence of humic acid, tryptone, and cleaned river sand but cells were not viable within five weeks in the absence of sand.

Although viable counts of *A. salmonicida* could not be detected after four weeks in loamy sediment and after seven weeks in sandy sediment, naked DNA and DNA from released cells were still present after 13 weeks (Deere et al. 1996). Viable cells of *A. salmonicida* were observed microscopically even when the bacterium could not be cultured on agar plates (Morgan et al. 1993; Effendi and Austin 1994). These viable but nonculturable cells underwent major morphological changes and lost pathogenicity for Atlantic salmon (Effendi and Austin 1995a) and were considered to be dormant (Ferguson et al. 1995). Rose et al. (1990b) have argued against dormancy and suggested that a small number of cells, fewer than the sensiticvity of detection by culture, remain viable and can be resuscitated by the addition of nutrient broth. Perez et al. (1995) have also supported the argument against dormancy indicating that *A. salmonicida* remained viable in filtered sea water for 23 to 90 days at 10°C and for 12 to 69 days at 20°C. After the addition of nutrients, Husevåg (1995) showed that a small percentage of *A. salmonicida* cells, previously starved for six months in a nutrient-free medium at 15°C, could be revived in liquid but not on agar media. Whether or not *A. salmonicida* enters a true dormancy, it is evident that nutrient flow within the environment could rejuvenate the growth and replication of this bacterium so that, even a small number of non-culturable cells might become a potential reservoir of infection for naïve fish.

TRANSMISSION

Fish that harbor latent infections of *A. salmonicida* and can transmit the pathogen horizontally by either physical contact or shedding the bacterium into the water column *represent* a significant threat for horizontal transfer of the pathogen to naïve fish within freshwater (Horne 1928) and marine environments (Scott 1968; Nomura 1993). Epizootics are particularly troublesome at the beginning of marine culture if undetected *A. salmonicida* carrier smolts are transported into pens (Hammell 1995). Under such conditions, transport often facilitates contagion and the physiological stress encountered with osmoregulation may enhance disease (Ezura et al. 1984). Contagion within natural waterways may be further enhanced by the escape of infected individuals from culture facilities or sea farms and the subsequent movements of these infected fish within the feral environment (Johnsen and Jenson 1994). Large aggregations of fish in deep pools or beneath waterfalls in response to favorable water temperatures may facilitate contagion (Johnsen and Jensen 1994).

There is probably no one way in which *A. salmonicida* enters a susceptible host, but the bacterium is readily transferred by the horizontal contact among infected fish that are cohabited with naïve individuals (Nordmo et al. 1998). Although some workers have failed to transmit the disease via a gastrointestinal route (Krantz et al. 1964; McCarthy 1977b), Klontz and Wood (1973) observed clinical furunculosis in sablefish that seemingly resulted from the ingestion of

infected coho salmon *(Oncorhynchus kisutch)*. Miyazaki and Kubota (1975a,b) have provided histopathological evidence for both perbranchial and percutaneous routes of infection.

Present information suggests that the source and reservoir of infection and transmission of atypical *A. salmonicida* is probably similar to that which has been described for typical strains (Wiklund and Dalsgaard 1998). Tuffery and Dehand (1979) were not able to isolate bacteria from tissues of live or dead carp that had been injected with a Carp Erythrodermatitis variant of *A. salmonicida*. Elliott and Shotts (1980b) also found that goldfish injected with *A. salmonicida* isolated from clinical cases of Goldfish Ulcer Disease died without developing ulcers. However, ulcers were produced if the goldfish were either exposed to the pathogen in the water or after scarification in which a sample of scales was removed and the exposed dermis was swab inoculated with bacteria. In studies conducted at the National Fish Health Research Laboratory, we also found that rainbow trout *(Salmo gairdneri)*, brook trout (*Salvelinus fontinalis*), and Atlantic salmon died after injection with atypical variants of *A. salmonicida* isolated from goldfish, without the development of dermal ulcerations. Removal of mucus, followed by skin inoculation, produced ulcers in brook trout and Atlantic salmon, but not in rainbow trout. Atypical isolates obtained from ulcerated flounders (*Platichthys flesus*) could reproduce dermal ulcerations only if the bacterium was injected subdermally or if the skin of fish had been scarified prior to waterborne challenge (Wiklund 1995). The importance of dermal scarification is further emphasized by Takahashi et al. (1975), who concluded that the natural transmission of Goldfish Ulcer Disease was most severe among fish whose dermis had been severely eroded by either ectoparasites or handling. *Aeromonas salmonicida* has been isolated from the sea louse *Lepeophtheirius salmonis* (Nese and Enger 1993) in the marine environment and from *Argulus corregoni* (Shimura et al. 1983), and *Tetrahymena pyriformis* (King and Shotts 1988) within the freshwater environment. Because each of these parasites is capable of inducing severe branchial or dermal damage, it has been theorized but not substantiated that infected ectoparasites may act as vectors in the spread of furunculosis.

Horizontal transmission of *A. salmonicida* has also been demonstrated where mollusks have been co-cultured with susceptible hosts (Bjoershol 1999; Starliper 2001). Another novel application of husbandry techniques that may effect the transmission of infection involves the use of wrasse as cleaner fish to clear sea lice from Atlantic salmon in net pen culture. The wrasse may themselves become infected with atypical variants of either by orally ingesting infected sea lice or through horizontal exposures with other infected fish. Infected wrasse may, therefore, act as a further reservoir of contagion for salmon with which they are co-cultured (Collins et al. 1991; Laidler et al. 1999). As a consequence of such infection, it was suggested that wrasse should not be released into the environment or transferred between facilities at the end of specific production cycles (Treasurer and Laidler 1994).

In addition to horizontal transmission via cohabitation or fish to fish contact, Wooster and Bowseer (1996) found that viable cells of *A. salmonicida* can be dispersed in aerosol droplets at least 104.1 cm (limits of the test chamber used) in the air. Viable *A. salmonicida* was also recovered from the water of tanks that were exposed to an aerosol spray downwind of the contaminant source. This study indicates that aerosol transmission of *A. salmonicida* may be particularly problematic where culture systems utilize spray bars within raceways or aquaria in close proximity to one another. These findings are also consistent with the report of Enger et al. (1992) which indicated that concentrations of *A. salmonicida* are selectively enriched within in the lipid rich surface microlayer at the air-water interface.

Mackie et al. (1930) noted the presence of *A. salmonicida* within the ovaries and testes of infected fish, but failed to experimentally induce intra-ovum infections and vertically transmit furunculosis between parent and offspring. Consequently, Mackie et al (1933) concluded that *A. salmonicida* contaminated the surface of the eggs and recommended that such eggs should be

disinfected to minimize or prevent further contagion (Mackie et al. 19933). McCarthy (1977b) indicated that vertical transmission was not a significant route of infection because *A. salmonicida* cells from infected parents were unlikely to survive in the eyed-egg stage. Similarly, Bullock and Stuckey (1987) were unable to document vertical transmission of furunculosis among the progeny of parental stocks that had either survived furunsulosis expizootics or had been experimentally injected with *A. salmonicida* prior to spawn.

VIRULENCE FACTORS

Because clinical signs of furunculosis are readily produced in fish injected with extracellular products produced during the growth of *A. salmonicida* (Ellis et al. 1981), an extensive body of research exists on mechanims of virulence associated with this pathogen. Such virulence has been associated with structural components of the bacterial cell and with exotoxins that are secreted during bacterial metabolism. Although it is phenotypically difficult to predict virulence *in vitro* based upon the presence or absence of an individual bacterial component (Olivier 1990), the synergistic effect of each of these elements is indeed significant to the cumulative expression of furunculosis *in vivo*. The nature of virulence in *A. salmonicida* is indeed complex and apparently varies between strains.

A-Layer Protein

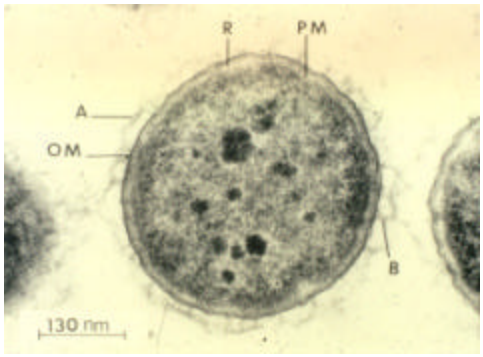

Figure 10: Transmission electromicrograph of a virulent *Aeromonas salmonicida* showing the presence of the A-layer (A) in relation to the bacterial outer membrane (OM), rigid layer (R), plasma membrane (PM) and pili-like appendages (B).

