Demonstration of Sustainable Cod Farming from Egg to Grow-out in Maine

Final Report

Saltonstall Kennedy Grant # NA03NMF4270167

Principal Investigator
Dr. Nick Brown
University of Maine
Center for Cooperative Aquaculture Research
33 Salmon Farm Rd
Franklin, ME 04634
npbrown@maine.edu
Telephone: (207) 422-9096
Fax: 207-422-8920

Co-investigators
Dr Linda Kling,
Associate Professor, School of Marine Sciences,
207 Rogers Hall,
University of Maine, Orono, ME 04469-5763
Telephone: (207) 581-2735
lkling@maine.edu

Dr Denise Skonberg,
Food Science and Human Nutrition
101 Hitchner Hall,
University of Maine, Orono, ME 04469-5763
Telephone: (207) 581-1639
Denise_Skonberg@umit.maine.edu

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1. Executive Summary

This report describes a two year project aimed at investigating and demonstrating the feasibility of commercial cod aquaculture in Maine. A cohort of hatchery reared fish was produced at the Center for Cooperative Aquaculture Research (CCAR) and combined with fish from a commercial hatchery, Great Bay Aquaculture, in New Hampshire. In May 2004, a total of 18,696 juvenile cod, at an average weight of 134 g were stocked out to a 50 m circumference, 7 m deep net pen at the Johnson Cove site in Eastport, Maine. The fish were fed daily with pelleted feed (52% protein, 14% fat) to appetite. Growth, survival, gonadosomatic (GSI) and hepatosomatic index (HSI) were measured on a regular basis. Water quality (O_2, NH_4, NO_2, NO_3, and turbidity) was also measured upstream and downstream from the cod pen and adjacent salmon pens. By November 2005, 18 months after stocking out, the final weight was around 800 g. Feed conversion was poor (2.96) and survival was relatively low (60.15 %). The main reason for poor growth was the high incidence of cataract in the population. This had first manifested itself at the nursery stage and may have been related to high stocking densities combined with water quality problems due to delays with transferring the fish to the net pen site. The low feed intake also resulted in low average HSI (5.2%). The final GSI was also low (0.42%), although the incidence of fish showing signs of maturation was high (70%).

Despite the poor growth, health checks revealed no pathogens of major concern. Most importantly, the cod tested negative for ISAv although they had been heavily exposed to the virus from infected salmon in the adjacent net pens.

A quality evaluation was conducted on the farm raised cod compared to wild caught cod. The overall quality of the farm raised Atlantic cod was comparable to the quality of the wild caught product. Positive attributes of the farm raised cod included its lack of parasitic worms and excellent nutritional profile. It had significantly higher protein, potassium, and phosphorus than the wild caught fish, less sodium, and similar levels of omega-3 fatty acids. The farmed cod fillets were significantly darker than the wild fillets, which was noted by the consumer panelists, and resulted in a lower acceptability score for color and appearance of the raw product. Although the raw farmed cod fillets exhibited slightly lower maximum shear values, the difference was not significant and did not affect consumer acceptability scores for cooked fillet texture. Consumers enjoyed the cooked fillets from the farmed cod and wild cod treatments equally. Given that consumers consider fish appearance vital for acceptability; future Atlantic cod rearing studies should emphasize dietary, rearing, or processing methods that may increase the whiteness or "L" color values of the resultant fillets.

With the aim of progressing towards the supply of disease free eggs for the industry, the remaining two aspects of the project looked at testing egg disinfection methods for cod eggs and establishing a quarantine system and set best management practices (BMPs) in order to safely recruit new wild fish for broodstock.

Disinfection of cod eggs with ozonated water (~1 mg/l for 3 min) maintaining an ORP reading of 800-900 mV gave comparable results to disinfection with glutaraldehyde (400 ppm for 10 min) and Perosan (diluted to 180 ppm peroxyacetic acid and 780 ppm hydrogen peroxide). No bacterial growth was observed on the eggs and no reduction in survival was observed with any of the treatments. Given that ozone concentrations as

low as 0.3mg/l for 0.5 min are known to be effective against nodavirus, treatment of newly fertilized cod eggs with ozonated water maintained at an ORP reading between 800-900 mV is a recommended cod hatchery procedure to minimize the transfer of pathogens.

A 20,000 gallon system with four 12 ft diameter tanks was adapted to serve as a quarantine system. A new recirculation system was built to enable minimal exchange (5% per day) of water and therefore minimize waste treatment. The system consists of a bead filter, two drum filters, a foam fractionater, a U.V. sterilizer, pump sump, header tank, surge tanks and separate settlement tank. All water leaving the system is ozonated and U/V sterilized. The BMPs described include strict hygiene and traffic rules, maintenance procedures, record keeping and auditing procedures, fish husbandry protocols, a 90 day quarantine period and rigorous health testing.

The results of the project and their dissemination in the context of continuing developments in the cod farming industry worldwide and in the New England region are discussed.

2. General Introduction

As stated in the recent 10 year plan for the NOAA aquaculture program, the US imports 70% of all seafood consumed in the country, or 4 million metric tons, creating a trade deficit of nearly $8 billion. Nutritionists are encouraging Americans to increase their seafood intake which is already growing rapidly. According to the UN Food and Agriculture Organization, while capture fisheries will not yield any more seafood than currently harvested, global demand is increasing and any further increased production will come from aquaculture. Aquaculture already supplies around 50% of the world's seafood.

The aquaculture industry in Maine is one of the most important in terms of value by State in the US. The industry here has experienced serious challenges over the last decade. Salmon farmers have had to respond to concerns of the potential environmental impacts of near shore aquaculture and to legislation regarding such issues as discharge of wastes, carrying capacities, fallowing periods and acreage caps. In addition, since the U.S. Fish and Wildlife Service listed the native Atlantic salmon as an endangered species, the industry is now limited to only North American strains. Resulting changes in management practices have often meant elevated production costs. In addition, increased competition has resulted in decreasing market share both nationally and internationally and tightening profit margins. Since 2001 the salmon industry has also been trying to combat the threat of infectious salmon anemia (ISA) and has suffered heavy losses from this disease as well as from super-chill caused by some recent severe winter conditions.

According to the Maine Dept of Marine Resources (DMR) commissioned report for the Governors Aquaculture Task Force (2004) the value of the aquaculture industry in Maine fell from $80m in the late 1990's to $57m by 2003 with 700 job losses, mainly in Washington County, due to challenges facing the salmon industry.

These problems have been encountered elsewhere in the world; particularly for companies growing only one type of crop and it has become a business strategy of the biggest players in the aquaculture industry to diversify. Marine finfish species feature widely in the list of alternative species to be cultured and one of the latest candidates in which countries such as Norway, UK and Canada are investing very heavily is the Atlantic cod. Investigation into the techniques to produce juvenile cod was started early last century in Norway and Scotland. Although juvenile production always seemed feasible, farming of cod was not really started until relatively recently due to minimal profit margin based on market price. However, recent drastic declines in the cod fisheries, which reached almost 4 million MT in the late 60's, have resulted in limited supplies and a significant change in the supply/demand balance.

Canada embarked on cod farming following the collapse and then closure of the cod fishery which had been at almost 0.5 million MT until the beginning of the 1990's. Juvenile capture started in 1997 and these were fattened in 8 sites producing a total of 33 MT. By 2000, 18 sites were in operation producing 188 MT worth $575, 000. Hatchery production was also started in Newfoundland with what was at the time, the largest producer of cod in the world.

Cod farming development in Norway is well underway with over 15 million juveniles produced in 2005 and 200,000 MT in harvests predicted by 2013 in Norway alone (Rosenlund and Skretting, 2006). In Scotland, 1 million juveniles were reared in 2005 and the British Marine Finfish Association predicts 30,000 MT/yr production by 2007 (around 80% of the current wild catch; source www.bmfa.org). The major industry players in the New England/Maritimes region, in particular Cooke Aquaculture, are showing interest in farming cod and now have some 200,000 cod stocked into cages in New Brunswick and are applying for licenses to grow cod in sites in Maine. The company showcased the first farmed cod at the 2006 Boston Seafood show and the product was very well received (Frank Powell, Cooke Aquaculture pers comm.). The Atlantic cod is a species with a traditionally strong market but a fishery that is in decline despite repeated cuts in the fishing effort. The goal of the newest set of regulations proposed by the New England Fishery Management Council is to further reduce the catch of Gulf of Maine cod by at least 32 %.

