1.2.14 Photobacteriosis

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A. Name of Disease and Etiological Agent

Photobacteriosis refers to two distinct disease syndromes affecting a variety of marine species and is caused by two different subspecies of the gram negative bacterium *Photobacterium damselae*. The two subspecies are different in biochemical phenotype and clinical signs of diseases they cause. *Photobacterium damselae* subsp. *piscicida*, (formerly known as "*Pasteurella piscicida"*) is the causative agent of photobacteriosis or the previously recognized disease name "pasteurellosis" of marine fish. *Photobacterium damselae* subsp. *damselae* (formerly *Vibrio damsela*) is the causative agent of "Vibrio damsela infection", a hemorrhagic septicemia with accompanying skin lesions in marine fish.

B. Known Geographical Range and Host Species of the Disease

1. Geographical Range

Photobacterium damselae subsp. *piscicida* has been isolated from wild and cultured fish obtained from the Atlantic Coast and Gulf Coast of the United States, Japan, Taiwan, and the Mediterranean. *Photobacterium damselae* subsp. *damselae* has been isolated from wild and cultured fish from Japan, Taiwan, the Mediterranean, and the Pacific and Gulf Coasts of the United States.

2. Host Species

Photobacterium damselae subsp. *piscicida* has been reported in wild white perch *Morone americanus,* wild and cultured striped bass *Morone saxatilis* and cultured hybrid striped bass *Morone saxatilis* x *M. chrysops* in the U.S. (Snieszko et al. 1964; Robohm 1983; Hawke et al. 1987; Hawke et al. 2003). The bacterium has remained an important pathogen of cultured yellowtail (*Seriola quinqueradiata*) in Japan since the late 1960's (Kubota et al. 1970, Egusa 1980). Other affected species of commercial importance in Japan are; the ayu *Plecoglossus altevelis*; the black seabream *Mylio macrocephalus*; the red seabream *Pagrus major*; the oval filefish *Navodan modestus*; the redspotted grouper *Epinephelus okaara*; the yatabe blenny *Pictiblennius yatabei* and the striped jack *Pseudocaranx dentex*. The disease has recently emerged as a serious pathogen of cultured fish in Europe and the Mediterranean having been documented from cultured seabream *Sparus aurata* in Spain (Toranzo et al. 1991), Portugal (Baptista et al. 1996), Malta (Bakopoulos et al. 1997), and Italy (Magariňos et al. 1992) and from cultured sea bass *Dicentrarchus labrax* in France (Magarinos et al. 1992), Turkey (Candan et al. 1996), and Greece (Bakopoulos et al. 1995). Photobacteriosis has caused serious economic losses in hybrid striped bass reared in ponds in Israel (Nitzan et al. 2001). The importance of *P.*

damselae subsp. *piscicida* as a pathogen of wild and farmed fish in Japan and in Europe has been established in several reviews (Magariños 1996; Austin and Austin 1993; Plumb and Hanson 2007). A case has been reported from the snakehead *Channa maculata*, a freshwater fish reared in Taiwan, which had been fed contaminated marine fish products (Tung et al. 1985). Most recently, the disease has been reported from cultured cobia *Rachycentron canadum* (Lopez et al. 2002) and paradise fish *Macropodus opercularis* (Liu et al. 2011) in Taiwan.

Photobacterium damselae subsp. *damselae* is pathogenic for a wide variety of aquatic animals such as fish, crustaceans, mollusks, and cetaceans. The organism is also a human pathogen causing necrotizing fasciitis, and is considered a zoonotic agent. Specific fish hosts are the blacksmith damselfish *Chromis punctipinnis* (Love et al. 1981), various species of sharks (Grimes et al. 1984, Grimes et al. 1985, Han et al. 2009), yellowtail *Seriola quinqueradiata* (Sakata et al. 1989), seabream *S. aurata* (Vera et al. 1991), European seabass *Dicentrarchus labrax* (Botella et al. 2002), turbot *Scophthalmus maximus* (Fouz et al. 1992), barramundi *Lates calcarifer* in Australia (Renault et al. 1994) and rainbow trout *Oncorhynchus mykiss* (Pedersen et al. 2009). The bacterium has also been isolated from a variety of newly cultured marine fish species in Spain *Pagrus auriga, Pagrus pagrus, Diplodus sargus, Argyrosomus regius* (Labella et al. 2011). In addition to fish, *P. damselae* subsp. *damselae* has been isolated from diseased shrimp *Penaeus monodon* (Wang and Chen 2006), octopus *Octopus joubini* (Hanlon et al. 1984), turtles *Dermochelys coriacea* (Obendorf et al. 1987), dolphins *Tursiops truncatus* (Fujioka et al. 1988) and wound infections in humans (Love et al. 1981; Clarridge et al. 1985).