Udey (1978) showed that virulent *A. salmonicida* possessed an additional layer (A-layer) associated with the external surface of the membrane of the cell wall. Evenberg et al. (1981) determined that the A-layer consisted of a major additional cell envelope protein (ACE) that was immunologically similar among virulent isolates. The protein was water-insoluble, hydrophobic, and similar to the K88 adhesive fimbriae of enteropathogenic *Escherichia coli*. The protein was described to have a molecular weight of 49 kilodaltons, which was expressed in a regularly repeating tetragonal array beyond the bacterial cell wall (Kay et al 1981), and was present within typical and atypical strains of the pathogen (Hamilton et al. 1981). Kay and Trust (1991) additionally showed that the tetragonal array is tethered to the cell surface via the O-antigen side chain of the bacterial lipopolysaccharide (LPS). The A-layer protein array apparently blocks bacteriophage receptors on the outer membrane of *A. salmonicida*, presents a chemically refractile and somewhat impermeant barrier (Trust et al. 1981) that buffers the underlying outer

membrane and its associated proteins from chemical modification involved with host defense including elements of both the immune response and cytotoxic effects of the complement pathway (Kay and Trust 1991). Garduno et al (2000) have also shown that A-layer positive strains of *A. salmonicida* can efficiently adhere to, enter, and survive within macrophages.

Glycerophospholipid cholesterol acyltransferase

When injected into fish in its purified form, bacterial endotoxin or lipopolysaccharide (LPS) that is produced by *A. salmonicida* is not pathogenic (Wedemeyer et al. 1969; Paterson and Fryer 1974). Within the matrix of extracellular products produced by *A. salmonicida*, however, LPS may be aggregated with bacterial glycerophospholipid cholesterol acyltransferase (GCAT; molecular weight = 25 kilodaltons). Lee and Ellis (1990) have indicated that the GCAT/LPS complex (molecular mass = 2000 kilodaton) is hemolytic for fish erythrocytes, leukocytolytic, cytotoxic, and lethal for Atlantic salmon when injected at a concentration of 0.045 µg protein/g of body weight. The LPS did not affect the enzymatic activity of free GCAT with egg yolk or phosphatidylcholine (lecithin). When complexed with LPS, GCAT was more heat stable and both hemolytic activity and lethal toxicity was about eightfold higher in the complexed form. Unlike earlier studies, therefore, the authors suggested that the LPS moiety of the aggregate GCAT/LPS complex is indeed an important element in the pathogenicity of *A. salmonicida*.

Bacterial proteases

Early studies indicated that as a consequence of septicemia bacterial utilization of the host's blood sugar induced hypoglycemia and subsequent death (Field et al. 1944). At that time very little was known about the potent action of bacterial exotoxins produced by *A. salmonicida* until Griffin (1954) suggested that this bacterium produced a leucocidin that was responsible for the severe leukopenia observed histopathologically in lesion development. Klontz et al. (1966) would initially show that this suppression of the inflammatory response could be produced by injecting trout with cell-free extracts from *A. salmonicida*. That work would initiate additional investigations of virulence associated with the exotoxic substances produced by *A. salmonicida* that continue to this day.

Fuller et al. (1977) extracted a leukocytolytic factor from the extracellular products (ECP), which not only was cytotoxic for leukocytes but also synergistically enhanced bacterial virulence when injected in concert with viable cells. A protease was also extracted from the extracellular material (Shieh and MacLean 1975) that induced the development of furuncle-like lesions in fish (Sakai 1977). Cipriano et al. (1981) determined that the extracellular material was actually a composite of substances; one analogous to the leukocytolytic factor described by Fuller (1977), another was proteolytic and induced lesion development similar to that reported by Sakai (1977), and yet others expressed cytotoxic activity to rainbow trout (*Oncorhynchus mykiss*) gonad cells (RTG-2) in tissue culture or were hemolytic for trout erythrocytes and lethal in brook trout (*Salvelinus fontinalis*) regardless of the virulence of the strain from which they were extracted.

Shieh (1985) found that the proteolytic activity inherent among virulent isolates was quantitatively more toxic for Atlantic salmon than that isolated from avirulent isolates. Production of a protease deficient mutant which lost virulence but maintained autoagglutinative,

serum resistant, adhesive, hemolytic, and leukocytolytic activities provided further indication that extracellular protease was an important element of bacterial pathogenicity (Sakai 1985). Based upon chymotryptic properties, inhibition by diisopropylfluorophosphate (DFP) and tosyl-phenylalanine-chloromethyl ketone (TPCK) and hydrolyzed N-benzoyl-L-tyrosine ethyl ester (BTEE), Tajima et al. (1984) characterized a heat labile alkaline serine protease from the ECP of *A. salmonicida* with a molecular mass of 71 kilodaltons. Proteolytic activity was stable from pH 5.0 to 10.0, but maximum activity was observed at pH 9.4 and 50^{o} C. Price et al. (1989) showed that strains of *A. salmonicida* actually produced at least two types of proteases; a serine protease of molecular weight 70 kilodaltons (similar to that described by Tajima) which is active against casein and gelatin and another of molecular weight 20 kilodaltons which is active against gelatin but not casein. Because no difference was observed in the toxicity of extracellular products produced by caseinase deficient mutant and its wild-type *A. salmonicida*, Drinan et al. (1989) inferred that the caseinase enzyme has no major role in the pathogenesis of *A. salmonicida* infections. Gudmundsdottir et al. (1990) has described a 20 kilodalton protease within *A. salmonmicida* subsp. *achromongenes* that was lethal for Atlantic salmon and identified as a metallo-protease based upon its inhibition by EDTA. A 35 kilodalton metalloprotease was also isolated from *A. salmonicida* subsp. *salmonicida* with optimum acticvity at pH 7.5 and 40^{o}C against gelatin and azocoll but not against casein (Arnesen et al. 1995).

Siderophore Production and Iron Regulation

Most typical and atypical isolates of *A. salmonicida* can grow under conditions of iron-restriction as evidenced *in vitro* by multiplication in the presence of iron chelators. However, siderophore production was detected only among typical strains and it behaved as a 2,3-diphenol-catechol (Hirst et al. 1991). Consequently, it was inferred that atypical and typical isolates regulate iron-uptake by different mechanisms. Among typical strains, utilization was siderophore-dependent and transferrin was digested by the 70 kilodalton serine protease. The presence of the catechol siderophore and major iron-regulated outer membrane proteins (IROMPs) were consistent and homogeneous within *A. salmonicida* subsp. *salmonicida* (Fernandez et al. 1998). Consequently it would appear that the siderophore-independent utilization of transferrin within atypical isolates is a function of proteolytic digestion by the metalloprotease.

CONTROL

Because furunculosis infects such a wide variety of hosts in a diversity of habitats, the bacterium is predominantly managed as a geographically ubiquitous, but obligate pathogen. The pandemic distribution and prevalence of this bacterium, however, should never diminish the gravity of its pathogenic ramifications in the minds of fish culturists and resource managers. Reference to the factoral interplay of host, pathogen, and environment is used so often that it becomes an abstract conceptualization of the disease process. Avoidance of *A. salmonicida* within populations of fish is critical to the prevention of furunculosis. Jarp et al. (1993) have found that the migration of anadromous fish into hatchery water supplies, a dense concentration of other infected fish culture stations proximate to uncontaminated stations, and sharing personnel or equipment between stations are among the most significant risks associated with the introduction of furunculosis

(Jarp. et al. 1993). Managers have some degree of control over each of these parameters. Ultraviolet irradiation (Bullock and Stuckey 1977) or ozonation (Wedemeyer and Nelson 1977; Colberg and Lingg 1978) of incoming culture water, fallowing of net pen sites, and education of personnel can alleviate significant sources of potential contamination and prevent disease. Regular monitoring programs that detect *A. salmonicida* in the water supply and provide early non-lethal detection on mucus can be coupled with topical disinfection or antibiotic regimens that either preclude or minimize infection (Cipriano 1997). Furthermore, only fertilized eggs or stocks of fish that have been certified to be free of *A. salmonicida* infection should be transferred between facilities attempting to maintain a specific pathogen free status. In practice, conduct of the stress induced furunculosis assay and regulatory confinement of infected smolts have reduced the number of furunculosis outbreaks associated with early marine culture (Smith 1991; Olivier 1992).

Eyed eggs from sources not certified to be specific pathogen free should be disinfected upon arrival, and must be isolated from subsequent contact with other eggs or with contaminated packing material and containers. Povidone iodine has become the compound of choice for disinfecting trout and salmon eggs (McFadden 1969), because it possesses greater bacteriocidal activity than merthiolates (Gee and Sarles 1942) or acriflavine (Atkinson 1930, Blake 1930). The compund is available commercially as an iodophor (e.g., - Betadine and Wescodyne) that generally contains approximately 1.0% to 1.6% active iodine in inert organic solvents (McFadden 1969; Amend 1974). Although Ross and Smith (1972) extended the effective use of povidone iodine against a number of bacterial fish pathogens including *A. salmonicida,* they also noted that disinfection was incomplete when 25 mg/L of iodophor was used for 15 to 120 minutes with certain strains of this pathogen. McCarthy *(1977b)* also *found* that a 10-minute treatment of artificially infected green or eyed eggs with 50 or 100 mg/L of active iodine (as Wescodyne) may not kill all *A. salmonicida* cells, but that a 30-min exposure to 1,000 mg/L acriflavine was completely efficacious. Consequently, Piper et al. (1982) recommended that salmonid eggs should be disinfected in a solution containing 100 mg of active iodine/L for 10 minutes, but the bacteriocidal activity of iodophor may still be influenced by the concentration of A. *salmonicida* present and the degree of organic contamination in the disinfectant solution (Sako et al. 1988).