The Atlantic cod is a good candidate for aquaculture, being relatively straightforward to rear in the hatchery and with good growth rates in farmed conditions. In fact, the propagation of Atlantic cod has been under development since the late 1800's. The basic technology for rearing cod was developed in the early 1980's (Howell, 1984; Oiestad *et al*., 1985; Rosenlund *et al*., 1993) but was shelved because the industry was not expected to be able to compete economically with the wild catches of cod from what were still viable fisheries. Now that cod stocks have collapsed on both sides of the Atlantic, interest in cod farming has reemerged. Atlantic cod tend to have high fecundity and are batch spawners. The broodstock adapt to captivity well and will spawn naturally in the tanks with little to no intervention. Also, they can easily be conditioned to spawn out of phase by putting them on varied photoperiod and temperature regimes (Norberg *et al*., 2004, van der Meeren and Ivannikov., 2006). Hatchery techniques are already quite well developed (Baskerville Bridges and Kling, 2000; Brown *et al*., 2003) and the supply of juveniles from commercial hatcheries is increasing.

Recent research has also confirmed that the species is not susceptible to Infectious Salmon Anemia (ISA), the disease that has severely impacted the salmon industry in Maine and New Brunswick (Snow and Raynard, 2005). Cod are also able to make antifreeze proteins (Purchase *et al*., 2001) making them less susceptible to super-chill. This may also enhance their potential for farming in the region.

In a report commissioned by the Maine DMR for the Governor's Aquaculture Task Force, cod was identified as one of the most promising species for the Maine aquaculture industry (Gardner Pinfold, 2003).

The finfish aquaculture industry in Maine has undergone a major consolidation in the last few years. Since December 2005, most of the salmon farming infrastructure in Maine has been owned and operated by Cooke Aquaculture. This company has stated a commitment to rebuild the industry in Maine. It also has a track record of interest in alternative marine species and already grows cod in net pens in New Brunswick. We expect these developments to continue in Maine.

3. Evaluation of Growout of Atlantic Cod (*Gadus morhua*) in Net Pens in Maine

3. A. Introduction

Salmon farmers in Maine are the most likely stakeholders to participate in the development of a cod industry, having the infrastructure and workforce in place. Since their infrastructure is geared to the farming of fish in net-pens, it is clear that this methodology should be examined for cod farming. It is most likely that a version of this practice will also be used to farm cod. To evaluate the use of net pens for ongrowing of cod, this part of the project involved moving a cohort of hatchery raised cod to a net pen in Cobscook Bay, Maine, the epicenter of the salmon industry. These fish were raised prior to the start of the project in the winter and spring of 2003 at the CCAR in our pilot recirculation hatchery. The objectives of this part of the project were to assess performance of the cod in this environment in terms of growth, survival, feed conversion and overall health. Any modifications required in feeding practices, fish handling protocols etc would be documented as the trial progressed.

At the time of writing the original proposal to NOAA, our industry partner was to be Atlantic salmon of Maine (ASM). However, the parent company, Fjord Seafood of Norway, decided in the fall of 2003 to restructure the company and sell off operations in Maine due to various factors, including lawsuits related to the endangered species listing of Maine salmon and discharge permits. This meant that ASM had to pull out of the project having just obtained their permits to grow cod.

A new industry partner, Stolt Seafarms, also Norwegian owned and a worldwide leader in salmon farming with a great deal of marine finfish farming experience in Europe was identified in October 2003. A subcontract was drawn up with this company and they effectively replaced ASM in all aspects. However, it became too late in the year to move the cod out to net pens given the time taken for the permit application process and the fact that water temperatures would be too low for stocking out small juvenile cod. It was decided that the cod would remain at the land-based nursery at the CCAR until the spring 2004. Further delays resulted in the cod remaining in a land-based nursery system of limited capacity until May 2004 at exceedingly high densities. This caused serious long term health issues and was ultimately to have a major impact on the outcome of the project.

3. B. Materials and Methods

A large proportion of the cod juveniles used for this study were reared at the CCAR. An overview of the rearing process is given in the following description.

Broodstock and eggs

Broodstock were caught by hook and line in Eastport, Maine during the spring of 2002. Forty broodstock were held in one 11m^3 tank in a recirculation system. The fish were fed cut herring and squid, and they doubled or even tripled in size prior to spawning in 2003. An airlift u-tube with a mesh bag was mounted in the tank to collect fertilized floating eggs. Eggs were first seen on 12/2/02. Eggs were collected at intervals until 3/15/03. No eggs were seen after this date. Most of the eggs used to produce fry were collected 1/17/03 through 3/1/03. Broodstock tank temperatures ranged from 3°C to 7°C

during this period. Egg batch volume ranged from 100 mls to 1 l. Early batches were not disinfected, but later batches were disinfected in 35 mls of Perosan (peracetic acid) in 10 l seawater for 1 minute, before stocking into the incubators. The eggs were incubated in 250 l conical bottomed tanks in a recirculating system consisting of a sump, moving bed biofilter/header tank, foam fractionator, and U.V. Temperatures were maintained between 4°C and 7.5°C during incubation. Dead eggs were removed and enumerated as needed, and the system was given a 10% water change on a daily or two day schedule. Hatching times in degree-days varied between each batch, from 65 to 94.8 degree days. The average time to hatch was 79.3 degree-days. Eight batches of eggs were successfully incubated (over 50% hatch) and stocked into the first feeding system. Hatch rates ranged from 50% to 90% for eggs used to raise fry.

Larval Rearing

The larvae were reared in eight 2000 l tanks connected to a recirculation system. This system comprised a moving bed biofilter, a sand filter, a foam fractionator, 10 μm cartridge filter a pump sump, a degassing trickle tower, and a U.V. sterilizer. The system typically received a makeup rate of 10% per day fresh seawater.

Six tanks were stocked with newly hatched larvae and/or hatching eggs at densities ranging between tanks from 39 to 56 larvae/l. Tanks were static (no flow) on day 1; flows were set to 1.0 l/m on day 2, and gradually increased to 5-6 l/m by 60 days post hatch (DPH). Stocking temperatures were 7°C and were slowly raised to 12°C by the end of the first feeding larval phase. Gas saturation levels were measured daily, and were typically at 100.5 % to 102 % total gas saturation. Water chemistry was tested weekly; ammonia and nitrite levels were well within the range considered safe for marine fish larvae. Oxygen levels were in the range of 7.0 to 9.0 mg/l.

Live Feed

Rotifers (L strain) were added to the tanks at 3 DPH at prey densities ranging from 2/ml to 6.25/ml. Subsequent additions of rotifers were based on consumption rates as determined with a visual inspection of tank water through a clear beaker. Rotifers were added twice per day in the morning and afternoon. The morning rotifers had been enriched overnight using Algamac (an algae based enrichment), and the afternoon rotifers were enriched for 6-8 hours in INVE selco products. Consumption rates peaked at 70 million rotifers fed daily to the tank with the highest larval density. Algal paste (*Nannochloropsis oculata*) was added to obtain densities of approximately 1 million algal cells/ml of tank water. Algae paste was used from 1 (DPH) to between 10 DPH and 20 DPH. Larvae were transitioned to Artemia when they were 10-12 mm in total length. This was at 330 to 360 degree-days, or about 35 DPH. Larvae were then co-fed rotifers and artemia for approximately 2 weeks until they were completely weaned off of rotifers at about 50 DPH. Artemia nauplii were enriched for 24 hours in either an Algamac or Inve Selco enrichments, and were fed to the tank twice daily. Artemia were initially added at a density of about 1/ml, with increasing numbers added daily based on consumption. Consumption peaked at about 30 million enriched artemia per day in the tank with the highest larval density (120,000 larvae initial stocking density).

Weaning onto dry diets was started at about 430 to 460 degree-days (40 to 45 DPH). A variety of weaning diets were utilized, including Lansy, Nippai, Dana and Gemma. Diets were initially handfed until it was ascertained that the larvae were accepting them, at which time weaning diets were added continuously with belt feeders. Acceptance usually occurred within 2 to 3 days after introduction. Larvae were co-fed weaning diets and diminishing quantities of artemia until about 80 to 85 DPH, after which they were considered fully weaned.

Larvae were transferred from the first feeding tanks to nursery tanks (1200 l) at about 560 degree-days (60 to 70 DPH). The nursery system was also a recirculating system employing a drum filter, moving bed biofilters, an oxygen contactor, a protein skimmer, and a U.V. sterilizer. Make-up flows during the time the fry were held in this system (June 03 to May 04) varied between 10% total system volume to 40%/day total volume.