C. Epizootiology

Photobacterium damselae* subsp. *piscicida. The causative agent of the disease previously known as "fish pasteurellosis", was originally described following a massive fish kill on Chesapeake Bay, USA, which destroyed approximately 50% of the natural populations of white perch *M. americanus* and striped bass *M. saxatilis* (Snieszko et al.1964). An isolate from this outbreak was deposited in the American Type Culture Collection, Manassas, Virginia (ATCC 17911). Based on a variety of physical and biochemical characteristics, the bacterium was tentatively placed in the genus *Pasteurella*. The pathogen was studied morphologically, physiologically and serologically by Janssen and Surgalla (1968), who concluded that the bacterium was a new species and proposed the name *Pasteurella piscicida*. The name was never given validity by bacterial taxonomists due to physiological inconsistencies with those described for the genus *Pasteurella*, such as: lack of nitrate reductase, tolerance of pH values outside the normal range, halophilia, lower optimum growth temperature and unusual host range. The bacterium was not included in Bergey's Manual of Systematic Bacteriology (Mannheim 1984) or the "Approved List of Bacterial Names" (Skerman et al. 1989). Nevertheless, the name was used in the literature until 1995 when the organism was formally renamed *Photobacterium damsela* subsp. *piscicida* based on 16S ribosomal RNA sequences (Gauthier et al. 1995). The name was later corrected to *P. damselae* subsp. *piscicida* (Truper and DeClari 1997). Subsequent to the original outbreak, the bacterium was found to be responsible for smaller fish kills involving natural populations of striped bass in Chesapeake Bay (Paperna and Zwerner 1976) and in western Long Island Sound (Robohm 1983). The disease was first documented from cultured fish in the U.S. in striped bass reared in earthen brackish water ponds on the Alabama Gulf Coast at the Claude Peteet Mariculture Center, Alabama Marine Resources Division (Hawke et al. 1987) and later from hybrid striped bass cultured in net pens located in shallow lakes in the Louisiana Coastal marshes (Hawke et al. 2003).

Outbreaks of photobacteriosis may occur in the temperature range of 14-29°C and at salinities of 3-21 ppt however the optimum range for acute disease is 18-25°C and 5-15 ppt. *Photobacterium damselae* subsp. *piscicida* is considered an obligate pathogen and its survival is short lived outside the host

even in salt water. In their original descriptive work, the Chesapeake Bay isolate was found to survive for only 3 days in sterile brackish water (Janssen and Surgalla 1968). There is evidence, however, that viable but non-culturable forms may exist for extended periods in both sea water and sediments (Magariños et al. 1993). It has been suggested that another species of fish or invertebrate present in the environment where susceptible species are cultured, may serve as an asymptomatic carrier and/or reservoir of infection (Robohm 1983). Louisiana Gulf Coast isolates of *P. damselae* subsp. *piscicida*, when compared with representative isolates from Chesapeake Bay USA, Greece, Japan, and Israel, were found to be almost identical in biochemical phenotype and enzyme activity however the isolates differed in their plasmid profiles and antimicrobic susceptibilities. Louisiana isolates were found to possess a unique plasmid banding profile when compared to strains from other geographic locations. The Louisiana isolates typically produced two large plasmid bands >30 kb and two smaller bands, 8.0 kb and 5.0 kb in size. Isolates from Israel and Greece exhibited bands corresponding to 10 and 8 kb and Japanese isolates possessed plasmids of 5 and 3.5 kb. (Hawke et al. 1996). Resistance to Romet® and/or Terramycin® by some Louisiana strains was the result of acquisition of an R-plasmid (Hawke et al. 2003, Kim et al. 2008). The Louisiana Gulf Coast strains, when analyzed by random amplified polymorphic DNA (RAPD) analysis, were found to belong to clonal lineage group 2, displaying a fingerprint similar to Japanese strains (Hawke et al. 2003; Magariños et al. 2000).

***Photobacterium damselae* subsp. *damselae*.** The etiologic agent of a disease causing cause skin ulcers in damsel fish *Chromis punctipinnis* inhabiting the coastal waters of southern California and wound infections in humans was originally named *Vibrio damsela* (Love at al. 1981). The organism has gone through a number of name changes over the years including: *Listonella damsela* (MacDonell and Colwell 1985), *Photobacterium damsela* (Smith et al. 1991), *P. damsela* subsp. *damsela* Gauthier et al. 1995 and finally *P. damselae* subsp. *damselae* (Truper and De'Clari 1997).