United States Fish and Wildlife Service regulations recommend that eggs should be disinfected by submersion for 30 minutes in 50 mg iodine/L immediately after fertilization. If eggs are then shipped to another facility for incubation, policy requires that those eggs undergo a secondary disinfection in 100 mg iodine/L for 10 minutes (USFWS 1995). Disinfection should be conducted in 10 to 15°C pathogen-free water at a pH of 7.0 (6 to 8). Soft water in which the normal acidity of iodophors can reduce effectiveness may be alkalized by adding 0.5 g sodium bicarbonate per liter of water. The eggs should be rinsed immediately after treatment, unless they are placed into a flowing-water incubator within a few minutes after disinfection.

SELECTION AND BREEDING

Selective breeding programs have been developed in salmonids to enhance experimental and field resistance to furunculosis. Brook trout (*Salvelinus fontinalis*) at the Hackettstown (NJ) state fish hatchery were selected on the basis of growth, coloration and the ability to survive natural epizootics of furunculosis. Subsequent progeny were continually evaluated for acquired resistance to disease and furunculosis mortality was reduced from 98% to 30.8%, after four successive generations of selection (Embody and Hayford 1925). Resistant progeny grew faster than non-selected fish, which corresponded with a rise in the average number of eggs obtained per female at first and second spawns but this was also accompanied by a gradual decrease in egg viability to the eyed-egg stage. Although the brook trout had acquired resistance to furunculosis, selection did not

enhance resistance to Bacterial Gill Disease nor infestations by *Gyrodactylus* and *Chilodonella* (Hayford and Embody 1930). Probably the most successful selection study involves the development of furunculosis-resistant strains of trout by the New York State Department of Environmental Conservation (Rome, NY). Wolf (1953) collected 11 strains of brook trout and seven strains of brown trout (*Salmo trutta*) from the New England area that were inbred for a minimum of eight previous generations. The fish were constantly subjected to *A. salmonicida* via bath, horizontal, and dietary challenges. Mortality within strains ranged from 36% to 66% for brook trout and from 5% to 99.7% for brown trout and Wolf concluded that enough variation existed among the resistance of various lots to warrant further evaluation through selective breeding. Ehlinger (1964) continued these studies and crossbred survivors between strains with intermediate or low mortality. Potential brood stock was further selected on the basis of size, coloration and egg yield. Resistance was monitored within strains as a function of eliminating susceptible breeders within a line and by experimental challenge on subsamples of the resultant progeny. During the next ten years, one resistant strain of brook trout and one strain of brown trout were produced which had practical application to the culture of salmonids throughout New York (Ehlinger 1977). Acquired resistance to furunculosis of the Rome trout is widely accepted and these fish are currently used throughout many hatchery programs in the New England and the Mid-Atlantic Regions (Kincaid et al. 1997) for their survival and performance in recreational fisheries where furunculosis is enzootic (Hulbert 1985).

Detailed genetic studies have shown that heritability of mortality among families of Atlantic salmon challenged by cohabitation with infected cohorts was high, h^2 = 0.32 +/- 0.10 and 0.48 +/- 0.17, for the sire and dam components, respectively (Gjedrem et al. 1991), suggesting that resistance to furunculosis can be effectively improved by selective breeding (Gjedrem and Gjoen 1995). These observations were consistent with those of Bailey et al. (1993) who also indicated that heritabilty (h^2 = 0.32 +/-6) among Atlantic salmon for resistance to furunculosis was sufficient to warrant further selection. Langefors et al. (2001) screened MHC class IIB genotypes established exon 2 sequences encoding the major part of the peptide-binding region among families of Atlantic salmon that demonstrated either high resistance (HR) or low resistance (LR) to *A. salmonicida*. Three alleles were discerned from these studies, which showed some correlation with resistance to furunculosis. One allele, e, was significantly more prevalent in HR families than in LR families. Brood fish carrying the e allele had a 12-fold greater chance of being HR than cohorts without the e allele. A second allele, i, had a significantly higher frequency among those fish that either remained uninfected or survived experimental challenges than it did among dead individuals. A third allele, j, was prevalent in LR families and among individuals that died from infection.

Variable degrees of resistance have also been noted among non-salmonids in response to atypical isolates of *A. salmonicida* infections. Dubois-Darnaudpeys and Tuffery (1979) have shown that tench are more resistant to carp erythrodermatitis than the common carp, grass carp and silver carp, whose degree of resistance is listed in descending order. Sovenyi et al. (1988) found that three-month-old progeny of hybrid crosses between common carp from Hungary and Japanese coloured carp had a body weight (43.6 +/- 22.4 g) and condition factor (8.85 +/- 1.05 g/cm^3) were significantly higher than those of the common carp purebreds (23.5 +/- 12.3 g body weight; 8.53 +/- 0.62 g/cm^3 condition factor). Furthermore, the hybrids sustained only half the mortality than that which was observed among the purebred carp after experimental challenge with an atypical *A. salmonicida*. Houghton et al. (1991) showed that a Polish strain of carp, inbred for six generations) was significantly more susceptible to carp erythrodermatitis than a Hungarian strain that had been inbred for five generations. Wiegertjes et al. (1993) further demonstrated that even

within the same strain of fish, resistance to erythrodermatitis increases with age as indicated by a shift from subacute (85% at 3 and 5 months) to chronic (40% at 10 months) mortality.

IMMUNIZATION

Vaccines of *A. salmonicida* emulsified in oil adjuvants and delivered by intraperitoneal injection provide long-lasting protection and their use is promoted in commercial aquaculture (Lillehaug et al. 1992; Ellis 1997). Both aqueous and oil-adjuvanted vaccines are commercially produced and may induce immunity at water temperatures as low as 2°C. Eggset et al. (1997) noted that even though an antibody response for *A. salmonicida* was delayed or strongly suppressed by this temperature, protection was still satisfactory at 18 weeks after vaccination. After 18 weeks, these workers further noted that protection was severely reduced among those fish reared at 10°C and given the aqueous vaccine, but a similar reduction was not apparent among those fish that were vaccinated with the oil-emulsion vaccine (Killie et al. 1997). Although commercial furunculosis vaccines administered in mineral oil yield significantly higher protection than aqueous-based vaccines, the mineral oil adjuvant may produce a higher degree of abdominal adhesions between the internal organs and the abdominal wall near the site of injection (Midtlyng 1993). If severe, these adhesions may decrease growth rate (Midtlyng 1994), the severity of which is inconsistent between farms and may or may not be significant when compared with the final growth weight of non-vaccinated fish (Midtlyng and Lillehaug 1998). It must be remembered, however, that vaccination is not an absolute barrier treatment that guarantees further expression of furunculosis within – or transmission of furunculosis from covertly infected vaccinated carriers (Hiney 1999).

The serologic relatedness that exists among strains of *A. salmonicida* (Popoff 1969; Paterson et al. *1980)* suggests that immunization of fishes against atypical forms of this pathogen is also a realistic possibility. However, Atlantic salmon vaccinated with a commercial vaccine against *A. salmonicida* subsp *salmonicida* or with this vaccine and another prepared against an autogenous strain of *A. salmonicida* subsp. *achromogenes* were equally protected against *A. salmonicida* subsp *salmonicida* by cohabitation challenge. However, salmon vaccinated only with Aeromonas salmonicida subsp. achromogenes were not protected against classical furunculosis (Gudmondsdottir and Gudmondsdottir 1997). Furthermore, Evenberg et al. (1988) showed only moderate to slight protection against an atypical variant of *A. salmonicida* causing carp erythrodermatitis, when carp were immunized with either cell envelope preparations, purified LPS, purified A-layer protein, and even formalinized whole cell bacterins. When detoxified ECP was used as the immunogen, carp were protected against a subsequent lethal challenge.

TREATMENT

Furunculosis of salmonids was the first disease of fishes to be treated with modern drugs including both sulfonamides and nitrofurans (Gutsell 1948). Although other drugs effectively control this disease (Herman 1970), the U.S. Food and Drug Administration enforces stringent requirements for drugs used on food animals in the United States, and only sulfamerazine (no longer registered), oxytetracycline, and the potentiated sulfonamide Ro5-0037 or ROMET® (Bullock et al. 1974) may be used. Although most sulfonamides are no longer used, sulfamerazine American Cyanamid, Co.) has been approved to treat furunculosis in rainbow

trout, brook trout, and brown trout at a dose of 200 mg of drug per kilogram of fish per day (10 g per 45.3 kg of fish weight) for 14 days. Treatment must terminate at least 3 weeks before fish are to be marketed or stocked. Sulfisoxazole (Gantrisin) was preferred for treating brown trout whose growth may be inhibited by sulfamerazine (Snieszko and Wood 1955). Oxytetracycline (Terramycin®, Pfizer, Inc.) is cleared for use among all species of salmonids, at the rate of 50 to 80 mg of drug per kilogram of fish per day (2.5 to 3.75 g per 45.3 kg of fish) for 10 days. Again, treatment must terminate at least 3 weeks before fish are released. ROMET® (Hoffman-LaRoche, Inc.), which consists of five parts sulfadimethoxine and one part ormetoprim, controlled furunculosis when fed at the rate of 50 mg per kilogram of fish per day (2.5 g per 45.3 kg) for 14 days (Bullock et al. 1974) and has been cleared for use at the same dosage when administered for five days with a 42 day withdrawal period.

