Report of the NJDEP Science Advisory Board

Re-use and Disposal of Hatchery Fish Diseased by Furunculosis

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Ecological Processes Standing Committee Report

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I. Introduction

The Pequest Trout Hatchery experienced an outbreak in the bacterium, *Aeromonas salmonicida*, in September of 2013. The outbreak manifested itself as a disease called furunculosis that spread and caused chronic mortality throughout the hatchery. Since furunculosis is easily spread through the water to other fish and asymptomatic fish can be carriers, a decision was made not to stock the affected surviving hatchery fish but to eradicate the diseased fish from the hatchery by euthanizing them and treating unaffected fish with antibiotics. Eradication of diseased fish resulted in the death of 229,000 trout and the need to dispose of approximately 14 tons of diseased fish carcasses. The carcasses were buried at the Warren County landfill (http://www.nj.com/warrenreporter/index.ssf/2014/03/trout.html). This event generated concern about how best to dispose of diseased fish in the future and led to the charge posed to the SAB Ecological Processes Standing Committee:

> *Provide options for beneficial reuse of hatchery fish that cannot be used for stocking. What is the risk to humans of the bacterium that causes furunculosis getting into the food chain for these options?*

The committee met and discussed the charge and their own concerns pertaining to diseased fish at NJ hatcheries. Safe disposal as well as public perception of what constitutes safe disposal was discussed. The committee decided to investigate standard methods for disposal of diseased fish and transmission of *A. salmonicida* to other bioreceptors including humans. In addition, the committee decided to enlarge the scope of the charge and consider ways to reduce the likelihood of a furunculosis outbreak at NJ hatcheries in the future. Prevention is the best way to eliminate the need for disposing of dead fish and possible stocking of diseased carrier fish. While Pequest Hatchery has taken increased biosecurity measures and is working to eradicate the bacterium, it is unlikely that future outbreaks can be completely avoided. Furunculosis is endemic in NJ waters as well as those of surrounding states and can be carried by fish and other wildlife that may or may not be symptomatic. Visiting birds likely introduced the disease that led to the outbreak at Pequest Hatchery and preventing this type of encounter in the open raceways of the hatchery is challenging. The Pequest Hatchery is investigating options for covering the raceways to improve biosecurity and eliminate this bird threat.

The report is broken down into the following sections:

II. Overview of Furunculosis and *A. salmonicida*
III. Fish to Fish Transmission of *A. salmonicida* and Disease Resistance
IV. Transmission of *A. salmonicida* to Organisms Other than Fish
V. Disposal Alternatives
VI. Committee Recommendations
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II. Overview of Furunculosis and *A. salmonicida*

Etiology of the disease

Furunculosis is a historically important fish disease; its causative pathogen *A. salmonicida* was the first fish bacterial pathogen to be identified and studied (Post 1987; Menanteau-Ledouble et al. 2016). *Aeromonas salmonicida* is a Gram-negative bacterium endemic to fresh and saltwater habitats in North America, Europe, and Japan (McCarthy and Roberts 1980). The bacterium is non-spore forming and a capsule is atypical (Post 1987). The species can survive without its host for up to three weeks in water and six months in sediments (Munro and Hastings 1993).

Infection of fish is likely through the gills or skin (Iwama and Nakanishi 1996). Four subspecies of *A. salmonicida* occur and the disease may manifest in three different characteristic forms associated with these subspecies. The peracute form of the disease occurs predominately in juveniles and is characterized by darkened integument followed by rapid death. The acute variant of the disease (furunculosis) is systemic and is associated with the "typical" subspecies *salmonicida*. This form debilitates and kills the fish quickly by bacterial hemorrhagic septicemia, rapidly producing high mortality in the population. Lesions are rarely found in acutely infected individuals (Iwama and Nakanishi 1996, Noga 1996). Fish chronically infected with "atypical" subspecies such as *achromogenes*, *masoucida*, and *smithia* produce ulcerated lesions (furuncles) in the body musculature (Iwama and Nakanishi 1996). Most major organ systems are affected and the fish will typically become anemic. Although prolific rapid mortality is not typical of this form, sublethal effects do reduce the overall condition of the fish. The association between causative bacterial subspecies and characteristic disease forms is not always delineated, as the typical subspecies *salmonicida* has been isolated from ulcerated lesions (Noga and Berkhoff 1990), while the atypical subspecies have been observed to cause furunculosis (Munro and Hastings 1993). Other atypical subspecies have recently been identified, and current research questions this system of classification (Cipriano and Austin 2011).