Photobacterium damselae subsp. *damselae* is a normal inhabitant of seawater and marine sediments and prefers warm water conditions (20°-30°C). Disease outbreaks have occurred on fish farms in earthen ponds, net pens and sea cages as well as indoor tanks and aquaria. The organism is transmitted through the water and highly susceptible species may be infected by this route. Less susceptible fish may require stress or injury as predisposing factors. Some authors anticipate disease problems caused by this organism to increase in light of global climate changes (Pedersen et al. 2009). On Danish mariculture farms where rainbow trout are cultured in seawater, the bacterium was found to cause disease at higher water temperatures. In laboratory trials it was 1000 times more virulent at 20°C than at 13°C in trout. In turbot, spikes in mortality may occur when water temperatures are increasing from 18°C to 25°C (Fouz et al. 1992). Mortality rates during different outbreaks on Spanish fish farms ranged from 22% in December to 94% in August. Strains of *P. damselae* subsp. *damselae* show a high level of heterogeneity in biochemical phenotype and serological characteristics (Smith et al. 1991; Fouz et al. 1992; Pedersen et al. 2009). Genetic variation, as determined by amplified fragment length polymorphism (AFLP), is also great with 24 of 33 strains collected from European seabass and gilthead seabream showing different banding patterns. PCR-based typing methods confirm high variability within the subspecies and a relationship between the geographic origin of the isolate or the host fish species cannot be established (Botella et al. 2002).

Extracellular products (ECPs) from *P. damselae* subsp. *damselae* have been shown to display cytotoxic activity for different fish and mammalian cell lines. Only virulent strains produce toxic ECPs and the cytotoxic components are thermolabile (Labella 2010). The primary virulence factor has traditionally been listed as damselysin, which has been characterized as a phospholipase D, however other phospholipases may participate in virulence since some virulent strains lack the *dly* gene for phospholipase D but still show phospholipase activity in their ECPs (Osorio et al. 2000).

D. Disease signs

1. Behavioral Signs

Clinical signs in fish with photobacteriosis vary greatly depending on the subspecies involved. Fish infected with *Photobacterium damselae* subsp. *piscicida* suffer from an acute septicemia, are lethargic, swim slowly near the surface and ultimately sink and rest on the bottom prior to death. Increased ventilation rates and loss of equilibrium may also be evident. Behavioral signs have not been adequately described for fish infected with *P. damselae* subsp. *damselae* in most species. In moribund Australian snapper *Pagrus auratus* diseased fish float on the surface in lateral recumbency for several hours prior to death (Stephens et al. 2006).

2. Gross Signs

Photobacteriosis caused by *P. damselae* subsp. *piscicida* is characterized as an acute bacterial septicemia. The disease rarely takes on a chronic form when fish are outside the optimal temperature range or when antibiotic intervention has occurred (Thune 1993). In the acute form, very little gross pathology is observed regardless of the species affected (Bullock 1978, Toranzo 1991). Affected striped bass and hybrid striped bass appear normal with the exception of pallor of the gills, petechae in the opercular region, and darker than normal pigmentation. Internally, an enlarged and friable spleen provides the only clearly visible gross clinical sign (Figure 1). Other organs and physical features appear normal with the exception of the liver which may be slightly mottled and the kidney which may be hemorrhagic. Yellowtail show evidence of edema and failure to regulate pigmentation. White perch show only slight hemorrhage of the operculum and base of fins. Diseased gilthead seabream exhibit no apparent external clinical signs except rare individuals that display hemorrhage around the head and opercular region. The chronic form of the disease may vary depending on the species affected, the temperature range and whether or not antibiotic feeds have been administered. In striped bass and white perch, small white “miliary” lesions may be seen in the swollen spleen and kidney (Bullock 1978; Wolke 1975). Similar lesions may be visible in cultured hybrid striped bass following antibiotic therapy (Figure 2). In yellowtail, chronic lesions are typified by1-2 mm granuloma-like lesions that are composed of masses of the causal bacterium, epithelial cells and fibroblasts. The lesions grossly resemble granulomas and result in the disease often being referred to as “pseudotuberculosis” in Japanese yellowtail (Kubota et al. 1970).