LITERATURE CITED

Adams, A. and K. Thompson. 1990. Development of an enzyme-linked immunosorbent assay (ELISA) for the detection of *Aeromonas salmonicida* in fish tissue. Journal of Aquatic Animal Health. 2: 281-288.

Altman, K., M. Marshall, S. E. Nicholson, P. J. Hanna, and N. Gudkovs. 1992. Glucose repression of pigment production in atypical isolates of *Aeromonas salmonicida* responsible for goldfish ulcer disease. Microbios. 72: 292-293.

Amend, D. F. 1974. Comparative toxicity of two iodophors to rainbow trout eggs. Trans. Am. Fish. Soc. 103(1):73-78.

Arnesen, J. A., G Eggset, and T. O. Jorgensen.1995. Partial purification and characterization of extracellular metalloproteases from *Aeromonas salmonicida* spp. *salmonicida*. Journal of Fish Diseases. 18: 283 - 295.

Atkinson, N. J. 1932. A bacterial disease of trout eggs and fry and its prophylaxis with acriflavine. Transactions of the American Fisheries Society. 62: 152 – 155.

Austin, B. 1993. Recovery of 'atypical' isolates of *Aeromonas salmonicida*, which grow at 37°C, from ulcerated non-salmonids in England. Journal of Fish Diseases. 16: 165 – 168.

Austin, D.A., D. McIntosh, and B. Austin. 1989. Taxonomy of fish associated *Aeromonas* spp. with the description of *Aeromonas salmonicida* subp. *smithia* subsp. nov. Systematic and Applied Microbiology. 11: 277 – 290.

Austin, B., D. A. Austin, I. Dalsgaard, B. K. Gudmondsdottir, S. Hoie, J. M. Thornton, J. L. Larsen, B. O'Hici, and R. Powell. 1998. Characterization of atypical *Aeromonas salmonciida* by different methods. Systematic and Applied Microbiology. 21: 50 – 64.

Bailey, J. K.; Olivier, G.; Friars, G. W. 1993. Inheritance of furunculosis resistance in Atlantic salmon. Bulletin of the Aquaculture Association of Canada. 4: 90-92.

Belland, R. J. and T. J. Trust. 1988. DNA-DNA reassociation analysis of *Aeromonas salmonicida*. Journal of General Microbiology. 134: 307 – 315.

Bjoershol, B., R. Nordmo, K. Falk, and S. Mortensen. 1999. Cohabitation of Atlantic salmon (*Salmo salar* L.) and scallop (*Pecten maximus*) – challenge with Infectious Salmon Anemia (ISA) Virus and *Aeromonas salmonicida* subsp. *salmonicida*. 12th International Pectinid Workshop, Bergen (Norway), 5 – 11 May 1999.

Blake, I. 1930. The external disinfection of fish ova with reference to the prophylaxis of furunculosis. Fisheries Board, Scotland Salmon Fisheries, 1930 No. II. is Majesty's Stationery Odffice, Edinburgh. 10 pp.

Bootsma, R., and J. Blommaert. 1978. Zur Aetiologie der Erythroder- matitis beim Karpfen *Cyprinu8 ca1'pio* L. Pages 20-27 *in* Neuere Erkenntnisse uber Fischinfektionen. Gustav Fischer Verlag, Stuttgart and New York.

Bootsma, R., N. Fijan, and J. Blommaert. 1977. Isolation and preliminary identification of the causative agent of carp erythrodermatitis. Vet. Arh. 47(6):291-302.

Boyd, E. F., M. P. Hiney, J. F. Peden, P. R. Smith and D. A. Caugant. 1994. Assessment of genetic diversity among Aeromonas salmonicida isolates by multilocus enzyme electrophoresis. Journal of Fish Diseases. 17: 97 – 98.

Bruno, D. W. 1986. Furunculosis in sea-reared Atlantic salmon, *Salmo salar* L. Colonization of gill epithelium. Bulletin of the European Association of Fish Pathologists. 6: 76 - 80.

Bullock, G. L., and H. M. Stuckey. 1975. Aeromonas salmonicida: detection of asymptomatically infected trout. Progressive Fish-Culturist. 37: 237 – 239.

Bullock, G. L., and H. M. Stuckey. 1977. Ultraviolet treatment of water for destruction of five gram-negative bacteria pathogenic to fishes. Journal of the Fisheries Research Board of Canada. 34:1244 - 1249.

Bullock, G. L. and H. M. Stuckey. 1987. Studies on vertical transmission of *Aeromonas salmonicida*. The Progressive Fish Culturist. 49: 302 – 303.

Bullock, G. L., H. M. Stuckey, D. Collis, R. L. Herman, and G. Maestrone. 1974. In vitro and in vivo efficacy of a potentiated sulfonamide in control of furunculosis in salmonids. Journal of the Fisheries Research Board of Canada. 31: 75 - 82.

Calabrez, M.C.T., P. Lintermans, and J. Vosjan. 1993. The polymerase chain reaction (PCR) technique as a specific and sensitive detection method for *Aeromonas salmonicida* and *Aeromonas sobria* in natural ecosystems (water, sediment and fish). World Aquaculture '93 Int. Conf., Torremolinos (Spain), 26-28 May 1993. Special publication. European Aquaculture Society. Bredene; no. 19, p. 328; 1993; (FROM DISCOVERY TO COMMERCIALIZATION; Carrillo, M.;Dahle, L.;Morales, J.;Sorgeloos, P.;Svennevig, N.;Wyban, J. (eds.)

Calkins, G. N. 1899. Report upon the resent epidemic among brook trout (Salvelinus fontinalis) on Long Island. Fourth Annual Report of the Commissioners of Fisheries, Game, and Forests. State of New York Fish and Game Commission. Pp. 175 – 190.

Carballo J., R. M. Seoane, and T. P. Nieto. 2000. Adhesion of *Aeromonas salmonicida* to materials used in aquaculture. Bulletin of the European Association of Fish Pathologists. 20: 77-82.

Carson, J. and J. Handlinger. 1988. Virulence of the aetiological agent of goldfish ulcer disease in Atlantic salmon, *Salmo salar* L. Journal of Fish Diseases. 11: 471 – 479.

Chapman, P. F., R. C. Cipriano, and J. D. Teska. (1991. Isolation and phenotypic characterization of an oxidase-negative *Aeromonas salmonicida* causing furunculosis in coho salmon (Oncorhynchus kisutch). Journal of Wildlife Diseaseas. 27: 61 – 67.

Cipriano, R. C. 1997. Strategies for management of furunculosis in Atlantic salmon effected by non-lethal detection of *Aeromonas salmonicida*: a review. Bulletin of the European Association of Fish Pathologists. 17: 215 – 219.

Cipriano, R. C. and J. Bertolini. 1988. Selection for virulence in the fish pathogen Aeromonas salmonicida using Coomassie brilliant blue agae. Journal of Wildlife Diseases. 24: 676 – 678.

Cipriano, R. C. and A. R. Blanch. 1989. Different structural characteristics in the cell envelope of the fish pathogen Aeromonas salmonicida. Microbios Letters. 40: 87 – 95.

Cipriano, R. C., B. R. Griffin, and B. C. Lidgerding. 1981. *Aeromonas salmonicida:* relationship between extracellular growth products and isolate virulence. Canadian Journal of Fisheries and Aqutic Sciences. 38: 1322 - 1326.

Cipriano, R. C., L. A. Ford, D. R. Smith, J. H. Schachte, and C. J. Petrie. 1997. Differences in detection of *Aeromonas salmonicida* in covertly infected salmonid fishes by the stress-inducible furunculosis test and culture-based assays. Journal of Aquatic Animal Health. 9: 108-113.

Colberg, P. J. and A. J. Lingg. 1978. Effect of ozonation on micro- bial fish pathogens, ammonia, nitrate, nitrite, and BOD in simulated reuse hatchery water. Journal of the Fisheries Review Board of Canada. 35: 1290 - 1296.

Collins, R. O., D. A. Ferguson, and M. A. Bonniwell. 1991. Furunculosis in wrasse. Veterinary Record. 128: 43

Dalsgaard, I., B. Nielsen, and J. L. Larsen. 1994. Characterization of *Aeromonas salmonicida* subp. *salmonicida*: a comparative study of strains of different geographic origin. Journal of Applied Bacteriology. 77: 21 – 30.

Dalsgaard, I., B. K. Gudmundsdsottir, S. Helgason, S. Hiie, O. F. Thoresen, U. P. Wichardt, and T. Wiklund. 1998. Identification of atypical *Aeromonas salmonicida*: inter-laboratory evaluation and harmonization of methods. Journal of Applied Microbiology. 84: 999 - 1006.

Deere, D., J. Porter, R. W. Pickup, and C. Edwards. 1996. Survival of cells and DNA of *Aeromonas salmonicida* released into aquatic microcosms. Journal of Applied Bacteriology. 81: 309-318.

Drinan, E. M., J. Donlon, S. Molloy, and P. R. Smith. 1989. Observations on the role of the caseinase enzyme in the pathogenesis of furunculosis. Journal of Fish Diseases. 12: 45 - 50.