Prevalence in hatcheries and aquaculture

Furunculosis is one of the most important diseases in juvenile and adult salmonids (Evelyn 1996, (Noga 1996). It has also been observed in other species, e.g. cod, carp, koi, American eel, catfish, pike, smallmouth bass, hybrid striped bass, and yellow perch (Sindermann 1989, Noga 1996, Wiklund and Dalsgaard 1998). The most susceptible species appear to be Atlantic salmon and brown trout, while rainbow trout exhibit some resistance (Cipriano 1983, Noga 1996). Fish kept at higher temperatures are more susceptible than those reared at colder temperatures (Noga 1996). Susceptibility and resistance to *A. salmonicida* appear to be heritable traits (Gjedrem et al 1991, Gjedram and Gjocen 1995, Fjalestad et al. 1991). Inherited resistance is maintained throughout the life of the fish (Snieszko et al. 1959); although, individual resistance varies in a population (Fjalestad et al. 1991). Selective breeding has produced resistant populations of brook trout and brown trout (Illyassov 1986, Cipriano et al 2002).

In addition to heritable susceptibility/resistance, physical condition and health of the fish are important parameters in determining likelihood of infection (Iwama and Nakanishi 1996). Disease outbreaks appear more frequently in cultured fish than in wild stocks. Although diseased fish and mortalities are more likely to be observed in cultured populations because the opportunity for encounter is greater, captive fish are also held in higher densities with concomitant stress from overcrowding and increased opportunity for disease transmission (Sindermann 1989, Noga 1996). There is evidence that both aquaculture and wild stocks of salmonids and other fish species harbor latent *A. salmonicida* infections. When fish are subject to environmental stressors (e.g. increase in temperature, transfer between holding facilities), some form of the disease typically manifests (Sindermann 1989, Noga 1996). Smith et al. (1982), studying Atlantic salmon aquaculture stocks, found that all smolts carried *A. salmonicida* latent infection, with subsequent mortality of 50% within two weeks of transfer from the hatchery to a sea cage. Some success has been achieved at preventing the disease with a bacterin vaccine, but side effects are prevalent (Midtlyng 1997a, Koppang et al 2005, Villumsen et al 2015). The vaccine has met limited success in non-salmonids (Bjornsdottir et al. 2005).

III. Fish to Fish Transmission of *A. salmonicida* and Disease Resistance

Mechanisms of transmission between fish

Beneficial reuse of furunculosis contaminated fish must consider the risk of transmitting the disease to other biota including humans. Studies have shown that furunculosis can be transmitted from contaminated to naïve fish through water and feed. One article provided data indicating that humans could transport the bacterium from one hatchery to the next. Details of these studies and mechanisms of fish to fish transmission are described below.

Regional contamination of Norwegian fish farms with *A. salmonicida* presented the opportunity to study the complex etiology of furunculosis. Jarp et al (1993) performed a matched case-control study based on answers to a survey filled out by staff of Atlantic salmon (*Salmo salar L.*) freshwater hatcheries. They collected information from a total of 96 Norwegian hatcheries, 30 that had *A. salmonicida* contamination and 66 that did not (controls). Ten factors related to transmission of the disease to fish were considered such as source of water supply, sharing of equipment or personnel with other fish farms and number of fish farms within 10 km radius. Of those factors, three stood out as associated with increased risk. The most risk came from the water supply (11.5x higher). Apparently, wild or "freed" fish migrating in water supplied to the hatchery spread the bacteria to farmed fish. The second highest risk was for sites located within 10 km of two other sites containing infected fish (3.2x higher). The third highest risk was for sites sharing personnel with other hatcheries (2.8x higher). This indicated that humans could transfer the disease on their person from one site another. The finding was supported by another study showing transmission of infectious salmon anemia by shared personnel in Norwegian seawater farms (Vagsholm et al. 1991). Overall, the results indicated a multifactorial etiology of the disease implicating water supply as a critical factor and perhaps, surprisingly, transmission of the disease to fish by humans. The results must be viewed as possibly biased as they were generated by a survey.