Fish with *P. damselae* subsp. *damselae* show evidence of a chronic disease characterized by external skin ulceration that may progress to a hemorrhagic septicemia. Fish affected by systemic infection show a fatty liver often with petechiae, abdominal swelling from ascites and splenomegaly (LaBella et al. 2011).

3. Microscopic Signs

The histopathology of photobacteriosis in naturally infected white perch and striped bass from Chesapeake Bay was first reported by Wolke (1975). He described in what was apparently a more chronic form of the disease, collections of necrotic lymphoid and peripheral blood cells in the spleen, focal areas of hepatocytes undergoing coagulation necrosis and a conspicuous lack of inflammatory cell responses. In the acute form of the disease in striped bass and hybrid striped bass there is acute multifocal necrosis of the lymphoid tissue of the spleen characterized by loss of cells, coagulation necrosis, karyorrhexis, and large colonies of the causal bacterium (Figure 3). In the liver, acute multifocal necrosis with prominent karyorrhexis is common. Inflammatory cellular accumulations are absent (Hawke et al. 1987). Similar microscopic lesions were reported in gilthead seabream (Toranzo et al. 1991). In acute disease, bacterial numbers in the blood of hybrid striped bass approach 1 million bacteria per ml of blood (Figure 4). The response in yellowtail is somewhat different with bacterial colonies forming in the spleen surrounded by inflammatory cells. Granuloma-like structures “pseudotubercles” form in the spleen containing

eosinophils and surrounded by epithelial cells. In all species, masses of bacteria (bacterial emboli) form in the capillaries blocking blood flow in interstitial spaces of the internal organs. In experimentally infected hybrid striped bass, death was attributed to bacterial emboli in the gill capillaries and asphyxiation due to inability to achieve blood flow and gas exchange (Hawke 1996).

Histopathological descriptions of *P. damselae* subsp. *damselae* are rare in the literature. Skin infections are similar to those caused caused by *Vibrio* spp and probably are secondary to skin abrasion or environmental stress (Figure 5). Skin lesions are shallow with exposed muscle with infiltrates of inflammatory cells. Internally necrotic lesions may occur in the liver, spleen and kidney with accompanying granulomatous inflammation. These infections may be indistinguishable from other infections caused by opportunistic bacteria. Extracellular products have been demonstrated to be more important with *P. damselae* subsp. *damselae* than *P. damselae* subsp. *piscicida* which seems to overwhelm the fish with sheer numbers. The intraperitoneal inoculation of ECPs from *P. damselae* subsp. *damselae* into redbanded seabream resulted in mortality 2-4 hr post inoculation.(LaBella et al. 2011). The main virulence factor of P. *damselae* subsp. *damselae* is “damselyn”, a thermolabile extracellular cytotoxin of 69 kDa.

Figure 1: Hybrid striped bass, collected in a moribund state, with acute photobacteriosis caused by *Photobaterium damselae* subsp. *piscicida*. The spleen is swollen and friable and lacks visible signs of necrosis (arrow), otherwise clinical signs are minimal. Photo by Dr. Joe Newton.