Dubois-Darnaudpeys, A., and G. Tuffery. 1979. Etude experimentale de la Sensibilite de differentes especes pisciaires a L'agent de L'erythrodermatite de la carpe (E.C.). Bull. Acad. Vet. Fr. 52(4):561-566.

Eggset, G., H. Mikkelsen, and J. E. A. Killie. 1997. Immunocompetence and duration of immunity against *Vibrio salmonicida* and *Aeromonas salmonicida* after vaccination of Atlantic salmon (*Salmo salar* L.) at low and high temperatures. Fish & Shellfish Immunology. 7: 247 - 260.

Ehlinger, N. F. 1964. Selective breeding of trout for resistance to furunculosis. New York Fish and Game Journal. 11: 78-90.

Ehlinger, N. F.1977. Selective breeding of trout for resistance to furunculosis. New York Fish and Game Journal. 24: 25 - 36.

Effendi, I. and B. Austin. 1994. Survival of the fish pathogen *Aeromonas salmonicida* in the marine environment. Journal of Fish Diseases. 17: 375 - 385.

Effendi, I.and B. Austin. 1995a. Dormant/unculturable cells of the fish pathogen *Aeromonas salmonicida*. Microbial Ecology. 30: 183 - 192.

Elliott, D., and E. B. Shotts, Jr. 1980a. Aetiology of an ulcerative disease in goldfish *Carassius auratus* (L.): microbiological examination of diseased fish from seven locations. Jornal of Fish Diseases. 3: 133 - 143.

Elliott, D., and E. B. Shotts, Jr. 1980b. Aetiology of an ulcerative disease of goldfish, *Carassius auratus* (L.); experimental induction of the disease. Jornal of Fish Diseases. 3:145 - 151.

Ellis A. E. 1997. Immunization with bacterial antigens: furunculosis. Developments in Biological Standardization. 90: 107 - 116.

Ellis, A. E., T. S. Hastings, and A. L. S. Munro. 1981. The role of *Aeromonas salmonicida* extracellular products in the pathology of furunculosis. Jornal of Fish Diseases. 4: 41 - 51.

Emmerich, R., and E. Weibel. 1890. Uber eine durch Bakterien verursachte Infektionskrankheit der Forellen. Allg. Fisch. Ztg. 15: 85 - 92.

Enger, Ø, B. Gunnlaugsdottir, B. K. Thorsen, and B. Hjeltnes. 1992. Infectious load of *Aeromonas salmonicida* subsp. *salmonicida* during the initial phase of cohabitant infection experiment with Atlantic salmon, *Salmo salar* L. Journal of Fish Diseases. 15: 425 – 430.

Eurell, T. E., D. H. Lewis, and L. C. Grumbles. 1979. Stained bacterial antigens for use in microagglutination procedures. Frogressive Fish-Culturist. 41: 55 - 57.

Evenberg, D., R. Bootsma, and B. Lugtenberg. 1981. Detection, purification and characterization of a major cell envelope protein, related to virulence, of autoagglutinating Aeromonas salmonicida strains. Society for General Microbiology. 8: 253-254.

Evenberg, D., P. de Graaff, B. Lugtenberg, and W. B. Van Muiswinkel. 1988. Vaccine-induced protective immunity against *Aeromonas salmonicida* tested in experimental carp erythrodermatitis. Journal of Fish Diseases. 11: 337 - 350.

Ezura, Y, H. Yamamoto, M. Yoshimizu, K. Tajima, H. Sannohe, K. Ikeda, H. Sako, T. Hara, and T. Kimura. 1984. An outbreak of furunculosis in coho salmon (*Oncorhynchus kisutch*) at the beginning of marine pen culture. Fish Pathology. 19: 75 – 80.

Ferguson, Y., L. A. Glover, D. M. McGillivray, and J. I. Prosser. 1995. Survival and activity of lux-marked *Aeromonas salmonicida* in seawater. Applied and Environmental Microbiology. 61: 3494-3498.

Fernandez, A. I. G., A. F. Fernandez, M. J. Perez, T. P. Nieto, and A. E. Ellis. 1998. Siderophore production by *Aeromonas salmonicida* subsp. *salmonicida*. Lack of strain specificity. Diseases of Aquatic Organisms. 33: 87 - 92.

Field, J. B., L. L. Gee, C. A. Elvehjem, and C. Juday. 1944. The blood picture in furunculosis induced by *Bacterium salmonicida* in fish. Arch. Biochem. 3: 277 - 284.

Fijan, N. N. 1972. Infectious dropsy of carp, a disease complex. Pages 39-51 *in* L. E. Mawdesley-Thomas, ed. Symposia of the Zoological Society of London. Academic Press, New York.

Fish, F. F. 1934. Ulcer disease of trout. Transactions of the American Fisheries Society. 64: 252 - 258.

Ford, L. A. 1994. Detection of *Aeromonas salmonicida* from water using a filtration method. Aquaculture. 122: 1-7.

Fuller, D. W., K. S. Pilcker, and J. L. Fryer. 1977. A leukocyto- lytic factor isolated from cultures of *Aeromonas salmonicida*. Journal of the Fisheries Research Board of Canada. 34: 1118 - 1125.

Garcia, J. A., J. L. Larsen, I. Dalsgaard, and K. Pedersen. 2000. Pulsed field gel elctrophoresis analysis of *Aeromonas salmonicida* subsp. *salmonicida*. FEMS Microbiology Letters. 190: 163 – 166.

Garduno, R. A., A. R. Moore, G. Olivier, A. L. Lizama, E.Garduno, and W. W. Kay. 2000. Host cell invasion and intracellular residence by *Aeromonas salmonicida*: role of the S-layer. Canadian Journal of Microbiology. 46: 660-668.

Gayer, E. K., L. Bekesi, and G. Csaba. 1980. Some aspects of the histopathology of carp erythrodermatitis (CE). Pages 127-136 *in* w. Ahne, ed. Fish diseases. Third COPRAQ-Session. Springer- Verlag, Berlin, Heidelberg, New York.

Gee, L. I. And W. B. Sarles. 1942. The disinfection of trout eggs contaminated with *Bacterium salmonicida*. Journal of Bacteriology. 44: 111 - 126.

Gjedrem, T.and H. M. Gjoen. 1995. Genetic variation in susceptibility of Atlantic salmon, *Salmo salar* L., furunculosis, BKD and cold water vibriosis. Aquaculture Research. 26: 129 - 134.

Gjedrem, T., R. Salte, and H. M. Gjoen. 1991. Genetic variation in susceptibility of Atlantic salmon to furunculosis. Aquaculture. 97: 1 - 6.

Griffin, P. J. 1953. Pigment formation by Bacterium salmonicida Journal of Bacteriology. 65: 652 – 659.

Griffin, P. J. 1954. The nature of bacteria pathogenic to fish. Transaction of the American Fisheries Society. 83: 241 - 253.

Griffin, P. J., S. F. Snieszko, and S. B. Friddle. 1953. A more comprehensive description of *Bacterium salmonicida.* Transactions of the American Fisheries Society. 82: 129 - 138.

Gudmundsdottir, B. K. and S. Gudmundsdottir. 1997. Evaluation of cross protection by vaccines against atypical and typical furunculosis in Atlantic salmon, *Salmo salar* L. Journal of Fish Diseases. 20: 343 - 350.

Gudmundsdottir, B. K., T. S. Hastings, and A. E. Ellis. 1990. Isolation of a new toxic protease from a strain of *Aeromonas salmonicida* subspecies *achromogenes*. Diseases of Aquatic Organisms. 9: 199-208

Gustafson, C. E., C. J. Thomas, and T. J. Trust. 1992. Detection of *Aeromonas salmonicida* from fish by using polymerase chain reaction amplification of the virulence surface array protein gene. Applied and Environmental Microbiology.. 58: 3816 -3825.

Gutsell, J. S. 1948. The value of certain drugs, especially sulfa drugs, in the treatment of furunculosis in brook trout, *Salve- linus fontinalis*. Transactions of the American Fisheries Society. 75: 186 - 199.

Hamilton, R.C., H. Kalnins, N. R. Ackland, and L. D. Ashburner. 1981. An extra layer in the surface layers of an atypical *Aeromonas salmonicida* isolated from Australian goldfish. Journal of General Microbiology. 122: 363 - 366.

Hamilton-Miller, J, M, T. 1975. Possible misidentification of the fish pathogen Aeromonas salmonicida. British Veterinary Journal. 131: 663 - 664.

Hammel, K. L. 1995. An overview of furunculosis in Atlantic Canada. Bulletin of the Aquaculture Association of Canada. 95: 8 – 11.

Hanninen, M. L., J. Ridell, and V. Hirvela-Koski. 1995. Phenotypic and molecular characteristics of *Aeromonas salmonicida* subsp. *salmonicida* isolated in Southern and Northern Finland. Journal of Apllied Bacteriology. 79: 12 – 21.

Hayford, C. O., Embody, G. C. 1930. Further progress in the selective breeding of brook trout at the New Jersey State Hatchery. Transactions of the American Fisheries Society. 60: 109 - 113.