A laboratory-based study published in 1998 investigated mechanisms of *A. salmonicida* fish to fish transmission in Atlantic salmon (*Salmo salar L.*) (Ferguson et al 1998). The stated aims were "to determine the survival of *A. salmonicida* in freshwater, to assess its ability to colonize different regions of Atlantic salmon parr and to determine the relative importance of these factors in transmission of *A. salmonicida* between fish". They took a unique approach by utilizing a genetically engineered strain of *A. salmonicida* (MT463 *lux*AB) that contained a luciferase gene. This gene can be induced to produce light allowing visualization of the bacteria's location on fish tissues and detection in water. Fish were challenged with *A. salmonicida* through intraperitoneal (I.P.) injection or co-habitation with infected fish. Results showed that maximum mortality occurred within 6 days for fish receiving I.P. injections. Uninfected fish held in the same tank began to die within 12 days. This indicated a 5-day incubation period between infection and disease manifestation and showed that the disease could spread from the injected fish to naïve fish living in the same tank. The naïve fish appeared to contact the bacteria through the water as the *lux*-marker was detected initially in the water column on day 2 and again on day 8. Interestingly, it was not detected in the water thereafter- out to day 23. This indicated that survival of bacterial cells in freshwater was poor. Co-habitation studies also showed that water-borne infection of naïve fish occurred by attachment of bacteria to their gills and skin/mucus but not their gastrointestinal track. Overall, the authors concluded that *A. salmonicida* could spread through freshwater, although poorly, to other fish and infect these fish by adhering to their gills and skin/mucus.

A more recent study investigated fish to fish transmission of *A. salmonicida* while evaluating the effectiveness of vaccination in rainbow trout (*Onchorhychus mykiss*) (Chettri et al, 2015). Fish were vaccinated I.P. with *A. salmonicida* and their immune response was evaluated 3, 4, 5 and 6 months later by reinjecting with *A. salmonicida* or by exposing them to infected fish in freshwater or saltwater. Endpoints for the immune response included expression of inducible genes such as IL1b (detects inflammation), antibody titers, and immunohistochemistry. The effect of saltwater was also investigated by culturing the bacteria in tap water, saline tap water (15 ppt), culture medium and saline culture medium (15 ppt). Results showed infiltration of white bloods cells into the injection site of fish as well as elevated levels of antibodies and IL1b. There was a strong, statistically significant correlation between % survival and antibody titer, showing that the immune response was protective. Saltwater (15 ppt) significantly reduced the growth of *A. salmonicida* as indicated by poor growth in saline tap water and saline culture medium and by low fish mortality in co-habitation experiments when performed in saltwater. The authors concluded that vaccinated fish were better protected from *A. salmonicida* when exposed to the bacteria through water as opposed to I.P. injection. Also, transmission of the disease was reduced when fish were exposed in saline solutions (15 ppt) likely due to poor growth of *A. salmonicida* in salt water. This paper supported the work of Ferguson et al (1998) by demonstrating fish to fish transmission of the disease through water.

While fish to fish transmission of *A. salmonicida* through contaminated water has now been well established (Austin and Austin, 2007), questions remain about other sources of the disease. Kim et al investigated fish feed as a possible factor (2013). Rockfish (*Sebastes schlegeli*) from multiple sea farms in Korea were fed low “commercial” value fish including Konoshiro gizzard shad (*Konosirus punctatus*), big head croaker (*Collichthys niveatus*), Pacific sand eel (*Ammodytes personatus*), and Japanese anchovy (*Engraulis japonicus*). The low value fish were stored frozen and thawed by microwave oven prior to feeding. Bacteria in the rockfish were detected by culturing swabs of kidney, spleen and or gastrointestinal mucus and analyzing the colonies by PCR using *vapA* gene primers. Rockfish chosen for PCR analyses showed signs of furunculosis such as “ulcerated lesions over their body surface, and hemorrhages around the opercula and at the base of the pectoral and pelvic fins” as well as “a swollen anus and hemorrhages in the gastrointestinal tract, with an abnormally induced thick exudate of mucus and cells”. Results showed the presence of an atypical strain of *A. salmonicida* in affected rockfish. This occurred even though the low-value fish comprising their feed did not show signs of infection. Overall, the authors concluded that using low-value fish as feed could transmit *A. salmonicida* to aquaculture fish. The results would not support the reuse of hatchery fish diagnosed with furunculosis as feed; although, it does not eliminate this reuse if they are first sterilized to destroy the bacterium.

Fish resistance to *A. salmonicida*

Infection of the Pequest Hatchery and others by *A. salmonicida* is a constant risk. This disease is endemic in the wild and can be released into the hatchery by visiting wildlife or even shared hatchery personnel as mentioned above. For many decades, aquaculture facilities have explored ways to increase the disease resistance of their fish. This has included studying the immune function of fish, development of more disease resistance fish strains as well as how best to vaccinate fish. Currently, the Pequest Hatchery does not have a vaccination program and depends on "good management practices and biosecurity to avoid the major obligate pathogens" (Jan Lovy, NJDEP-Div. of Fish and Wildlife, personal communication August 2016). A review of the literature on methods for increasing hatchery fish disease resistance follows, including some interesting new findings on probiotics that might be economically feasible for NJ hatcheries.