Larval survival to the time of transfer to the nursery varied between the six larval rearing tanks from 0% to 10%. Out of approximately 678,000 total larvae initially stocked, a total of 21,021 fry were removed from the first feeding tanks to the nursery system, giving an overall survival of 3.1%. The first grading occurred on 5/14/03 with the oldest batch of fish, at 92 DPH. A 7 mm floating bar grader was used for this initial grade. All batches were subsequently graded, and the fry were hand counted. Survival rates from the time of transfer to the nursery system to the first grading varied from 62% to 95%, and averaged 74.8%. Thus, a total of 15,731 fry survived to the first grading.

By June 10, the largest fry were distributed into five large rounded square tanks (3200 l) and the smaller fry were graded into five small round tanks (1200 l) in the nursery. Stocking densities at this time were 0.74 fry/l in the large tanks and 0.4 to 0.76 fry/l in the small round tanks. At this point, the fish were in very good condition, the weekly mortality rate was extremely low, and the fish were actively feeding on a crumble diet at about 2% body weight per day. On 7/3/03, the fish were weight sampled for the first time. There were 15,152 fish remaining, and the average weight was 4.7 g, with a range between size grades of 2.6 to 7.3 g. Sixty fry collected by Microtechnologies, Inc. on 7/10/03 for health testing showed no significant findings. On 7/16/03 and 7/17/03 the fry were vaccinated with a 30 min immersion in “Vibrio 2” vaccine manufactured by Aqua Health, USA Inc. The fry were graded and redistributed on 7/22/03, using 9 mm and 11 mm bar graders. In addition, 15,000 fry (average weight=11g) were obtained from GreatBay Aquaculture (GBA) and stocked into the remaining nursery tanks. These fry had also been vaccinated by immersion at GBA.

The combined population of cod, numbering approximately 30,000 fry, were fed by means of belt feeders supplemented with hand feeding once per day. The cod were fed at 2% to 3% body weight with extruded pellets manufactured by Dana feeds, Ziegler Brothers, and Corey Feed Mills. Pellet sizes were increased as the fish increased in size, based on monthly weight sampling, and using a table provided by Dana Feed as a guide. Thus, fry were fed 2 mm pellets from 12 g to 40 g, 3 mm from 40 g to 100 g, and 4 mm from 100 g to 200 g. Initially, feed from Dana was used in 2003, with some period of feeding with a feed from Ziegler Bros, and then predominately feed from Corey (Canada) in 2004. Temperatures during this period were held at 12°C.

Mortality rates were primarily a function of aggression and jumping, and no disease events resulting in mortality were observed during the entire holding time at CCAR. There was one large mortality event August 6 when two tanks of fish (1,800 fry) were lost due to a spike in water temperatures caused by a chiller failure. By March 1 the total number was 22,287 fry. As the density increased to higher than desirable levels, an increasing prevalence of eye problems was observed, commencing in September of 2003, and gradually worsening. These eye problems were characterized by an opaque cataract forming over the eye. An increasing percentage of fry experienced this problem, and by the time the fish were shipped to the net pens in May 04, over 90% of the fry were affected. Consulted veterinarians were unable to arrive at a definitive cause for this condition. Water quality parameters for ammonia and nitrite were within acceptable levels. However, pH was often at 6.6 to 6.8 during the first few months, and on some occasions gas super-saturation was measured at 102 to 104%. Daily additions of soda ash to the system helped maintain the pH at 6.9 to 7.1.

On 1/28/04, the tank densities ranged from 39 kg/m^3 to 90 kg/m^3, with an average density of 60 kg/m^3. Average fish weight ranged from 56 g to 142 g per tank, and averaged 84.5 g for the population as a whole.

The cod fry were held in the nursery until January 30th, 2004, at which time they were given a "booster" immersion vaccination of "Vibrio 2", graded, and redistributed between a second system in the adjacent greenhouse (larger fish) and the original system (smaller fish). This had the beneficial effect of lowering tank densities to 20-30 kg/m^3. During the week of 2/22/04 to 3/26/04, the fry were vaccinated for a third time, this time with an intra-peritoneal injection of 0.15 ml of an adjuvanted water in oil emulsion vaccine (Alphaject 4000 manufactured by Alpharma and distributed by Syndel, BC, Canada), consisting of inactivated bacterial cultures of *Aeromonas salmonicida* var salmonicida, *Vibrio anguillarum* (serotype 01 and 02), and *Vibrio salmonicida* and mineral oils and emulsifiers. The vaccine was administered by Coastal Vaccinations, Blacks Harbor, New Brunswick. Fish were anaesthetized prior to injection with immersion in a solution of 13 mg MS-222 in 75 l of seawater. Fry that were well below the average weight or with spinal deformities, "popeyes", or jaw defects were culled out at this time. Approximately 3500 fry were culled in this manner. The fry were divided into 2 populations of smaller and larger fish; the average fish weight of the smaller fish was 83 g and of the larger fish was 132 g at the time of vaccination.

The last weight sample before the fish were moved to the net pen site was done on 4/26/04. At this time, there were 5,466 of the smaller fish, averaging 114 g, and 13,230 of the larger fish, averaging 143 g, for a total population of 18,696 at an overall average weight of 134 g (2515 kg biomass). These fish were transferred to the cage site at the end of May.

Cage site and net-pen facility

Stolt Seafarms was permitted to grow cod through the lease permit issued by Maine Department of Marine Resources at the Johnson Cove, DMR designation - IAFI JK site in Eastport. The 10.34 acre site is shown in Figure 3.1 the southwest corner 44°55'35.54"N, Longitude 67°00'41.18"W. A picture of the grow out site is shown in Figure 3.2. The cod pen can be identified as the pen with the black shade cloth over it.

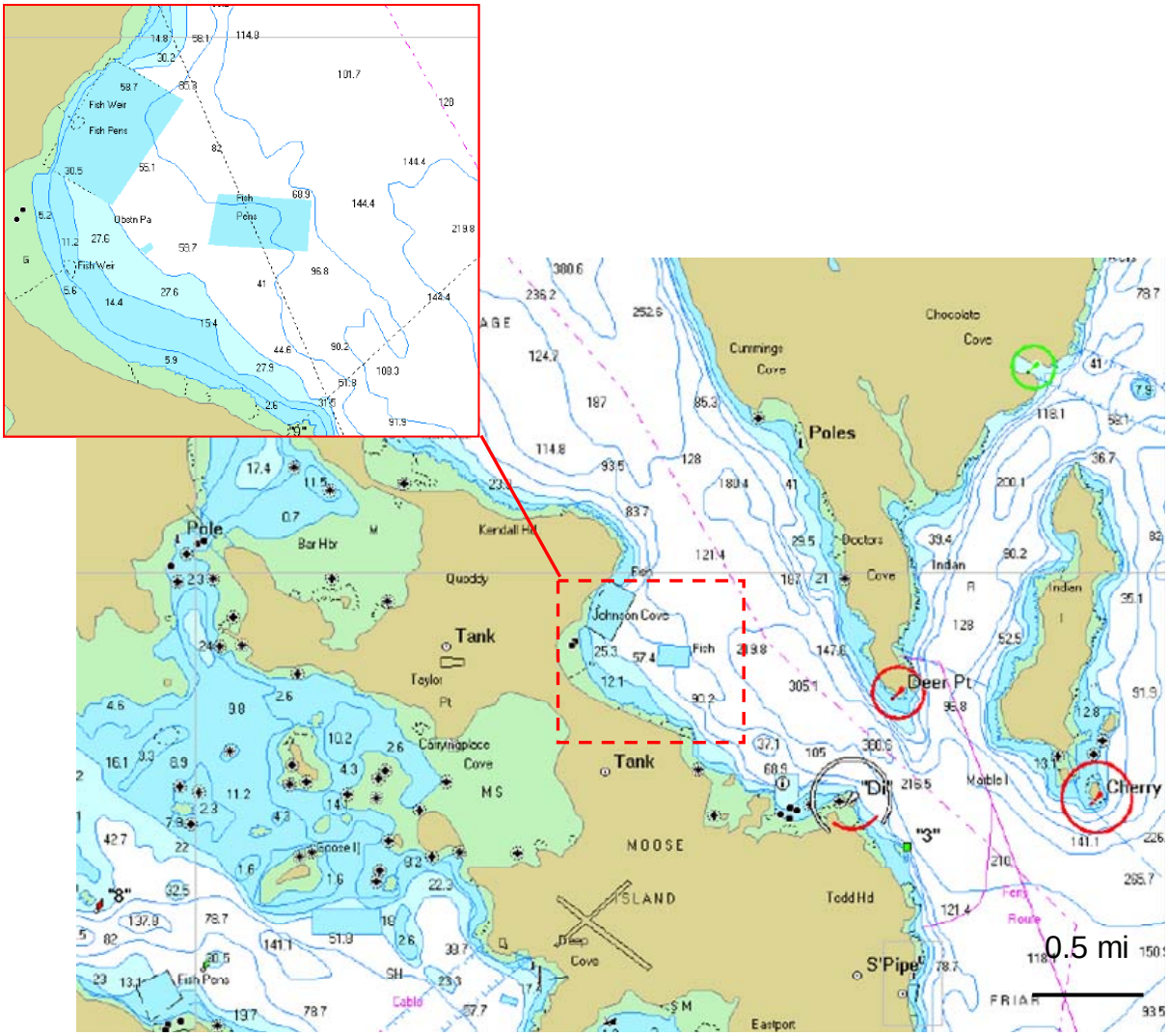


Figure 3.1. Chart showing lease site at Johnson Cove, Eastport, Maine. Depths are in feet.