Herman, R. L. 1970. Prevention and control of fish diseases in hatcheries. Pages 3-15 *in* S. F. Snieszko, ed. A symposium on diseases of fishes and shellfishes. American Fisheries Society, Special Publication No.5.

Hiney, M. 1999. Field observation of clinical furunculosis in Atlantic salmon smolts vaccinated with an oil-based furunculosis vaccine. Bulletin of the European Association of Fish Pathologists. 19: 66 - 69.

Hiney, M. P. and P. R. Smith 1998. Validation of polymerase chain reaction-based techniques for proxy detection of bacterial fish pathogens: Framework, problems and possible solutions for environmental applications; review. Aquaculture 162: 41 – 68.

Hiney, M., M. T. Dawson, D. M. Heery, P. R. Smith, F. Gannon, and R. Powell. 1992. DNA probe for *Aeromonas salmonicida*. Applied and Environmental Microbiology. 58: 1039 - 1042.

Hiney, M. P. J. J. Kilmartin, and P. R. Smith. 1994. Detection of Aeromonas salmonicidia in Atlantic salmon with asymptomatic furunculosis infections. Diseases of Aquatic Organisms. 19: 161 – 167.

Hiney, M. P., P. R. Smith, and E. M. Bernoth. 1997. Covert Aeromonas salmonicida infections, pp. 54 – 97, in: Bernoth, E. -M., A. E. Ellis, P. J. Midtlyng, G. Olivier, and P. Smith, eds. Furunculosis Multidisciplinary Fish Disease Research. Academic Press. San Diego, California. 529 pp.

Hirst, I. D., T. S. Hastings, and A. E. Ellis. 1991. Siderophore production by *Aeromonas salmonicida*. Journal of General Microbiology. 137: 1185 – 1192

Holt, J. G., N. R. Krieg, P. H. A. Sneath, J. T. Staley, S. T. Williams. 1994. *Bergey's Manual of Determinative Bacteriology, Ninth Edition*. Williams and Wilkins, Baltimore, Maryland. 787 pp.

Houghton, G., G. F. Wiergertjes, A. Groenveld, and W. B. van Muiswinkel. 1991. Differences in resistance of carp, *Cyprinus carpio* L., to atypical *Aeromonas salmonicida*. Journal of Fish Diseases. 14: 333 - 341.

Horne, J. H. 1928. Furunculosis in trout and the importance of carriers in the spread of the disease. Journal of Hygiene. 28: 67 – 78.

Humphrey, J. D. and L. D. Ashburner. 1993. Spread of the bacterial fish pathogen Aeromonas salmonicida after importation of infected goldfish, Carrasius auratus, into Australia. Australian Veterinary Journal. 70: 453 – 454.

Hulbert, P. J. 1985. Post-stocking performance of hatchery-reared yearling brown trout. New York Fish and Game Journal. 32: 1 – 8.

Husevåg, B. 1995. Starvation survival of the fish pathogen *Aeromonas salmonicida* in seawater. FEMS Microbiology Ecology. 16: 25 - 32.

Høie, S., M. Heum, and O. F. Thoresen. 1997. Evaluation of a polymerase chain reaction-based assay for the detection of *Aeromonas salmonicida* ss *salmonicida* in Atlantic salmon *Salmo salar*. Diseases of Aquatic Organisms. 30: 27-35.

Høie, S., I. Dalsgaard, I. L. Aase, M. Heum, J. M. Thornton, and R. Powell. 1999. Polymerase chain reaction (PCR)-based typing analysis of atypical isolates of the fish pathogen *Aeromonas salmonicida*. Systematic and Applied Microbiology. 22: 403 – 411.

Jarp, J., K. Tangen, F. V. Willumsen, H. O. Djupvik, and A. M. Tveit. 1993. Risk factors for infection with Aeromonas salmonicida in Norwewgian freshwater hatcheries. Diseases of Aquatic Organisms. 17: 81 – 86.

Johnsen, B. O.and A. J. Jensen. 1994. The spread of furunculosis in salmonids in Norwegian rivers. Journal of Fish Biology. 45: 47 - 55.

Kay, W. W. and T. J. Trust. 1991. Form and functions of the regular surface array (S-layer) of *Aeromonas salmonicida*. Experientia. 47: 412 – 414.

Kay, W. W., J. T. Bulkley, E. E. Ishiguro, B. M. Phipps, J. D. L. Monette, and T. J. Trust. 1981. Purification and disposition of a surface protein associated with virulence of *Aeromonas salmonicida*. Journal of Bacteriology. 147: 1077 - 1084.

Killie, J. E. A., G. Eggset, and H. Mikkelsen. 1997. Immunocompetence and duration of immunity against *Vibrio salmonicida* and *Aeromonas salmonicida* after vaccination of Atlantic salmon (*Salmo salar* L.) at low and high temperatures. Developmental and Comparative Immunology. 21: 181.

Kincaid, H. L., Gray, M. J., Mengel, L. J., Brimm, S. 1997. National Fish Strain Registry – Trout species tables of reported strains and broodstocks. U.S. Fish and Wildlife Service / U.S. Geological Survey. Washington, D.C. 165 pp.

King, C. H. and E. B. Shotts. 1988. Enhancement of Edwardsiella tarda and Aeromonas salmonicida through ingestion by the ciliated protozoan Tetrahymena pyriformis. FEMS Microbiology Letters. 51: 95 – 100.

Klontz, G. W., and J. W. Wood. 1973. Observations on the epidemiology of furunculosis disease in juvenile coho salmon *(Oncorhynchus kisutch)*. Pages 1-8 *in* W. A. Dill, ed. Symposium on the major communicable fish diseases in Europe and their control. European Inland Fisheries Advisory Commission. Technical Paper Number 17: Supplement 2.

Klontz, G. W., W. T. Yasutake, and A. J. Ross. 1966. Bacterial diseases of the Salmonidae in the Western United States: pathogenesis of furunculosis in rainbow trout. American Journal of Veterinary Research. 27: 1455 - 1460.

Krantz, G. E., J. M. Reddecliff, and C. E. Heist. 1964. Immune response of trout to *Aeromonas salmonicida*. Part I. Development of agglutinating antibodies and protective immunity. Progressive Fish-Culturist. 26: 3 - 10.

Laidler, L. A., J. W. Treasurer, A. N. Grant, and D. I. Cox. 1999. Atypical Aeromonas salmonicida infection in wrasse (Labridae) used as cleaner fish of farmed Atlantic salmon, Salmo salar L., in Scotland. Journal of Fish Diseases. 22: 209 – 213.

Langefors A. Lohm J. Grahn M. Andersen O. von Schantz T. 2001. Association between major histocompatibility complex class IIB alleles and resistance to *Aeromonas salmonicida* in Atlantic salmon. Proceedings of the Royal Society of London. 268: 479 – 485.

Lee, K. K.and A. E. Ellis. 1990. Glycerophospholipid: cholesterol acyltransferase complexed with lipopolysaccharide (LPS) is a major lethal exotoxin and cytolysin of *Aeromonas salmonicida*: LPS stabilizes and enhances toxicity of the enzyme. Journal of Bacteriology. 172: 5382 - 5393.

Lillehaug, A., T. Lunder, and T. T. Poppe. 1992. Field testing of adjuvanted furunculosis vaccines in Atlantic salmon, *Salmo salar* L. Journal of Fish Diseases. 15: 485 - 496.

Mackie, T. J., J. A. Arkwright, T. E. Pryce-Tannatt, J. C. Mot tram, W. D. Johnston, and and w. J. M. Menzies. 1930. Furunculosis Committee interim report. H. M. Stationery Office, Edinburgh. 65 pp.

Mackie, T. J., J. A. Arkwright, T. E. Pryce-Tannatt, J. C. Mot tram, . w. D. Johnston, and w. J. M. Menzies. 1933. Second interim report of the Furunculosis Committee. H. M. Stationery Office, Edinburgh. 81 pp.

Maheshkumar, S. M., S. M. Goyal, and P. P. Economon. 1990. Concentration and detection of Aeromonas salmonicida from hatcehery water. Journal of Fish Diseases. 13: 513 – 518.

Marsh, M. C. 1905. The Cold Spring Harbor epidemic among trout. Tenth Annual Report of the Commissioners of Fisheries, Game, and Forests. State of New York Fish and Game Commission. Pp. 125 – 139.

Mawdesley-Thomas, L. E. 1969. Furunculosis in the goldfish, *Carassius auratus* (L.). Journal of Fish Biology. 1:19 - 23.

McArdle, J. F., C. Dooley-Martyn, and F. McKierman. 1986. Histological examination of the gills as a method of detecting asymptomatic carriers of *A. salmonicida* in Atlantic salmon (*Salmo salar*). Bulletin of the European Association of Fish Pathologists. 6: 80 – 84.

McCarthy, D. H. 1975. Detection of *Aeromonas salmonicida* antigen in diseased fish tissue. Journal of General Microbiology. 88: 384 - 386.

McCarthy, D. H. 1977a. The identification and significance of atypical strains of *Aepomonas salnonicida.* Bull. Off. Int. Epizoot. 87: 459 - 463.

McCarthy, D. H. 1977b. Some ecological aspects of the bacterial fish pathogen *Aepomonas salmonicida.* Aquatic Microbiology. 6: 299 - 324.