The high density of fish in aquaculture systems, such as hatcheries, naturally increases the propensity for the spread of disease. Stimulating the immune system as a means of disease prevention has become a normal part of many aquaculture operations. Developing disease resistance has involved intra peritoneal (I.P.) injections of bacterium into the fish, immersion of the fish into a solution containing the vaccine and or dietary supplements. Although these approaches have proven successful for some bacteria, *A. salmonicida* has proven challenging. Studies have shown that neither I.P. injections nor immersion offer sufficient protection or enduring immunity in salmonid species (Gudding and Van Muiswinkel, 2013). The Pequest Hatchery did employ a vaccination program for a single population of brown trout during the furunculosis outbreak (Jan Lovy, personal communication). They boosted the immune system using a dietary supplement (beta-glucans) and immersed fish in an inactivated autogenous vaccine. The procedure appeared effective for 6 months after which the fish were released. No follow up was performed, i.e. recapture and examination of the released brown trout, so there is no means for determining the long-term effectiveness of the vaccination.

Vaccines are an expensive means of biosecurity. These costs might be reduced if only selected fish required vaccination. Research has shown that in most fish species, larvae and fry depend on their immune system for protection against disease and that their innate and adaptive immune systems can be inherited from their mother (Van Muiswinkel and Nakao, 2014). According to Zhang et al (2013), "mothers exposed to particular pathogens will synthesize more immune factors including both adaptive and innate immune components, these factors can then be transferred to offspring, helping them to mount rapid and efficient immune responses when challenged with the same pathogen". Unfortunately, evidence suggests that transfer of maternal antibodies in salmonid fish (such as trout) is insufficient to protect offspring. For example, in rainbow trout (*Oncorhynchus mykiss*), fry from mothers inoculated against infectious hematopoietic necrosis virus (IHNV) were protected from viral challenge out to 25 days but not 40 days (Oshima et al, 1996). In Atlantic salmon (*Salmo salar L.*), brood fish with specific antibodies against *Yersinia ruckeri* did transfer their antibodies to eggs and fry but not at levels that protected offspring from enteric redmouth disease (Lillehaug et al, 1996). This is unfortunate as vaccination of brood stock is one potential way of reducing the economic cost of instituting a vaccination program at hatcheries. The literature found for this report did not include studies on the transfer of maternal immunity against *A. salmonicida*.

An approach for reducing future furunculosis outbreaks at NJ hatcheries is to limit the types of species maintained there. Research has shown that some fish species are more likely to contract furunculosis than others. Testing for disease resistance frequently involves inoculating fish with the bacterium through injection or immersion and then measuring infection rate by subsequently culturing their tissues for the bacterium or performing PCR using bacterium-specific primers. One such comparison study was performed in burbot (*Lota lota)* and rainbow trout (*O. mykiss*). Five pathogens were tested: IHNV, infectious pancreatic necrosis virus, *A. salmonicida, F. psychrophilum* and *R. salmoninarum* (Polinski et al, 2010). Results for *A. salmonicida* showed that both burbot and rainbow trout where susceptible to the disease; however, survival was greater in burbot. Interestingly, surviving rainbow trout became asymptomatic carriers while burbot cleared the bacterium below detectable levels within 17 days post-challenge. Not only did these results show species differences in disease susceptibility to furunculosis, it also cautioned that some species exposed to the disease could be asymptomatic carriers and transmit it to wild fish once released.

Historically, species maintained at the Pequest Hatchery included rainbow trout, brown trout and brook trout. Although rainbow trout are more sensitive than burbot to *A. salmonicida* infection, they have proven more resistant than other salmonid species. Indeed, no rainbow trout were impacted at the Pequest Hatchery during the furunculosis outbreak (Jon Lovy, personal communication February 2017). Differences in species sensitivity were investigated in an article published by Cipriano et al. (1994). Their work was based on an outbreak of furunculosis at the Ed Weed State Fish Hatchery in south Hero, Vermont in 1992. The water supply to the hatchery became contaminated when equipment for UV radiation broken down. The hatchery maintained multiple species including brook trout (*Salvelinus fontinalis*), landlocked Atlantic salmon (*Salmo salar*), lake trout (*Salvelinus namaycush*), rainbow trout (*O. mykiss),* and steelhead (*Oncorhynchus mykiss*). They also had a furunculosis resistant strain of brown trout (*Salmo trutta*) from the New York State Department of Environmental Conservation Hatchery in Rome, New York (Ehlinger, 1977). Their investigations found that the furunculosis outbreak produced high mortality in brook trout, landlocked Atlantic salmon and lake trout but not rainbow trout, steelhead and the resistant strain of brown trout (Cipriano et al, 1994). The differential sensitivity of each species was associated with levels of *A. salmonicida* in mucus on external surfaces of fish such that resistant fish had lower levels of the bacterium in their mucus. Of the fish at the Ed Weed hatchery, rainbow trout and steelhead had the lowest number of infected individuals and the lowest levels of bacterium in their mucus (not detected in steelhead). Earlier research comparing brown trout, brook trout and rainbow trout indicated that this type of resistance was positively correlated with mucus precipitin activity (Cipriano and Heartwell, 1986). Other researchers have found that the high resistance of rainbow trout is associated with a major serum protease inhibitor, alpha 2- macroglobuin (Freedman, 1991).