Figure 3.2. Photograph of net pen site at Johnson Cove

This site was chosen for several reasons. It is typical of on-growing locations in Maine, known habitat of wild Atlantic cod and historic data for temperature, dissolved oxygen levels, turbidity, etc. are within acceptable limits for Atlantic cod. Stolt Seafarms were already stocking this site with salmon smolts that same spring and so it was expected that the time to growout for these cod would be similar and that the site would be occupied by both species for the same time period. This would make the operating costs for the cod as low as possible, much lower than if this was a stand alone project.

The cod were moved by road transport in sea water tanks. A 40' flatbed truck carried 4 tanks, 6' x 6' x 4'. The volume of each tank was 4 m^3. The tanks were fitted with a 12 volt pump and water was recirculated to mix oxygen and to help with degassing. Oxygen was supplied to each tank. The total biomass of fish moved was 2515 kg resulting in an average density of fish of 157 kg/m^3. The fish arrived in good condition after a 5 hour road/boat trip and were stocked into an approximately 1,400 cubic meter polar circle net pen which is shown in Figure 3.3.

Figure 3.3. Close up of cod net pen.

The net pen was 50 m in circumference and 7 m deep with a 10 mm grid containment net. There was also a predator net and bird net installed on the pen and the cod pen was situated within a standard 100 m circumference by 10 m deep polar circle net pen with only the predator net in place. A shade net was utilized during the summer months. The net volume allowed for an anticipated harvest density of 15-18 kg/m^3 assuming normal mortality rates and expected growth rates. There are 15 net pens located at the grow-out site and the cod pen was located on the outside middle of the seaward side. This was done in order to offer some protection from the water currents and also to maximize the flushing available to the pen to optimize water quality. The fish were reared simultaneously with Atlantic salmon in adjacent pens initially stocked with 22,000-25,000 salmon per pen.

The cod were fed to satiation 1-3 times per day as site and staffing conditions permitted so that feed would not be a limiting factor to growth. The fish were hand fed at a rate of 1-3% of body weight per day a pelleted fish feed manufactured by Corey feeds

(52 % protein, 14 % fat). The feed size was initially a 3 mm pellet which was increased to a 5 mm then 7.5 mm pellet as the fish increased in size. Feeding was observed visually on numerous occasions and notes made as to the feeding behavior of the cod. An underwater camera was also utilized to observe the behavior of the cod where they weren't visible from the surface. The fish were fed by hand one or two times per day as close to slack/ebb tides as possible so that the fish did not have to fight the currents for food during feeding.

It was observed that many fish had difficulty feeding, probably due to poor vision as a result of cataract. To facilitate feeding and to take advantage of the cods ability as an olfactory feeder, a mesh table , approximately 1 m by 2 m, was constructed and suspended within the pen in order to catch feed as it fell through the water column. The typical method to utilize the table was to throw the feed by a scoop during slack tide and concentrate it over the table so that any feed the cod didn't take out of the water column could be used by the cod who could not visually feed. Another method was to use the site diver to place feed on the table when they were present on site doing the weekly mort dives. The typical feed rate was 1% to 3% of the estimated biomass. The feed frequency was decreased during the winter months due to the low water temperatures and to minimize disturbance of the salmon in adjacent pens.

Fish health was monitored by regularly scheduled mortality dives by the site divers, picture below, as well as regularly scheduled site visits by a contracted veterinarian specialized in the needs of the aquaculture industry.

Site maintenance was performed by Stolt Seafarms personnel as well as the contracted diver (Figure 3.4) as needed.

Figure 3.4. Diver preparing to feed cod on the "feeding table"

Fish growth was monitored by collecting seven random samples of approximately 100 fish either collected by the site diver or by dip net over the course of the study period. After the cod were caught they were placed in aerated holding tanks for evaluation. Before the fish were handled for data collection they were anaesthetized

utilizing MS-222, dosage of 5-8 g per 80-100 l of seawater for 1-2 min, to prevent injury. The fish were individually weighed on an AccuWeigh Scale with a 10 kg x 10 g capacity. The depth (measured in centimeters from the point just behind the dorsal fin to the anal vent) was taken with a 30 cm ruler. The total length was measured with a 1 m stick in centimeters from the tip of the snout to the tip of the caudal peduncle.

Any deformities, lesions, parasites or other abnormalities were noted. Also, the cataracts, if present, were quantified and qualified according to the scale utilized by Wall and Bjerkas 1999. This is a scale that ranges from 0 to 4. With 0 being no observable occlusion or normal eye, 1 less than 10% occlusion, 2 being 10%-50% occlusion, 3 being 51%-75% occlusion, and 4 being greater than 75% occlusion.

Three hepatosomatic indexes (HSI) and gonadosomatic indexes (GSI) samples were taken over the course of the study period as well. This was done to compare the HSI of typical wild cod to that of the farmed cod to determine the impacts of artificial diets under farmed conditions. The fish were euthanized by a lethal concentration of MS-222 and then the fish were individually weighed. After weighing the fish were dissected and their liver tissue was removed and weighed. The HSI was calculated from :

[(Total liver weight (g))/(Total body weight (g))] x100 expressed as a percentage of body weight. The sample sizes ranged from 10 to 30 fish.

Similarly, from the same sample group the GSI was calculated from:

[(Total gonad weight (g))/(Total body weight (g))] x100 expressed as a percentage of body weight.

To compare water quality downstream from the cod pen and a comparably stocked salmon pen and upstream from the net pens, samples were collected, according to the DMR Maine Pollution Discharge Elimination System (MEPDES) permit requirements from three different sample locations using a LaMotte water collecting jar. Water samples were collected 5 m downstream from the cod pen, 5 m downstream from a comparably stocked salmon pen, and 5 m upstream from the site. Water was collected from 3 depths at each sample site; mid pen depth, mid water column depth, and 1 m off bottom. Water samples were collected from each location and depth at six specific times over the course of a sampling day. These times were high tide (0 hr), high tide +2 hr, high tide +4 hr, high tide +6 hr, 1 hr prefeeding, and 1 hr postfeeding.

The water quality parameters measured were as follows: turbidity in meters (using a secchi disc), temperature in Celsius and dissolved oxygen in mg/l (using an YSI 85 DO meter), pH, ammonia in mg/l, nitrite in mg/l, and nitrate in mg/l (analyzed using a Hach 5000 spectrophotometer).

3. C. Results

Feeding Behaviour

During feeding it was observed that approximately 2,000 fish would come to the surface to feed very actively at or just below the surface. Some fish were also observed to “graze” on fouling organisms attached to the net. It was observed both above and below the surface that the cod missed many of the pellets as they fell through the water

column most likely as a result of the high incidence of bilateral cataracts. The feeding table helped many more cod to feed, probably by using their olfactory sense rather than sight. Feed was placed on the table either by the site diver, when present, or else the feed was thrown in by scoop during slack/ebb tides so that it fell on the table. These methods met with the most success.

The cod feeding behavior was observed numerous times with differing results. The initial feeding behavior of the cod was to congregate on the bottom of the pen and pull feed from the water column as it fell through. After time, the cod were conditioned to come to the surface upon recognition of the feed boat. Upon commencement of feeding a strong response could be observed in the fact that many fish would actively boil on the surface actively seeking out feed. An even greater number learned to congregate on the feed table and take advantage of the trapped feed.

Growth and Feed Conversion

The growth in average weight of the population is shown in Figure 3.5. The final weight of the population was only 800g at harvest. The specific growth rate (SGR) varied drastically throughout the grow-out period. The highest SGR of 1.13 was observed over the warmer period of 6/9/05 to 7/18/05. The lowest SGR was observed during the colder months.