McCarthy, D. H. and C. T. Rawle. 1975. The rapid serological diagnosis of fish furunculosis caused by rough and smooth strains of *Aepomonas salmonicida*. Journal of General Microbiology. 86: 185 - 187.

McCarthy, D. H. and P. Whitehead. 1977. An immuno-India ink tech- nique for rapid laboratory diagnosis of fish furunculosis. Journal of Applied Bacteriology. 42: 429 - 431

McCarthy, D. H. and R. J. Roberts. 1980. Furunculosis of fish--the present state of our knowledge. Pages 293-341 *in* M. R. Droop and H. W. Jannasch, eds. Advances in Aquatic Microbiology, Vol. 2. Academic Press, London.

Midtlyng, P.J. 1993. Efficacy and side-effects in Atlantic salmon (*Salmo salar* L.) of injectable furunculosis vaccines. Special publication. European Aquaculture Society. Bredene; 19: 414; 1993; In: From Discovery To Commercialization, Carrillo, M.;Dahle, L.;Morales, J.;Sorgeloos, P.;Svennevig, N.;Wyban, J. (eds.)

Midtlyng, P.J. 1994. Growth of Atlantic salmon after intraperitoneal injection of oil-adjuvanted furunculosis vaccines. International Symposium on Aquatic Animal Health, Seattle, WA (USA), 4-8 Sep 1994. Univ. Of California, School Of Veterinary Medicine: Davis, Ca (Usa), Pp. P. W-24.4; 1994; (International Symposium On Aquatic Animal Health: Program And Abstracts)

Midtlyng, P. J. and A. Lillehaug. 1998. Growth of Atlantic salmon *Salmo salar* after intraperitoneal administration of vaccines containing adjuvants. Diseases of Aquatic Organisms. 32: 91 - 97.

Miyata, M., V. Inglis, and T. Aoki. 1996. Rapid identification of *Aeromonas salmonicida* subspecies *salmonicida* by the polymerase chain reaction. Aquaculture. 141: 13 - 24.

Miyazaki, T. and S. S. Kubota. 1975a. Histopathological studies on the furunculosis of the Amago. – II. Perbranchial infection. Fish Pathology. 9: 203 – 212.

Miyazaki, T. and S. S. Kubota. 1975b. Histopathological studies on the furunculosis of the Amago. – III.. Perbcutaneous infection. Fish Pathology. 9: 213 – 218.

Mooney, J., E. Powell, C. Clabby, and R. Powell. 1995. Detection of *Aeromonas salmonicida* in wild Atlantic salmon using a specific DNA probe test. Diseases of Aquatic Organisms. 21: 131 - 135.

Morgan, J. A. W, G. Rhodes, and R. W. Pickup. 1993. Survival of nonculturable *Aeromonas salmonicida* in lake water. Applied and Environmental Microbiology. 59: 874 - 880.

Nese, L. and ? . Enger. 1993. Isolation of *Aeromonas salmnicida* from salmon lice *Lepeophtheirus salmonis* and marine plankton. Diseases of Aquatic Organisms. 19: 79 – 81.

Nielsen, B., J. E. Olsen, and J. L. Larsen. 1994. Ribotyping of *Aeromonas salmonicida* subsp. *salmonicida*. Diseases of Aquatic Organisms. 18: 155 – 158.

Nomura, T. 1993. The epidemiological study of furunculosis in salmon propagation. Scientific Reports of the Hokaido Salmon Hatchery (Japan). 47: 1 – 99.

Nordmo, R., A. Ramstad, and J. M. H. Riseth. 1998. Induction of experimental furunculosis in heterogeneous test populations of Atlantic salmon (*Salmo salar*) by use of a cohabitation method. Aquaculture. 162: 11 – 21.

Oakey, H. J., J. T. Ellis, and L. F. Gibson. 1998. The development of random DNA probes specific for *Aeromonas salmonicida*. Journal of Applied Microbiology. 84: 37 - 46.

O'Brien, D., J. Mooney, D. Ryan, E. Powell, M. Hiney, P. R. Smith, and R. Powell. 1994. Detection of *Aeromonas salmonicida*, causal agent of furunculosis in salmonid fish, from the tank effluent of hatchery-reared Atlantic salmon smolts. Applied and Environmental Microbiology. 60: 3874 - 3877.

O'Hici, B., G. Olivier, and R. Powell. 2000. Genetic diversity of the fish pathogen Aeromonas salmonicida demonstrated by random amplified polymorphic DNA and pulse-field gel electrophoresis analyses. Diseases of Aquatic Organisms. 39: 109 – 119.

Olivier, G. 1990. Virulence of Aeromonas salmonicida: lack of relationship with phenotypic characteristics. Journal of Aquatic Animal Health. 2:119 - 127.

Olivier, G. 1992. Furunculosis in the Atlantic Provinces: An overview. Bulletin of the Aquaculture Association of Canada. 92: 4 – 10.

Paterson, W. D. and J. L. Fryer. 1974. Immune response of juvenile coho salmon (*Oncorhynchus kisutch*) to *Aeromonas salmonicida* cells administered intraperitoneally in Freund's complete adjuvant. Journal of the Fisheries Research Board of Canada. 31: 1751 – 1755.

Paterson, W. D., D. Douey, and D. Desautels. 1980. Relationships between selected strains of typical and atypical *Aeromonas sa~onicidaJ Aeromonas hydrophila,* and *Hemophilus piscium.* Canadian Journal of Microbiology. 26: 588 - 598.

Pavan, M. E., S. L. Abbott, J. Zorzopulos, and J. M. Janda. 2000. *Aeromonas salmonicida* subsp. *pectinolytica* subsp. nov., a new pectinase-positive subspecies isolated from a heavily polluted river. International Journal of Systematic and Evolutionary Microbiology. 50: 1119 – 1124.

Pedersen, K., H. Kofod, I. Dalsgaard, and J. L. Larsen. 1994. Isolation of oxidase-negative *Aeromonas salmonicida* from diseased turbot *Scopthalmus maximus*. Diseases of Aquatic Organisms. 18: 149 – 154.

Perez, M. J., A. I. G. Fernandez, L. A. Rodriguez, and T. P. Nieto. 1995. Importance of the sample size to determine the survival time of *Aeromonas salmonicida*. Bulletin of the European Association of Fish Pathologists.15: 45 - 48.

Piper, R. G., I. B. McElwain, L. E. Orme, J. P. McCraren, L. G. Fowler, and J. R. Leonard. 1982. Fish Hatchery Management. United States Department of the Interior, Fish and Wildlife Service, Washington DC. 517 pp.

Pol, J. M. A., R. Bootsma, and J. M. Berg-Blommaert. 1980. Pathogenesis of carp erythrodermatitis (CE): role of bacterial endo- and exotoxin. Pages 120 - 125 *in* w. Ahne, ed. Fish diseases. Third COPRAQ-Session. Springer-Verlag, Berlin, Heidelberg, New York.

Popoff, M. 1969. A study of *Aeromonas salmonicida.* I. Biochemical and antigenic characteristics. Ann. Rech. Vet. 3: 49 -57.

Powell, R., J. L. Larsen, S. Hoie, I. Dalsgaard, B. Gudmundsdottir, and B. Austin. 1998. Improved identification and taxonomy of atypical isolates of the fish pathogen *Aeromonas salmonicida*. European Commission DG 12 Science, Research and Devevelopment: Luxembourg. Pp. 110 – 113; Third European Marine Science and Technology Conference. Lisbon 23 – 27 May 1998. Volume 5. Fisheries and Aquaculture (AIR: 1990-94).

Price, N. C., L. Stevens, D. Duncan, and M. Snodgrass. 1989. Proteases secreted by strains of *Aeromonas salmonicida*. Journal of Fish Diseases. 12: 223 - 232.

Rabb, L., J. W. Cornick, and L. A. McDermott. 1964. A macroscopic slide agglutination test for presumptive diagnosis of furun- culosis in fish. Progressive Fish-Culturist. 26: 118 - 120.

Rose, A. S., A. E. Ellis, and A. L. S. Munro. 1990a. The survival of *Aeromonas salmonicida* subsp. *salmonicida* in sea water. Journal of Fish Diseases. 13: 205 - 214.

Rose, A. S., A. E. Ellis, and A. L. S. Munro. 1990b. Evidence against dormancy in the bacterial fish pathogen *Aeromonas salmonicida* subsp. *salmonicida*. FEMS Microbiology Letters. 68: 105 - 108.

Ross, A. J. 1962. Isolation of a pigment-producing strain of *Aeromonas liquefaciens* from silver salmon (*Oncorhynchus kisutch*). Journal of Bacteriology. 84: 590 – 591.

Ross, A. J., and C. A. Smith. 1972. Effect of two iodophors on bacterial and fungal fish pathogens. Journal of the Fisheries Research Bopard of Canada. 29: 1359 – 1361.

Sakai, D. K. 1977. Causative factor of *Aeromonas salmonicida* in salmonid furunculosis: extracellular protease. Scientific Reports of the Hokaido Salmon Hatchery. 32: 61 - 89.