Maintaining only rainbow trout at NJ hatcheries is one way to reduce the risk of future furunculosis outbreaks. For other species, maintaining stocks of disease resistant strains is a consideration. Research at the New York State Department of Environmental Conservation Rome Hatchery has shown resistant strains of brook trout better survive *A. salmonicidia* challenge experiments and have greater recovery post-stocking than disease susceptible strains (Cipriano et al, 2002). One concern for disease resistant fish is that they may be asymptomatic carriers and reservoirs of infection when released into the hatchery and eventually the wild. The Pequest Hatchery does not currently maintain furunculosis resistant strains of fish (Jan Lovy, personal communication January 2017). Introducing these strains has been discussed but concern remains about introducing carriers to the hatchery now that the disease appears to be eradicated. However, more information has become available in the literature on mechanisms of resistance (Van Muiswinkel and Nakao, 2014), and there may be molecular techniques for identifying genetic markers of resistance in fish that have never been exposed to furunculosis and would not be carriers.

Another current area of investigations on disease prevention is probiotics and immunostimulants. These strategies for fish health management offer an alternative to traditional approaches such as vaccines and antibiotics. The concern with vaccines is that they are pathogen-specific and costly, and the concern with antibiotics is the potential for development of antibiotic resistance. Several reviews on the use of probiotics and immunostimulants in fish are available (Harikrishnan et al, 2011, Newaj-Fyzul et al, 2014, Van Muiswinkel and Nakao, 2014, Banerjee and Ray, in press 2017). In general, immunostimulants include compounds such as β-glucan and plant extracts that are added to fish feed to enhance non-specific innate immunity. Some of these have shown success against *A. salmoncidia* (Pionnier et al, 2013, Thanigaivel et al, 2015) and others have not (Zanuzzo et al, 2015). Probiotics involve incorporating microorganisms, live or killed, into fish feed not only to enhance immunity but also to improve fish digestion, growth and reproduction. An 8-week study in juvenile rainbow trout (*O. mykiss*) investigated the effects of probiotics on general health parameters and measures of innate immune system function (Ramos et al, 2015). Fish were exposed to a diet containing 1) multiple species of bacterium (*Bacillus sp*., *Pedicoccus sp*., *Enterococcus sp*., *Lactobacillus sp*.), 2) a mono-species (*Pediococcus acidilactici*) or 3) an unsupplemented diet. Results showed improved growth rate and feed conversion rate in fish fed probiotics as well as significant increases over control in lysozyme activity. All of this occurred without damaging the morphology of the fish intestine. A closer look at the effects of probiotics on *A. salmonicida* infections showed that probiotic bacteria can colonize the gastrointestinal tract of Atlantic salmon (*S. salar* L.*)* and prevent cellular damage when challenged by *A. salmonicida* (Salinas et al, 2008) and that leukocytes (white blood cells) isolated from gut of rainbow trout (*O. mykiss*) fed a mixture of live bacteria had higher levels of *A. salmonicida* phagocytosis than controls (Balcázar et al, 2006). Some concerns surrounding the use of probiotics include the longevity of the health benefits and the need to use viable versus non-viable cells. Additionally, there are legal concerns about whether probiotics should be considered feed additives or veterinary medicines. To date, only *Pediococcus acidilactici* is authorized as a dietary fish probiotic in the European Union (Ramos et al, 2015).

IV. Transmission of *A. salmonicida* to Organisms Other than Fish

The genus *Aeromonas* contains numerous species of bacteria that are part of the typical bacterial community that occurs in most aquatic environments on a world-wide basis (Gauthier, 2015). Contamination of human wounds and fractures by *Aeromonas* containing waters may be associated with a variety of infections (e.g., eye, respiratory tract or other systemic infections) and *Aeromonas* bacteria are commonly isolated from patients with gastroenteritis (Janda and Duffey, 1988; Janda and Abbott, 1996; Nichols et al, 1996). Species of *Aeromonas* associated with many of these human health occurrences include *A. hydrophila, A. caviae,* and *A. sobria.* Drinking water contaminated with these motile species of *Aeromonas* bacteria have also been implicated as being associated with human cases of diarrhea. The various extracellular toxins (e.g., principally haemolysins) and cell-surface and secreted proteases found in these bacteria may contribute to their pathogenicity and virulence in humans, although additional work is needed to definitively support these associations.