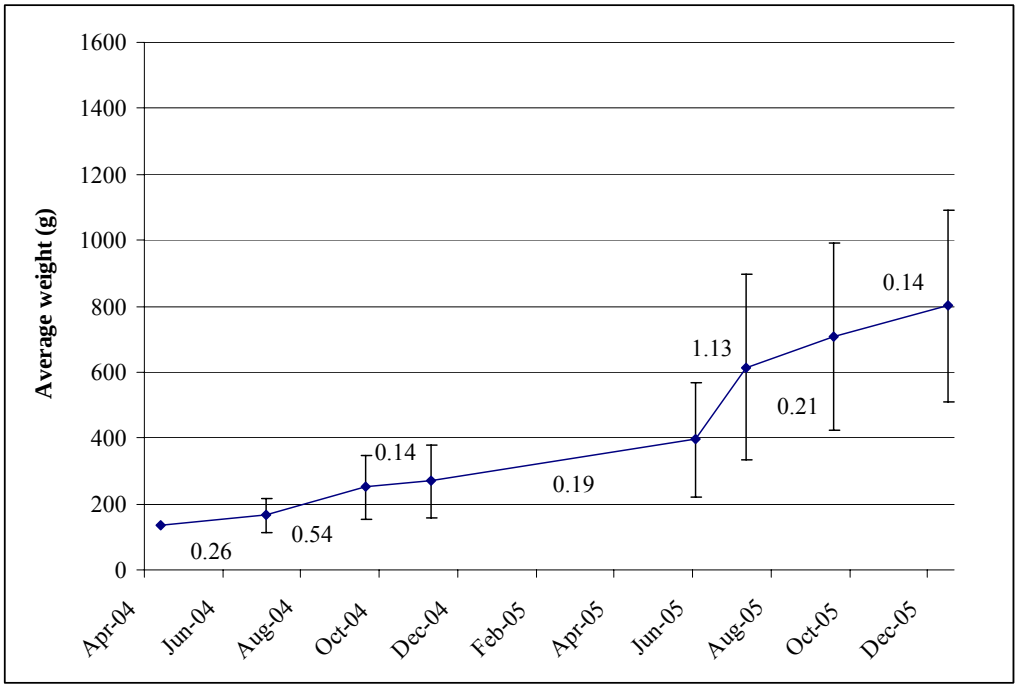


Figure 3.5 Graph showing growth rate of Atlantic cod between May 04 and November 05. Error bars represent S.E. Numbers for each period are specific growth rate (SGR) in % body weight/day.

The overall feed conversion ratio (FCR) (weight of feed fed (kg) ÷ change in biomass (kg) for the grow-out period was 2.96. The feed efficiency ratio (FER), calculated as the inverse of the feed conversion ratio was 0.34.

The observed growth data was recorded and broken down into quartiles in order to give a more detailed picture of the results and to account to the effects the cataracts might be having on the growth performance of the cod. It was anticipated that the 4th quartile would be closest to meeting the growth anticipations of having market size (3-5 kilogram) fish by the end of the grow-out period. In fact, the average weight of the upper quartile was 1.2 kg as shown in Figure 3.6.

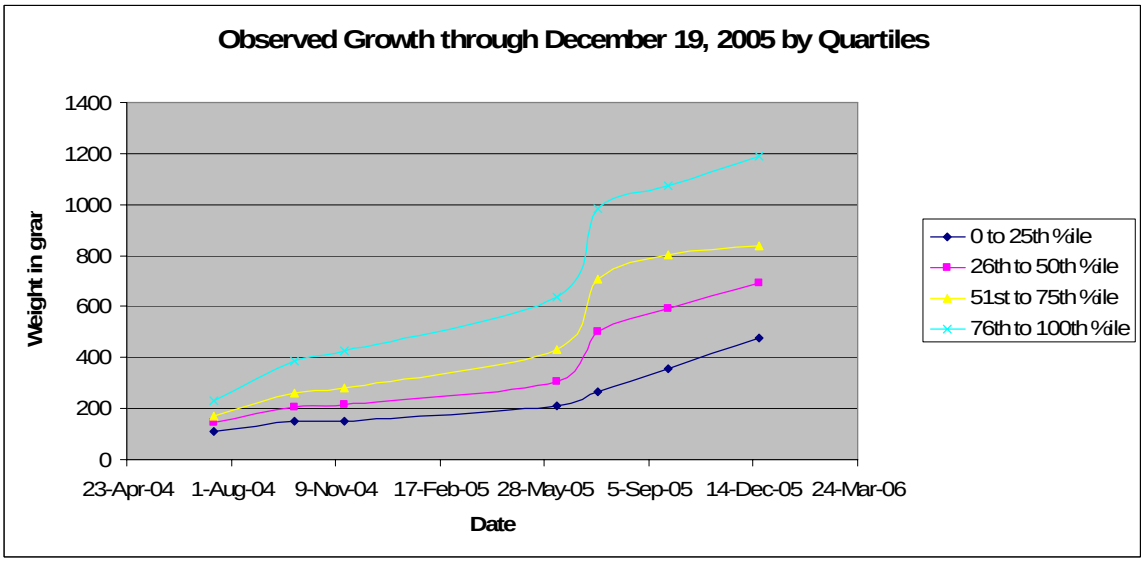


Figure 3.6. Change in average weight of cod by quartiles.

Gonadosomatic Index (GSI) and Hepatosomatic Index (HSI)

Three HSI measurements were performed over the experimental period (see Figure 3.7). The first was done after stocking out in July 2004. The second in November 2004, and the third was performed in July 2005. Three GSI measurements (Figure 3.7) were taken at the same time. The sample taken in July 2004 showed that approximately 30% of the population was exhibiting signs of sexual maturation. The final sample taken in July 2005 showed that approximately 70% of the population was experiencing sexual maturation.

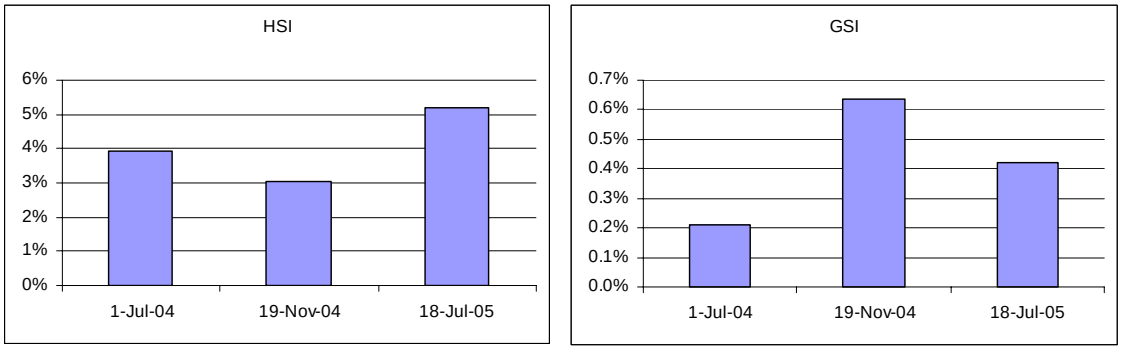


Figure 3.7. Graphs showing mean hepatosomatic index and gonadosomatic index on 7/1/04, 11/19/04 and 7/18/05.

Health

From the start of the project, the incidence of cataract was high. The incidence of cataract by severity near the beginning of the growout period (7/15/04) and then at the end (9/23/05) is shown in Figure 3.8. This shows that there was no reduction in severity and in fact, the opposite seems true, there were almost no fish with normal eye morphology at the end of the trial.

The health reports are given in the Appendix. These show that despite high densities through the nursery phase, high incidences of cataract and associated reduction in feed intake, as well as exposure to ISAv from salmon in adjacent net pens, the population did not succumb to any serious infection, either viral or bacterial. There was also no observation of external parasites. Total mortality was 39.9% over the growout period.