Sakai, D. K. 1978. Colliquative activity of purified protease for muscular tissue in Aeromonas salmonicida subsp. salmonicida. Scientific Reports of the Hokaido Salmon Hatchery. 33: 55 – 73.

Sakai, D. K. 1985. Loss of virulence in a protease-deficient mutant of *Aeromonas salmonicida*. Infection and Immunity. 48: 146 - 152.

Sakai, D. K. 1986. Electrostatic mechanism of survival of virulent *Aeromonas salmonicida* strains in river water. Applied and Environmental Microbiology. 51: 1343 – 1349.

Sako, H., N. Ishida, Y. Maeno, and M. Sorimachi. 1988. Bactericidal activities of five disinfectants on *Aeromonas salmonicida*, *Vibrio anguillarum* and *V. ordalii*. Fish Pathology. 23: 219 – 229.

Scallan, A. and P. Smith. 1993. Importance of sampling time in detecting stress inducible furunculosis in Atlantic salmon smolts. Bulletin of the European Association of Fish Pathologists. 13: 77 - 78.

Scallan, A., C. Hickey, and P. Smith. 1994. Evidence for the transient nature of strees indiucible asymptomatic *Aeromonas salmonicida* infections of Atlantic salmon. Bulletin of the European Association of Fish Pathologists. 13: 210 – 212.

Schultz, D. 1980. Erythrodermatitis of carp: studies of the mode of infection. Pages 137 - 144 *in* W. Ahne, ed. Fish diseases. Third COPRAQ-Session. Springer-verlag, Berlin, Heidelberg, New York.

Scott, M. 1968. The pathogenicity of *Aeromonas salmonicida* in sea and brackish waters. Journal of General Microbiology. 50: 321 - 327.

Shieh, H. S. 1985. Correlation between cell virulence and protease toxicity of *Aeromonas salmonicida* isolates. Microbios Letters. 28: 69 - 72.

Shieh, H. S. and J. R. MacLean. 1975. Purification and proper- ties of an extracellular protease of *Aeromonas salmonicida*, the causative agent of furunculosis. International Journal of Biochemistry. 6: 653 - 656.

Shimura, S., K. Innoue, M. Kudo, and S. Egusa. 1983. Studies on effects of parasitism of *Argulus corregoni* (Crustacea: Branchiura)on furunculosis of *Oncorhynchus masou* (Salmonidae). Fish Pathology. 18: 37 – 40.

Smith, P. R. 1991. Stress test picks up furunculosis carriers. Fish Farmer. 14: 18.

Snieszko, S. F. 1952. Ulcer disease in brook trout *(Salvelinus fontinalis):* its economic importance, diagnosis, treatment and prevention. Progressive Fish-Culturist. 14: 43 - 49.

Snieszko, S. F., and S. B. Friddle. 1948. A contribution to the etiology of ulcer disease of trout. Transactions of the American Fisheries Society. 78: 56 - 63.

Snieszko, S. F., and E. M. Wood. 1955. The effect of some sulfonamides on the growth of brook trout, brown trout, and rainbow trout. Transactions of the American Fisheries Society. 84: 86 - 92.

Snieszko, S. F., P. J. Griffin, and S. B. Friddle. 1950. A new bacterium *(Hemophilus piscium* n. sp.) from ulcer disease of trout. Journal of Bacteriology. 59: 699 - 710.

Sovenyi, J. F., M.Bercsenyi, and J.Bakos. 1988. Comparative examination of susceptibility of two genotypes of carp (*Cyprinus carpio* L.) to infection with *Aeromonas salmonicida*. Aquaculture. 70: 301 - 308.

Starliper, C. E. 2001. The effect of depuration on transmission of *Aeromonas salmonicida* between the bivalve *Amplema plicata* and Arctic char. Journal of Aquatic Animal Health. 13: 56 – 62.

Tajima, K., T. Takahashi, Y. Ezura, and T. Kimura. 1984. Enzymatic properties of the purified extracellular protease of *Aeromonas salmonicida* , Ar-4 (EFDL) Bulletin of the Japanese Society of Scientific Fisheries. 50: 145 - 150.

Takahashi, K., T. Kawana, and T. Nakamura. 1975. Studies on ulcer disease of goldfish – III. Infection experiments. Fish Pathology. 9: 187 – 192.

Teska, J. D. and R. C. Cipriano. 1993. Nonselective nature of Coomassie brilliant blue agar for the presumptive identification of Aeromonas salmonicida in clinical specimens. Diseases of Aquatic Organisms. 16: 239 – 242.

Thornton, J. M., D. A. Austin, B. Austin, and R. Powell. 1999. Small subunit rRNA gene sequences of *Aeromonas salmonicida* subsp. *smithia* and *Hemophilus piscium* reveal pronounced similarities to A. salmonicida subsp. salmonicida. Diseases of Aquatic Organisms. 35: 155 – 158.

Treasurer, J. W. and L. A. Laidler. 1994. *Aeromonas salmonicida* infection in wrasse (Labridae) used as cleaner fish on an Atlantic salmon, *Salmo salar* L., farm. Journal of Fish Diseases. 17: 155 – 161.

Trust, T. J. A. G. Khouri, R. A. Austen, and L. D. Ashburner. 1980. First isolation in Australia of atypical Aeromonas salmonicida. FEMS Microbiology Letters. 9: 39 – 42.

Trust, T.J., W. W. Kay, E. E. Ishiguro, J. T. Buckley, and T. W. Pearson. 1981. Properties of a protein, a virulence factor on the surface of *Aeromonas salmonicida*. "Conference on Immunology and Immunization of Fish," van Muiswinkel, W.B.;Cooper, E.L. (eds.) Wageningen (Netherlands), 22 - 24 Jun 1981. Developmental and Comparative Immunology. Suppl. 2: 175 - 180.

Tuffery, G., and C. Dehand. 1979. Experimental study of the conditions for isolation of the agent of carp erythrodermatitis. Bull. Fr. Piscic. 275: 83 - 89.

Udey, L. R. 1978. An additional cell wall layer associated with virulence of the fish pathogen, *Aeromonas salmonicida*. Abstracts of the Annual Meeting of the American Society for Microbiology. 78. (Abstract B-66)

Umelo, E. and T. J. Trust. 1998. Physical map of the chromosome of *Aeromonas salmonicida* and genomic comparison between *Aeromonas* strains. Microbiology. 144: 2141 – 2149.

USFWS. 1995. U.S. Fish and Wildlife Service, Fisheries: Fish Health Operations. U. S. Department of the Interior. Washington DC. FWM 170, Part 713 FW, pp. 1 - 4.

Wedemeyer, G. A., and N. C. Nelson. 1977. Survival of two bacterial fish pathogens *(Aeromonas salmonicida* and the enteric redmouth bacterium) in ozonated, chlorinated, and untreated water. Journal of the Fisheries Research Board of Canada. 34: 429 - 432.

Wedemeyer, G., A. J. Ross, and L. Smith. 1969. Some metabolic effects of bacterial endotoxins in salmonid fishes. Journal of the Fisheries Research Board of Canada. 26: 115 - 122.

Whittington, R. J., N. Gudkovs, M. J. Carrigan, L. D. Ashburner, and S. J. Thurstan. 1987. Clinical, microbiological and epidemiological findings in recent outbreaks of goldfish ulcer disease due to atypical *Aeromonas salmonicida* in southeastern Australia. Journal of Fish Diseases.10: 353 - 362.

Wiegertjes, G. F., J. G. Daly, and W. B. van Muiswinkel. 1993. Disease resistance of carp, *Cyprinus carpio* L.: identification of individual genetic differences by bath challenge with atypical *Aeromonas salmonicida*. Journal of Fish Diseases. 16: 569 - 576.

Wiklund, T. 1995. Virulence of 'atypical' *Aeromonas salmonicida* isolated from ulcerated flounder *Platichthys flesus*. Diseases of Aquatic Organisms. 21: 145 - 150.

Wiklund, T. and I. Dalsgaard. 1998. Occurrence and significance of atypical *Aeromonas salmonicida* in non-salmonid and salmonid fish species: a review. Diseases of Aquatic Organisms. 32: 49 - 69.

Wiklund, T., I. Dalsgaard, E. Eerola, and G. Olivier. 1994. Characteristis of 'atypical' cytochrome oxidase-negative *Aeromonas salmonicida* isolated from ulcerated flounders (*Platichthys flesus*L.). Journal of Applied Bacteriology. 76: 511 – 520.

Wolf, L. E. 1938. Observations on ulcer disease of trout. Transactions of the American Fisheries Society. 68: 136 - 151.

Wolf, L. E. 1953. Development of disease-resistant strains of fish. Transactions of the American Fisheries Society. 83: 342 - 349.

Wooster, G. A.and P. R. Bowser. 1996. The aerobiological pathway of a fish pathogen: survival and dissemination of *Aeromonas salmonicida* in aerosols and its implications in fish health management. Journal of the World Aquaculture Society. 27: 7 - 14.

Yamada, Y., Y. Kaku, and H. Wakabayashi. 2000. Phylogenetic intra-relationships of atypical Aeromonas salmonicida isolated in Japan as determined by 16S rDNA sequencing. Gyobyo Kenkyu/Fish Pathology. 35: 35 – 40.