Bacteria of the genus *Aeromonas* have been reported to occur in a variety of wildlife including fish, turtles, alligators, snakes, and frogs (Gosling, 1996). In addition, Lehane and Rawling (2000) reported that cellulitis, myositis and septicemia were aeromonad-associated zoonoses acquired by humans from injuries related to handling fish. Igbinosa et al (2012) provide a recent review of the significance to public health of emerging *Aeromonas* species infections. Based on their review, *A. salmonicida*, a non-motile species of *Aeromonas*, causes ulcerative disease, furunculosis, and septicemia in fish specifically, while *A. hydrophila* causes hemorrhagic disease, red sore disease, and septicemia in various species including humans. These authors also discussed the occurrence of *Aeromonas* species in the gastrointestinal tract of both symptomatic and asymptomatic humans. The rates of occurrence in fecal samples from residents of developed countries was 0-4% (c.f. Svenungsson et al, 2000) while the isolation rate from individuals with diarrheal illness ranged from 0.8-7.4% (c.f. Albert et al, 2000). However, *A. salmonicida,* the bacterial species responsible for furunculosis in fish, has almost never been associated with reports of human disease, and it is doubtful that the reported association with human disease found during preparation of the current document represents true transmission to a human subject.

The two occurrences of potential infection of a human subject by *A. salmonicida* reported in the open literature appears to be those from Tewari et al (2014) and Cremonesini and Thomson (2008). Tewari et al (2014) reported on the case of a 34-year old female who appeared at their clinic in Delhi, India. The patient was a hospital nurse whose initial stool and urine samples revealed no pathogenic organisms present. Her initial blood culture indicated the presence of a potentially pathogenic organism that could not be conclusively identified using conventional biochemical approaches. During a subsequent visit a second blood sample was collected with the subsequent identification of the pathogen as *Aeromonas salmonicida*. The investigators appeared skeptical of the organism identification for a number of reasons including the observation that *A. salmonicida* does not typically grow at the normal human body temperature of 37ºC and the reported misidentifications of *Aeromonas* species reported in the open literature when using the approach they used to attempt to identify the organism cultured from the patient's blood sample. Cremonesini and Thomson (2008, c.f. Gauthier, 2015) reported a single instance of a potential linkage of *A. salmonicida* to human disease that could not be confirmed by either biochemical or genetic testing.

In summary, it is possible that these instances of human disease were due to a species of motile *Aeromonas* bacteria (e.g., *A. hydrophila*); however, it appears likely that *A. salmonicida*, a non-motile species, is first and foremost a pathogen of fish but that it may also occur in other aquatic organisms and aquatic environments.

V. Fish Disposal Alternatives

This document has made clear that the primary means of addressing furunculosis in hatchery raised trout is by using preventative measures. Many of these measures have already been adopted at Pequest Hatchery. However, should future outbreaks occur that warrant euthanizing large numbers of fish then effective disposal methods may still be required.

This review covers disposal methods used by animal processing industries, with emphasis on trout. A considerable body of literature exists on methods used by food processing industries (see below), with four major alternatives identified for disposal of animal carcasses. These are: 1) landfill disposal, 2) beneficial reuse as fish meal for animal feed or reuse as fertilizer, and 3) composting. The fourth, called "rendering" is not considered here as it primarily refers to producing purified fats or lard from animal tissue.

There are several factors to consider in selection of disposal alternatives for trout exhibiting symptoms of, or suspected of having contracted, furunculosis. These include risk of spreading the disease (or other diseases), public perception, effectiveness of disposal/environmental impacts and cost. Each of the alternatives identified are evaluated below based on these factors.

Landfill disposal

Landfill disposal was previously used by Pequest Hatchery to handle an outbreak of furunculosis that required disposition of diseased fish. During the 2013-14 event, approximately 229,000 fish were disposed of at a local landfill; a press report (http://www.nj.com/warrenreporter/index.ssf/2014/03/trout.html) indicated over 14 tons of fish were buried with several feet of cover.