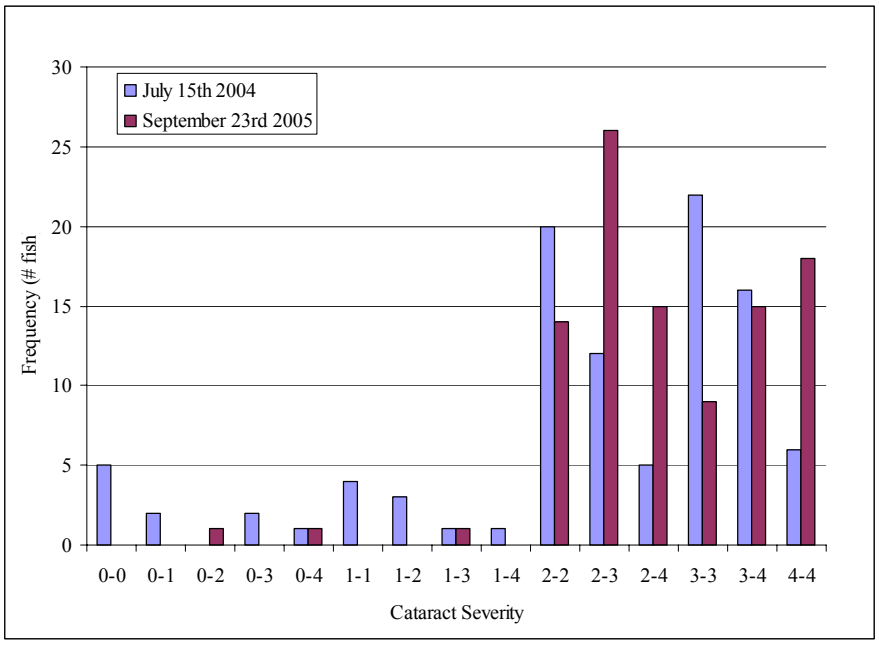


Figure 3.8. Frequency of cataract by severity category 0 to 1 for both eyes (not right or left specific) on 7/15/04 and 9/23/05.

Environmental monitoring

The water temperature profile is shown in Figure 3.9. This is typical of this site and well within suitable range of Atlantic cod.

The mean values (across all depths and sampling times) and standard deviations for pH, turbidity, dissolved oxygen, total ammonia nitrogen (TAN), unionized ammonia (UIA), nitrite and nitrate are shown in Table 3.1.

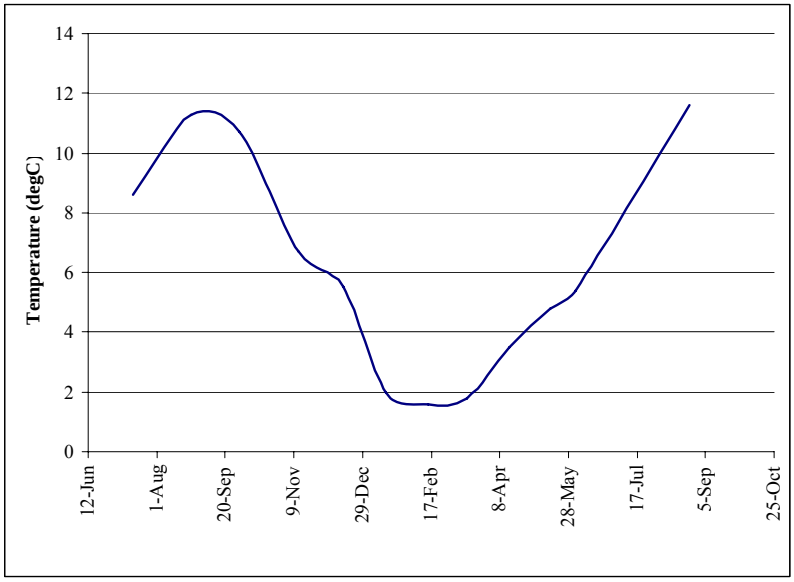


Figure 3.9. Water temperature at cod growout site during project period.

Table 3.1. Mean values for pH, turbidity, Secchi disc depth (m), dissolved oxygen (mg/l), total ammonia nitrogen (mg/l) (TAN), unionized ammonia (mg/l) (UIA), nitrite (mg/l) and nitrate (mg/l) across three depth sampling points and all sampling times.

Sampling Site		pH	Turbidity (meters)	O_2 (mg/l)	TAN (mg/l)	UIA (mg/l)	Nitrite (mg/l)	Nitrate (mg/l)
5 meters downstream from cod pen	Mean	8.02	5.30	10.07	0.0067	0.0001	0.0127	0.7085
	SD	0.14	0.612	1.56	0.0257	0.0004	0.0117	0.7094
5 meters downstream from salmon pen	Mean	8.02	5.27	9.60	0.0015	0.0000	0.0118	0.6499
	SD	0.14	0.62	1.47	0.0097	0.0001	0.0113	0.8703
5 meters upstream from site	Mean	8.02	5.22	9.95	0.0000	0.0000	0.0090	0.4940
	SD	0.14	0.65	1.68	0.0000	0.0000	0.0086	0.5705

Readings for nitrogenous compounds are all very low and well below permitted levels according to the MEPDES permit. The values of nitrate and nitrate are very slightly elevated downstream of the fish pens for both salmon and cod. Dissolved oxygen levels are similar across all sampling stations. Figure 3.10 shows oxygen concentrations

in more detail. This data is presented to show the high oxygen concentrations in late fall when the water temperature is cooler and when the biomass is still small in both salmon and cod pens, contrasted with the summer sampling shortly before harvesting when water temperature is highest and when biomasses are highest and impacts to oxygen are expected to be at their maximum. This figure shows that there is little impact from the net pens in summer or winter for either species. Surprisingly, the oxygen concentration is higher downstream from the cod pen than upstream in the summer. This could be due to hydrodynamic effect of the net pen mixing deeper water, or even a result of algae growth on the pen and photosynthetic activity.

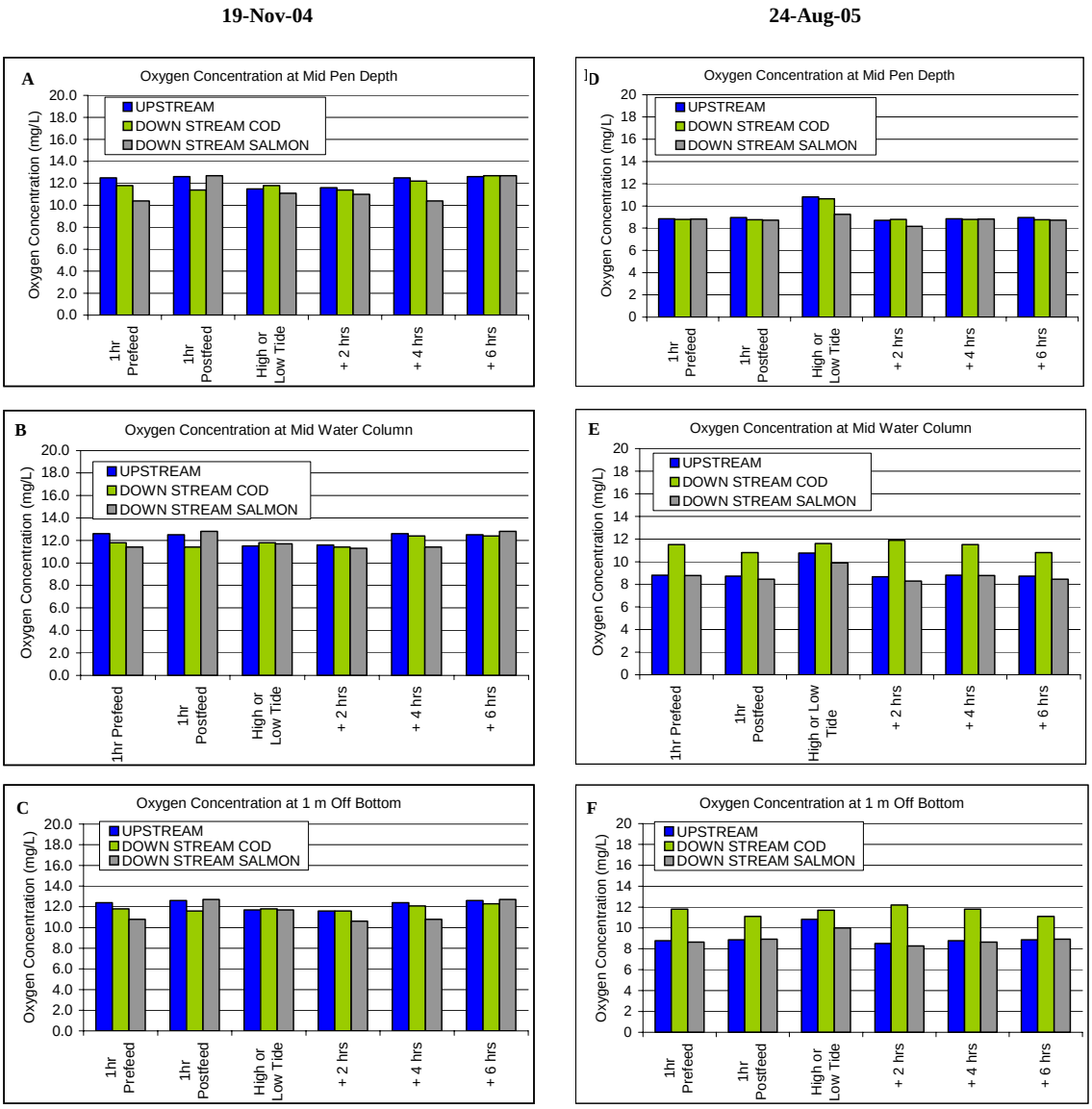


Figure 3.10 A-F. Graphs showing dissolved oxygen levels (mg/L) taken on 11/19/04 (A to C) and 8/24/05 (D to F) at mid pen depth (A and D), mid water column (B and E) and 1 meter off bottom (C and F). In each case, sampling locations are 5 m upstream (blue bars), 5 downstream from the cod net pen (green bars) and 5 downstream from the salmon net pen (grey bars) are shown.