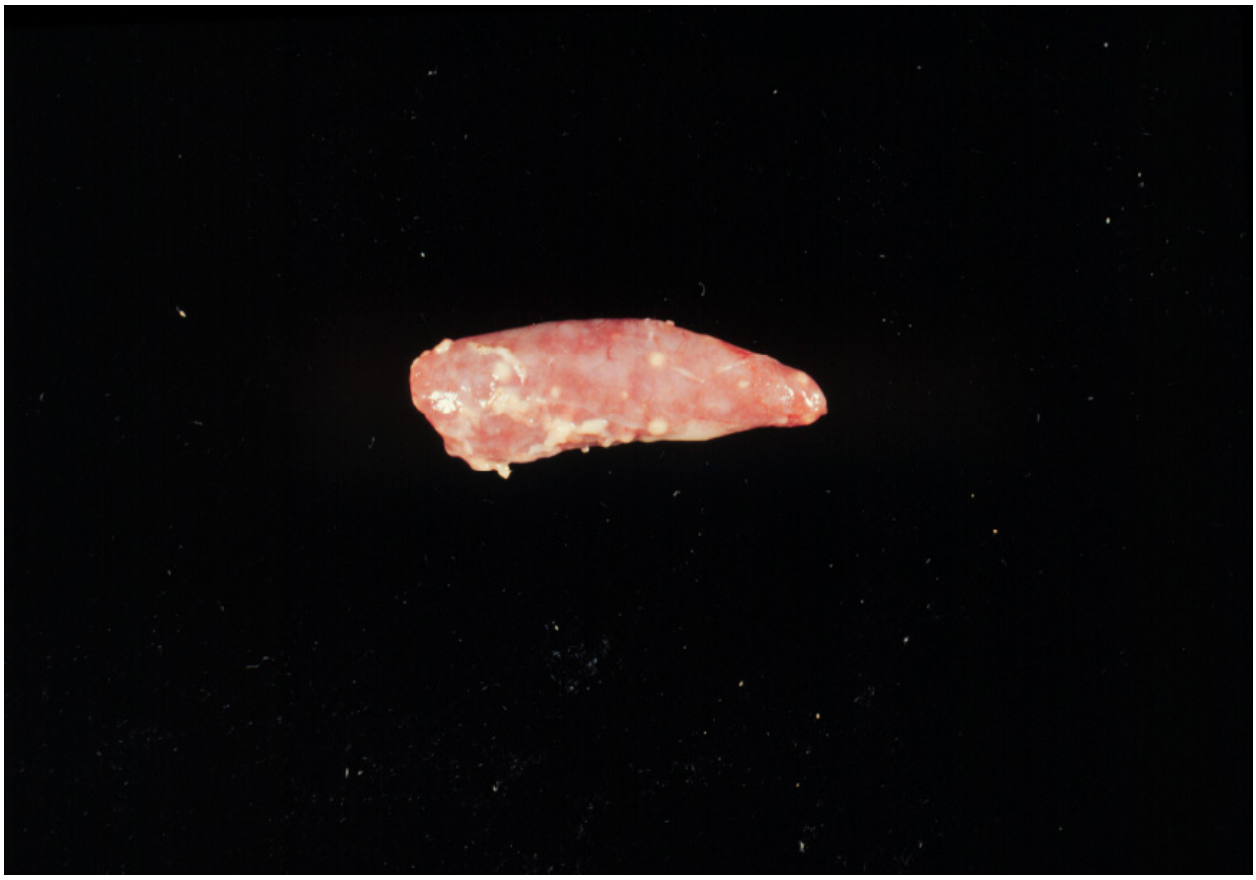

Figure 2: Chronic infection caused by *Photobacterium damselae* subsp. *piscicida* characterized by necrotic foci, bacterial colonies and granuloma-like lesions in the splenic parenchyma visible as white “miliary” lesions. Photo by Dr. Al Camus