This method is effective at limiting spread of the disease, which is transmitted from fish to fish within the aquatic environment, by water contaminated with the bacterium or by birds carrying the bacterium into hatcheries on their body. There are few public perception issues to address, since fish are buried deep within a lined landfill, where odors are managed using daily cover. A State of New Jersey dump truck was used to transport the fish and the state incurred no outside costs for disposal in the way of transportation or disposal. However, should outbreaks become a regular occurrence in the future, cost may eventually become an issue should the County require a tipping fee for disposal.

Hnath (1983) reviewed disposal options for diseased hatchery fish and recommended that infected fish stocks be reused if possible for other purposes, with burial or incineration only as necessary. He concluded that while incineration and steam sterilization are effective treatment measures, burial is the cheapest effective option. It is environmentally protective as long as a liner is used to prevent groundwater and surface water contamination; transporting the material to a licensed operating landfill is required.

Beneficial reuse by consumption

Beneficial reuse is desirable from the perspective of sustainability, in that the resource is not being wasted (as it is using burial as a disposal method). Beneficial reuse of unwanted fish could be in the form of donating unblemished fish to charity centers or prisons for consumption, processing the fish as fish meal or liquid fertilizer. Even though the bacterium is not known to be transmissible to humans and causes no documented health effects, donating the fish to others for consumption is impracticable from the perspective of public perception. While the fish may be harmless to the public, such a program would likely engender controversy, making it impracticable to implement. In addition, there would be transportation costs and logistical considerations involved with implementing such a program.

Another possibility would be to provide waste fish directly to mink farms for direct consumption. However, only one mink farm was located in New Jersey near the Pequest

Hatchery; the Eagle Ridge Farm, Ltd., in Kinnelon, NJ. They were not contacted by the Committee, but it is doubtful that a single farm could handle an influx of fish from a large-scale mortality event such as occurred at Pequest Hatchery in 2013-2014. Although this option could be environmentally safe, there would be significant logistical considerations involved with implementation, even if the capacity were available for handling all of the fish.

Fish meal refers to products that include dried fish (including offal and other byproducts of processing) that are fed to poultry, livestock or pets. If *A. salmonicida* infected fish were used for this purpose, the fish would have to first be pasteurized (Hnath 1983) in order to prohibit spread of the bacterium. Beneficial reuse as fish meal is a recommended and acceptable means of addressing byproducts from the fish processing industry (Windsor 2001). However, to address the issue of impacted hatchery fish, it would require the setup and implementation of a processing facility unless suitable buyers could be identified who would accept the fish. The State would still likely incur transportation costs to the processing facility for the impacted fish unless the buyer was willing to pick them up.

To evaluate this possibility further, the Ecological Services Committee contacted potential buyers (e.g. Purina) in the pet food industry, but there was little interest. From an economic standpoint, buyers rely on regular supplies to manufacture a product that meets their own quality assurance and control requirements. In our opinion, it would prove difficult for them to accommodate intermittent sources such as an outbreak that resulted in a sudden influx of 14 tons of fish.

<u>Beneficial reuse as fertilizer</u>

Fish processing waste may be reused as fertilizer, either after being composted, or from distillation of a liquid fertilizer product (Kinnunen 2016). This method would not result in spread of the disease assuming the heat of the distillation process killed the bacterium. Commercial fertilizer production is most applicable in situations where fish are being processed regularly, and therefore a significant stock source is available for generating product. While a commercial entity exists for producing liquid fertilizer for plants (Dramm Corporation, Manitowoc, Wisconsin, cited in Kinnunen 2016), it is unlikely to be feasible, either economically or logistically, for addressing sporadic outbreaks of furunculosis at NJ hatcheries.

<u>Composting</u>

Composting is often used as a method of disposal for mortality and waste fish at hatcheries (Jeff Eastman, Government of Manitoba, personal communication). Several references summarize effective means of composting fish (Archer and Baldwin 2006, USGS 1998) and animal waste in general (Bonhotal et al 2014, Bonhotal et al 2008, Gulliver 2001). In general, there are two types of composting: windrow (outdoor linear piles) and vessel composting (enclosed vessels in which compost and amendments are added). Due to the size of the fish populations requiring disposal, windrow composting would be more practical as it would require far less capital investment.

For commercial processing and agricultural industries, composting may provide a cheaper disposal alternative to burial or incineration (Bonhotal et al 2014). If practiced correctly, it is environmentally safe and would prevent transmission of the disease further into the fish population (assuming birds and fish-eating mammals were excluded from the facility). A typical approach for composting is to dispose of the carcasses in a pit and to cover them with material that will facilitate their degradation and breakdown. Typically, the amount of amendment is much greater than the tissue to be processed; for example, Archer and Baldwin (2008) tested the effectiveness of a mixture of one part seafood waste to three parts amendment. Amendments to the pile could include sawdust, leaves, hay, corn stubble, animal manure or other similar plant material that will facilitate breakdown.