3. D. Discussion

One of the key characteristics of the Atlantic cod, that make them a potential aquaculture species, is their fast growth rate. Growth rates should result in a 3-5 kg fish in less than a 24 month grow-out period (Moksness *et al*., 2004). The growth seen in this demonstration project was very disappointing. Comparisons with cod grown in farming conditions in the western Atlantic region are difficult. According to Murphy (2002) "Cod double their weight and are ready for market in approximately 100 days, providing a rapid return on investment". In this paper, the cod referred to are medium sized Atlantic cod, wild caught off Newfoundland, and fattened with a raw fish diet. Another study, assessing the suitability of Atlantic cod as a candidate for farming offshore in New Hampshire, shows faster growth than our study. Hatchery raised fish, raised from eggs spawned in late 2002 (so 4 months older than the fish in this study), stocked out at 45 g to a submersible cage in September 03, reached an average weight of 652 g by February 2005 after 537 days. The fish in this study, by contrast, were smaller at this age with an average weight of 396 g by June 04. The water temperatures in New Hampshire are higher on average and so growth would be expected to be faster. Norwegian and Icelandic literature also indicates higher potential for farmed cod growth e.g. Braaten (1984) and Bjornsson and Steinarsson (2002). In these studies, fish of 500 g should grow at a rate of between 0.51 and 0.59%/day. The fish of this size in our study only grew at a rate of around 0.21%/day at similar temperatures. The FCR for the grow-out period was also not in line with the expectations derived from current literature. FCRs in the range of 0.74 to 0.88 and should be consistently possible with cod under the right conditions (Bjornsson and Steinarsson, 2002; Rosenlund *et al*., 2004). This strongly indicates that much of the feed was uneaten in our study.

The mortality rate over the grow-out period was 39.9% with most of the losses occurring within 3 months of stocking them out into net pens. Most of the mortalities were thought to be due to the fact that many of the cod were never able to start back onto feed and thus wasted away. The main reason for the poorer than expected growth and feed conversion is clearly the high prevalence of severe cataract. Before stocking these fish to the net pen, they were feeding well in tanks, where the chance of capturing pellets is probably increased. The fish did not manage to maintain feed intake in the net pen environment. The cause of the cataract epidemic is unknown but several factors are implicated in the onset of cataracts in fish. These factors are nutritional (zinc, thiamine, riboflavin, tryptophan, methionine or histidine dietary deficiencies, (Bjerkas and Sveier, 2004)), environmental (gas supersaturation in conjunction with rapid temperature fluctuations, salinity variations, water treatment procedures such as ozone and U.V. sterilization (Bjornsson, 2004) or bacterial infections (Ersdal *et al*., 2001). Cod utilize vision and chemoreception in order to feed (Lokkeborg, 1998). They are primarily visual feeders during the day with the ability to see in light as low as 0.1 lux. but also utilize a well developed chemosensory system, found in the barbel and pelvic fins (Lokkeborg 1998; Jobling 2004; Yacoob *et al*., 2004). This characteristic probably enabled the semi-blind fish to locate and ingest at least enough to give a maintenance ration.

It has been shown that sometimes, depending on the underlying causes of the cataracts, as the fish grow they can accrete new tissue free of cataract (Ersdal *et al*., 2001). It was the expectation that this would be the case with the cod population in our

study since the likely cause was water quality (supersaturation or temperature fluctuation). However, following the development of the cataract over time showed that this was not the case. If anything, the severity increased. This is one of the most important findings of the study. Atlantic cod juveniles with cataracts are not worth stocking out to a net pen environment.

A consultant veterinarian at the Institute of Aquaculture in Stirling, Scotland, Dr Hugh Ferguson, confirmed that the cod were experiencing cataracts at various levels of severity. He further noticed that the cod were showing signs of photoreceptor damage in the retina. He felt that the receptor damage may have been light induced and could possibly be reversible. It was his suggestion that the shade net be reinstalled on the cod pen. However, the site operators felt that the increased load on site personnel was not warranted due to the increased time required to feed when the shade cover was in place and thus elected not to reinstall the shade cloth after the winter season. It is considered a real possibility that the cod could have been experiencing continued eye irritation due to ambient lighting conditions exaggerated by the relatively shallow, 7 m, depth of their pen and the inability to submerge to lower depths and avoid the more intense light in the shallows. The industry in the region is moving towards much deeper nets for cod (Alan Cooke, pers comm.). The reason for this is to enable the cod to move up and down in the water column, to avoid high light intensity in the summer, and to avoid temperature extremes and pressure changes that may perturb gas bladder function.

The second factor that may have slowed growth and increased mortality was the high current speed of the grow-out site location. The site experiences on average a 7.5 m tidal swing every 6 hr with an average current on site of approximately 1 mph but reaching speeds of 1.3 m/s. The slack tides average approximately 1 hr per tide swing. Water depth at the site was typically 13-21 m depending on the tide. There are several advantages to such a high energy site. The most obvious is improved flushing rate and good quality water. Excess feed and fish waste is dispersed over a much larger area decreasing the effects on the benthic environment below the net pens as well. The temperature tends to be very uniform throughout the water column, as well as the pH, dissolved oxygen levels, and turbity. However the relatively fast current may make the site unsuitable for cod farming. Some species such as salmon are strong swimmers and thus high energy sites may actually help promote growth. The cod is considered to have a slow swimming speeds (Bjornevik *et al*., 2003) and increased swimming speeds may retard growth (Davison, 1997; Johnston, 1999). It has also been shown that cod forced to swim at excessive speeds in cold water quickly become exhausted and unable to maintain position in the water column (He 1991). During the grow-out trial the cod were observed numerous times being pushed to the sides of the pen during feeding and giving the impression that they were too exhausted to swim. Thus, the lower than expected growth rates may be somewhat attributable to the increased energy cost of the cod in order to hold station under less than optimal conditions and leading to higher than expected maintenance energy costs.

It was considered important to closely monitor the nitrogenous waste stream as nitrogen is generally considered to be the limiting nutrient in marine environments (Black 2001). The results showed slight elevations in nitrogenous wastes, as expected, from both the cod and salmon cages. In such a high energy site as this, if the pens are properly

managed and stocked appropriately, there will be little impact and this is borne out by the data. However, little can be concluded from the relative impact of the cod versus the salmon in this study since the biomass of the cod did not reach the levels intended i.e. commercial scale densities. In a recent Norwegian study on nutrient retention in the Atlantic cod (Reftsie *et al*., 2006) showed nitrogen retention of consumed feed of over 40% with a fishmeal based diet and an apparent digestibility of 87.5%, similar to farmed salmon (Halver and Hardy, 2002). The feed conversion in the Norwegian study was around 0.8. This suggests that farmed Atlantic cod will have a similar environmental impact to Atlantic salmon and that recommendations for stocking densities and the location of net pens will be similar. However, if relatively low energy sites are required, this will affect flushing rates and this factor may have to be taken into account.