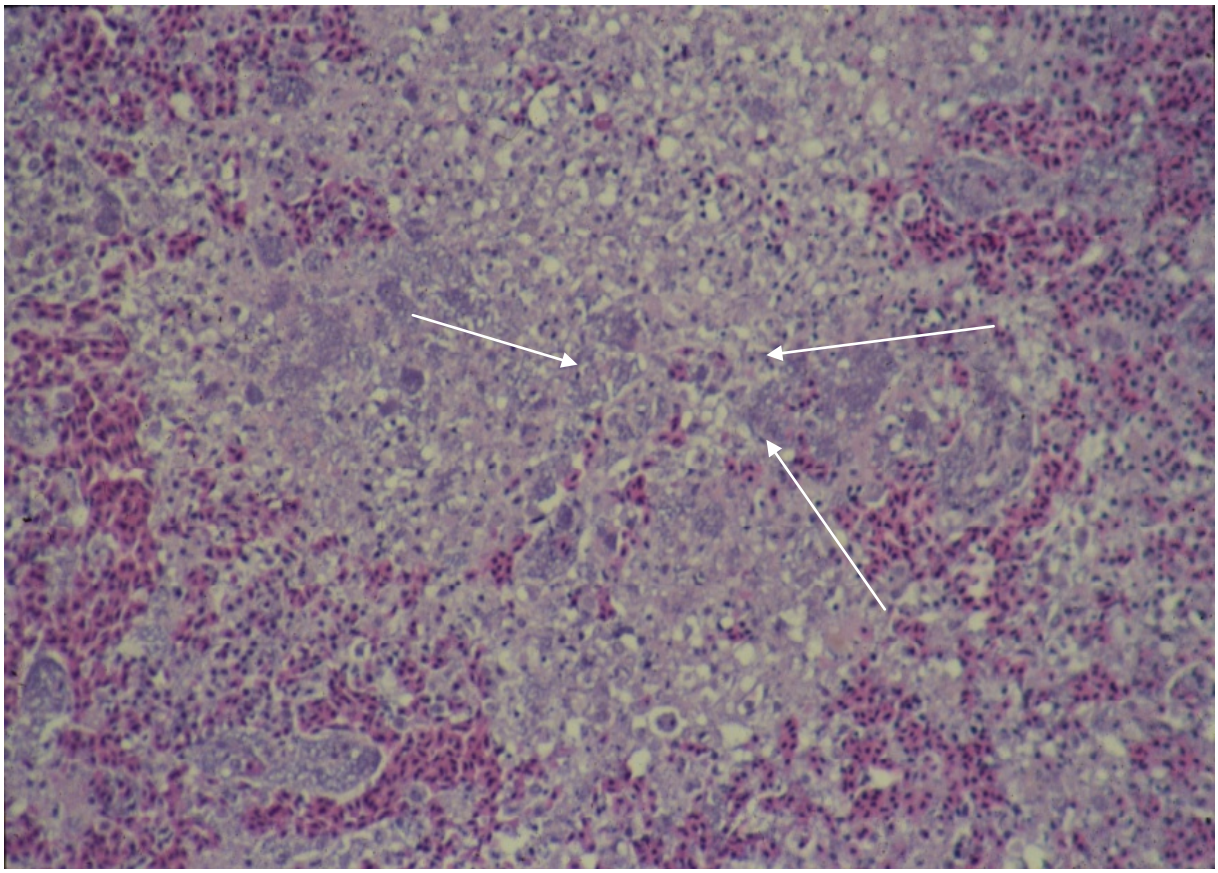

Figure 3: Focal necrosis in the spleen associated with numerous colonies of *Photobacterium damselae* subsp. *piscicida* (arrows). Inflammatory cell accumulations are lacking. Photo by Dr. John Hawke.

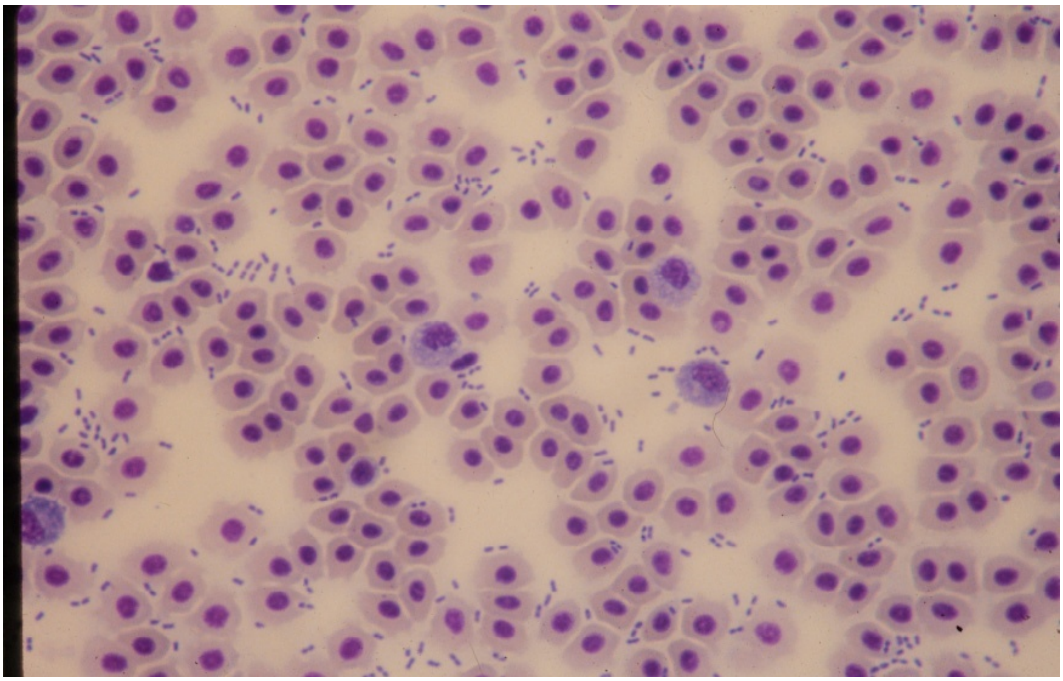

Figure 4. Blood smear from a moribund hybrid striped bass with approximately $1x10^6$ cfu/ml of *Photobacterium damselae* subsp. *piscicida* in the blood. Photo by Dr. John Hawke.