Composting carcasses differs from traditional composting in two major respects: the end goal is not necessarily a salable product, and the issues with managing odors, disease transmission and scavengers are greater (Bonhotal et al 2014). These authors describe in detail how an animal compost operation should be managed to ensure air flow through the pile, inhibit groundwater contamination by lining the pit, and ensuring breakdown by addition of carbonaceous inputs. In terms of performance, a well-managed pile can result in breakdown within six months.

Regarding the utility of this method at Pequest Hatchery, it appears that sufficient land area may be available to create a small-scale composting facility capable of handling occasional outbreaks of furunculosis, should this become a long-term problem at the facility. Computer programs are available to determine land requirements based on carcass weights and other variables (Keener et al 2000). Windrow composting was used effectively by the U.S. Geological Survey to compost approximately 25,000 fish infected with furunculosis (USGS 1998). If this method is adopted at the facility, care should be taken to site the composting area well away from property boundaries and the visitor area so that odors are not an issue. The area would also need to be lined with geotextile to minimize potential contamination of groundwater and ultimately surface water of any nearby streams.

Composting would require additional labor on the part of the State to manage the disposal issue. However, it would provide the additional value offering a beneficial reuse option as fertilizer for local landowners or farmers. The Hatchery already does provide fish waste as fertilizer for local farms (Jon Lovy, personal communication February 2017).

Based on review of the available literature and discussion with professionals in the industry, it appears that the most cost-effective method of addressing future outbreaks of furunculosis requiring fish disposal is continued disposal of dead fish in a local landfill. However, should outbreaks become more frequent, and if landfills begin to charge for disposal, the State may want to further consider the possibility of a small-scale composting facility near the site to address fish requiring disposal. These options appear to be the most cost effective means of addressing the problem without spreading the bacterium. While composting would provide a beneficial reuse for the discarded fish, it would require additional labor and logistical requirements beyond burial at a landfill. Other methods of disposal (e.g. beneficial reuse as animal feed or liquid fertilizer) would probably not be commercially viable for producers.

VI. Committee Recommendations

The charge to the committee was to "*provide options for beneficial reuse of hatchery fish that cannot be used for stocking*" and to ascertain "*what is the risk to humans of the bacterium that causes furunculosis getting into the food chain for these options?*" The committee's work focused on issues associated with hatchery fish contaminated with *A. salmonicida* due to the 2013 outbreak of furunculosis at the Pequest Hatchery.

Drawing from a review of the literature, the committee found no beneficial reuse for hatchery fish contaminated with *A. salmonicida* beyond generating fertilizer through composting. However, composting fish on site or near the hatchery raised logistical concerns in terms of cost, given the intermittent need to dispose of large numbers of fish, and other factors such as disruption of the compost pile by predators and bad odors. The committee thought that seeking collaboration with a nearby composting facility could be worthwhile if the need to dispose of fish carcasses in general occurred regularly.

Limited inquiries were made of commercial vendors, and there did not appear to be any interest in acceptance of the fish by these vendors. Additionally, there is evidence that *A. salmonicida* can be spread to other fish through contaminated feed if not first sterilized, which would be cost prohibitive.

The best option for disposal currently appears to be in a landfill. However, there was no study found that investigated spread of the disease from landfills into nearby water supplies. The literature did not raise concerns about transmission of the disease to humans, very few cases have ever been reported and no cases that could be definitively substantiated.

Given the pathogenic nature of *A. salmoncida* in fish populations, the committee also considered how future outbreaks might be reduced at NJ hatcheries through preventative strategies. The recent literature reports transmission of the disease from one fish to another through water and low-value fish feed. The reviewed studies do raise concerns that release of contaminated hatchery fish into waterways could spread the disease to wild fish. Reuse as feed is possible if the diseased carcasses are first sterilized. However, the likely cost is prohibitive. Ultimately, the best way to prevent massive fish kills and the need for disposal of diseased fish is prevention. The Pequest Hatchery has an admirable “track record” for biosecurity; although, it could be suggested that they consider implementing new strategies such as probiotics, immunostimulants, and genetically-based, disease-resistant fish for improving the resiliency of their hatchery fish populations. In addition, all available housekeeping and personal hygiene measures should be utilized to prevent transfer of *A. salmonicida* between hatcheries by hatchery personnel and equipment. Something else to consider is the status of the released fish as some of these fish could be asymptomatic carriers. Molecular techniques are available for testing fish for the presence of specific types of bacteria using a swap of their skin mucus. A study in this regard would be interesting.

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