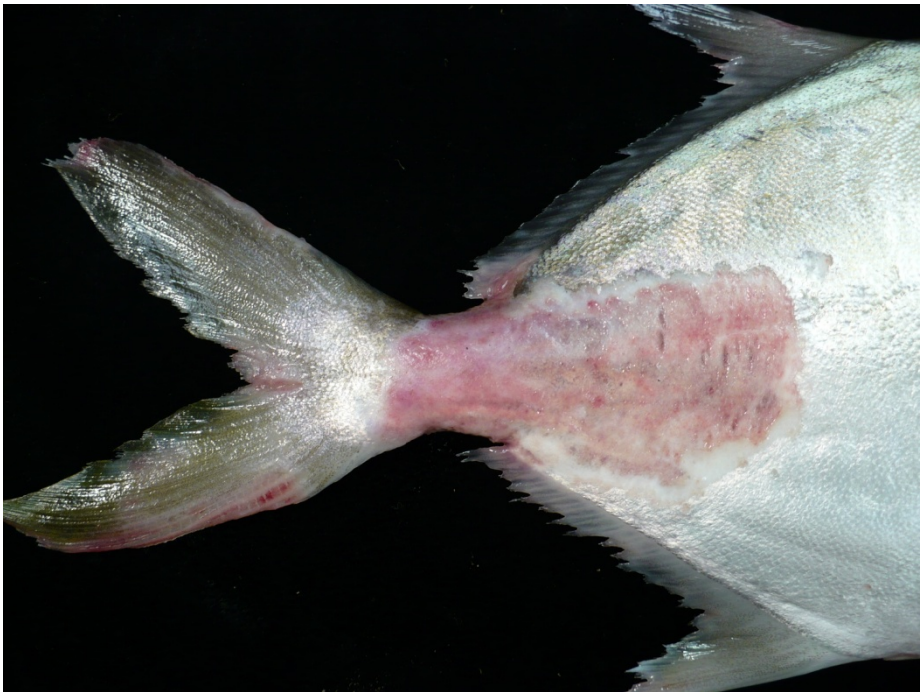

Figure 5. Skin lesion caused by *Photobacterium damselae* subsp. *damselae* on caudal peduncle of pompano. Photo by Dr. John Hawke.

D. Disease Diagnostic Procedures

Photobacterium damselae subsp. *piscicida* and subsp. *damselae* may be isolated from the liver, spleen, kidney or brain by streaking on on tryptic soy agar (TSA) with 5% sheep blood, brain heart infusion (BHI) with 2% NaCl or TSA with 2% NaCl. Incubation at 28°C for 48 hrs is necessary to obtain 1-2 mm isolated colonies of *P. damselae* subsp. *piscicida*. *Photobacterium damselae* subsp. *damselae* is faster growing and 1-2 mm colonies are visible after 24 hrs incubation. Biochemical tests may be performed in standard media however addition of 1-2% NaCl to the media may enhance growth.

1. **Presumptive Diagnosis**
   a. **Culture, Staining and Biochemical Phenotype.**
   Photobacterium damselae subsp. *piscicida*: Colonies of the bacterium are 1-2 mm, smooth, and non-hemolytic on blood agar at 48 hrs post inoculation. For presumptive diagnosis, the bacterium should be shown to be an oxidase positive (allow 60 seconds for color development), gram negative rod that exhibits pleomorphism and bipolar staining by Gram's or Giemsa staining. The bacterium is a non-motile facultative anaerobe that produces acid but no gas from glucose, mannose, maltose, fructose and galactose. In the majority of biochemical tests the organism is non-reactive but gives enough positive results to generate a unique code number in the API 20E system (bioMèrieux, Durham,NC) of 2005004. The bacterium is sensitive to vibriostatic agent (0/129) and fails to grow on thiosulfate citrate bile sucrose TCBS agar.

   Photobacterium damselae subsp. *damselae*: Colonies of the bacterium are 1-2 mm, smooth, and hemolytic on blood agar at 24 hrs post inoculation. Phenotypically the bacterium resembles its close neighbor *Vibrio* more than *P. damselae* subsp. *piscicida*. For presumptive diagnosis, the bacterium should be shown to be an oxidase positive, motile, gram negative rod; a facultative anaerobe that produces acid but no gas from glucose, mannose, and maltose; and is positive for arginine dihydrolase, Voges-Proskauer (VP), and urease. In the API 20E system (bioMèrieux, Durham,NC) a unique code number of 6015004 is generated. The bacterium is sensitive to vibriostatic agent (0/129) and grows on thiosulfate citrate bile sucrose TCBS agar producing a green colony.

   b. **Histology**
   The presence of lesions described in this chapter in gills, liver, and spleen, along with the overall absence of gross clinical signs is typical of photobacteriosis but could also be confused with other forms of bacterial septicemia and the diagnosis should be confirmed by bacteriology, serology and or PCR.

2. **Confirmatory Diagnosis**
   a. **Confirming a diagnosis made by bacteriology and biochemical phenotype (16S rRNA sequencing).** Universal primers to 16S rRNA can be utilized to generate PCR products. The products (complete or partial sequences) may be confirmed as *Photobacterium damselae* by sequencing the PCR products and comparing them to the sequences deposited in GenBank by NCBI BLAST analysis (www.ncbi.nlm.nih.gov/Blast). Note: This method can allow for identification of the species *Photobacterium damselae* but cannot differentiate between the two subspecies. Alternatively, novel sequences can be directly compared to GenBank reference sequence NR_040831.

   b. **Confirming a diagnosis by species-specific PCR and culture on TCBS agar.**
   A simple and rapid method of identification and differentiation between the subspecies of *P. damselae* is done by using primers designed to amplify the capsular polysaccharide gene. A pair of primers was selected to amplify a 410-bp fragment of capsular polysaccharide gene

derived from *P. damselae* subsp. *piscicida* (GenBank accession number AB074290). The forward primer, CPSF, is (5`-AGGGGATCCGATTATTACTG-3`) corresponding to positions 531–550 of the *P. damselae* subsp. *piscicida* gene for capsular polysaccharide and the reverse primer, CPSR, is (5`-TCCCATTGAGAAGATTTGAT-3`) corresponding to positions 921–940. Since both subspecies of *P. damselae* are detected using this PCR the subspecies must be differentiated by growth on thiosulfate citrate bile sucrose TCBS agar. *P. damselae* subsp. *damselae* grows on TCBS producing a green colony and *P. damselae* subsp. *piscicida* fails to grow (Rajan et al. 2003).

c. Multiplex PCR for identification and differentiation between subspecies of *Photobacterium damselae*. A multiplex-PCR approach, employing 2 primer pairs directed to internal regions of the 16S rRNA and *ureC* genes, has been utilized to identify and discriminate between subspecies of *Photobacterium damselae* (Osorio et al. 2000). With this procedure, *P. damselae* subsp. *damselae* strains yield 2 amplification products, one of 267 bp and the other of 448 bp, corresponding to internal fragments of the 16S rRNA and *ureC* genes, respectively. However, *P. damselae* subsp. *piscicida* isolates only show the PCR product of 267 bp (16S rRNA fragment), indicating the absence of the urease gene in its genome. Nucleotide sequence of partial *ureC* gene from *Photobacterium damselae* subsp. *damselae* was retrieved from GenBank database with accession number U40071. A forward primer, Ure-5' (20-mer 5'-TCCGGAATAGGTAAAGCGGG-3'), and a reverse primer, Ure-3' (22-mer 5'-CTTGAATATCCATCTCATCTGC-3'**),** were designed flanking a 448 bp-long stretch of the *ureC* gene. A forward primer, 118-mer 5'-GCTTGAAGAGATTCGAGT- 3') (positions 1016 to 1033 in *Escherichia coli* 16s rRNA gene), and a reverse primer, (18-mer 5'-CACCTCGCGGTCTTGCTG-3') (positions 1266 to 1283), flanking a 267 bp fragment of the 16s gene of strain ATCC 29690 of *Photobacterium damselae* subsp. *piscicida* (GenBank accession number Y18496) are used in conjunction with Ure-5' and Ure-3' in a multiplex PCR reaction (Osorio et al. 1999).

E. Procedures for Detecting Subclinical Infections

Subclinical infections *Photobacterium damselae* subsp. *piscicida* are detected in healthy fish by use of the Bionor Aquarapid Pp test kit. This test kit utilizes an antibody specific for *Photobacterium damselae* subsp. *piscicida* antigen in an enzyme-linked immunosorbent assay format. Bionor A/S Skein, Norway (Romalde et al. 1995a and 1995b). There are no methods for detecting subclinical infections of *Photobacterium damselae* subsp. *damselae* other than the above mentioned PCR tests.

F. Procedures for Detecting Prior Exposure

There are no published serological methods for detecting antibodies against *Photobacterium damselae* subsp. *piscicida or* subsp. *damselae*

G. Procedures for Transportation and Storage of Samples

Samples for histology should be collected and fixed according to standard methods. Tissues for PCR may be stored in 95% ethanol or frozen for later DNA isolation.

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