The quality of the juvenile cod used in this study was compromised by the conditions in the nursery, due to delays in moving out to the net pen and limitations in tank capacity. The industry must have access to high quality juveniles in adequate quantities in order to ensure that they can stock out and produce economically viable quantities of cod. The industry requires that juveniles selected for grow-out be healthy and easily adaptable to the grow-out environment. The juveniles must be free of diseases and defects such as lordiosis, scoliosis, cataracts, and parasites so that they can concentrate the majority of their energies towards somatic growth. Much work is now focused on juvenile quality and selective breeding programs are under way in Norway (Gjerde *et al*. 2004) and Iceland. The Norwegian program is already at the F2 generation stage with 86 families in the program (Terjesen pers comm.). Recently Canada announced the Cod Genome Project for the Maritime Provinces and expects to produce 90 F1 full sib families (Jane Symonds *pers comm*.). The group predicts an economic impact of $8 million per year from increased production efficiencies resulting from the stock improvements assuming a total harvest of 3000 MT/yr. A similar sized industry could be expected in New England.

Towards the end of the growout period, despite their relatively small size, there was a high prevalence of maturation in the population. These findings agree with recent studies. Under farmed conditions Atlantic cod will become sexually mature before the second year of growout resulting in decreased somatic tissue growth, degradation of flesh quality from a marketing standpoint, and increased time to market weight. Photoperiod manipulation (extending day-length using submerged lighting) can be utilized to delay sexual maturation beyond the grow-out phase (Davie *et al*. 2003; Karlsen *et al*., 2006; Taranger *et al*., 2006). It is expected that cod farming will need to incorporate photoperiod manipulation into the grow-out protocols in order to maximize the growth potential of cod and minimize the problems associated with the early onset of sexual maturation. It would have been of great interest to utilize a submersible lighting during the grow-out trial period but this was not feasible due to lack of infrastructure.

Atlantic cod do not utilize carbohydrates in the diet very readily and also tend to accumulate fat in the liver when fed with diets high in fat (>14%), (Lie *et al*., 1986). Typical wild cod have a hepatosomatic index (HSI) of 2%- 8% (Karlsen *et al*., 2006)) and amounts tend to be linked with seasonal variations and mobilization of lipid stores from the liver to meet energy requirements due to decreases in available food (Holdway, 1984). Typical cultured cod fed a commercial diet may have an HSI of approximately 12% due to the high energy and digestibility of commercial diets and what should be a

consistent availability of adequate feed (Jobling, 1988; Karlsen*et al*., 2006). The HSI values found in our study would be considered typical for wild cod which are typically in the range of 2% to 6%. According to Jobling (1988) the protein energy to total energy (PE:TE) ratio of the feed should be greater than 0.45 to 0.50 to reduce the buildup of fat in the liver and in the muscle. Cod typically have less than 1% fat stored within the muscle. The low HIS found in the cod in our study again points to low energy intake from poor feeding.

The potential for cod farming brings concerns over diseases and their transferability between pens of same species or different species and to and from wild stock. *Vibrio anguillarum* has been readily identified as a pathogen of concern as has *Aeromonas salmoncida*. However, there are good indications that vaccines already in use in the salmon industry are effective treatments in cod as well (Mikkelsen, 2004). A recent review paper (Bricknell *et al*., 2006) compared the susceptibility of cod and salmon to known pathogens and discussed how the two species may complement each other when grown at the same site by having variances in their susceptibilities to different pathogens. Importantly, the Atlantic cod is not susceptible to the ISA virus (Snow and Raynard, 2006) and this was demonstrated in our study.

A business appraisal of the economics of cod farming in Maine was to be one of the outputs of this project. To do this, information on costs of juveniles, feed, labor, materials, power and fuel, maintenance and the capital costs of infrastructure: net pens, boats, nets, machinery, shore side infrastructure etc are all required. Some of these costs can be estimated but many of the costs are company information and to make an accurate business model, these figures should be real. Unfortunately, as the project approached its conclusion, Stolt Seafarms was taken over by Cooke Aquaculture. Many of the personnel were laid off, including the accountant who was to work with us in providing this information. Despite many attempts to obtain accounting records, this information was not forthcoming.

During the course of this project, an opportunity was given to the site personnel to work with a new species. The staff at the site gained valuable experience which will no doubt be useful in the future. Most of the employees now work for Cooke Aquaculture who are steadily ramping up production of cod in the region. The site manager, Austin Dinsmore, accompanied Dr Brown and Bill Palmer to Norway to visit a number of cod farming sites. These included a commercial cod hatchery, Sagafjord in Stord which rears several million fish annually and a full scale net pen based cod farm at Smøla run by Stolt Seafarms. At the time of the visit there were around 200,000 fish at this site and yet more fish were being prepared for net pen transfer at the Stolt nursery site at Tustna nearby, which we also visited. The visit coincided with the 4 day ICES Symposium on Gadoid Mariculture held in Bergen in June 2004 which the whole party attended. As part of the exchange with Stolt Seafarms Norway, Thor Jonassen, Stolt's cod farming development manager hosted us in Norway and traveled to Maine to advise on vaccination protocols and fish transport techniques.

4. Quality Evaluation of Farm-Raised Atlantic Cod

4. A. Introduction

The goal of this study was to characterize various quality attributes and to assess the consumer acceptance of farm-raised Atlantic cod fillets. Product quality was assessed in terms of processing yield, proximate composition, mineral composition, fatty acid profile, instrumental texture and color analysis of the fillets, and sensory testing of the fillets by a consumer panel. Fillets from wild-caught Atlantic cod served as a control.

4. B. Methods and Results

Processing

Forty-two freshly slaughtered farmed Atlantic cod (weighing approximately 47 kg total) were received at the Department of Food Science & Human Nutrition Fish on 12/19/05. Each fish was individually weighed, gutted by hand, and then reweighed. They were then washed, filleted and skinned by hand, vacuum packaged, and then blast frozen at -35°C. Twenty-three fresh wild cod fillets were purchased from Harbor Fish Market in Portland on 1/5/06. The fish had been purchased the day before at the Portland Fish Exchange, from a fishing vessel that had just docked. These fish represent the freshest cod commercially available to an American consumer. The fillets were vacuum packaged and then blast frozen at -35°C. Both farmed and wild cod fillets were boxed and placed in a storage freezer (-25°C) to await analysis.

Average dress out yield ([gutted weight/whole fish weight]*100) for the farmed cod was 87.5%. The fish were lean, and ranged in weight from 776 – 1624 g, with an average weight of 1125g. Most of the fish had large quantities of seaweed in their gut contents. Skinned fillet yield was 18 %, which was less than expected. Average fillet weight was ~ 100g (3.5 oz). No worms were detected in either the gut contents or in the fillets. The wild cod fillets were considerably larger, having an average weight of ~474 g (16.7 oz). A number of cod worms were found in the wild fillets, but their overall occurrence was low.

Chemical analyses

Proximate and mineral composition

Nine fish per treatment were sampled for chemical analysis. For the farmed cod treatment, one fillet was taken from each of 9 fish different fish. The fillets were divided into three composite groups (having 3 fillets each), and then each composite group was processed for two minutes with a food processor until thoroughly homogenized. Nine fish fillets were randomly selected from the wild cod treatment, and then also grouped into three composite samples, and processed for two minutes until thoroughly homogenized. The pureed fish samples were placed in Ziploc freezer bags and stored at -20C until analyzed for proximate composition, fatty acid profile, and mineral composition.

Samples were subjected to moisture and ash analyses using AOAC (1998) methods 950.46 and 938.08, respectively. Crude lipid was determined by the acid

hydrolysis method (AOAC #922.06). Protein content was analyzed using an Elementar Nitrogen/Protein combustion analyzer. The ashed samples were dissolved in 2 ml of concentrated acid ($HCl:HNO_3$; 1:1), then diluted with distilled water (Shearer, 1984). The diluted mixture was analyzed for calcium, phosphorus, magnesium, sodium, potassium, iron, and zinc content with an ICP spectrophotometer (Jarrell-Ash AtomComp).

The proximate composition values were similar to typical values for cod fillets reported in the literature. There were no significant differences in moisture, ash, and crude lipid contents between the farmed and wild cod fillets (Table 1). The farmed cod fillets had a significantly higher protein content than the fillets from the wild fish, with values of 17.9 and 16.7 %, respectively.

Table 4.1. Proximate composition of cod fillets (g/100g)

	Farmed Cod	Wild Cod
Moisture	81.26 ± 0.68	82.33 ± 0.22
Ash	1.13 ± 0.04	1.06 ± 0.07
Crude lipid	0.49 ± 0.02	0.35 ± 0.14
Protein	17.89 ± 0.62**	16.74 ± 0.29

All values are on a wet weight basis. Each value is the average (± s.d.) of three composite samples (3 fish/sample), each analyzed in duplicate. ** = significant ($P < 0.05$) differences between treatments based on analysis of variance.

Figure 4.1. Selected mineral contents in farmed and wild Atlantic cod fillets

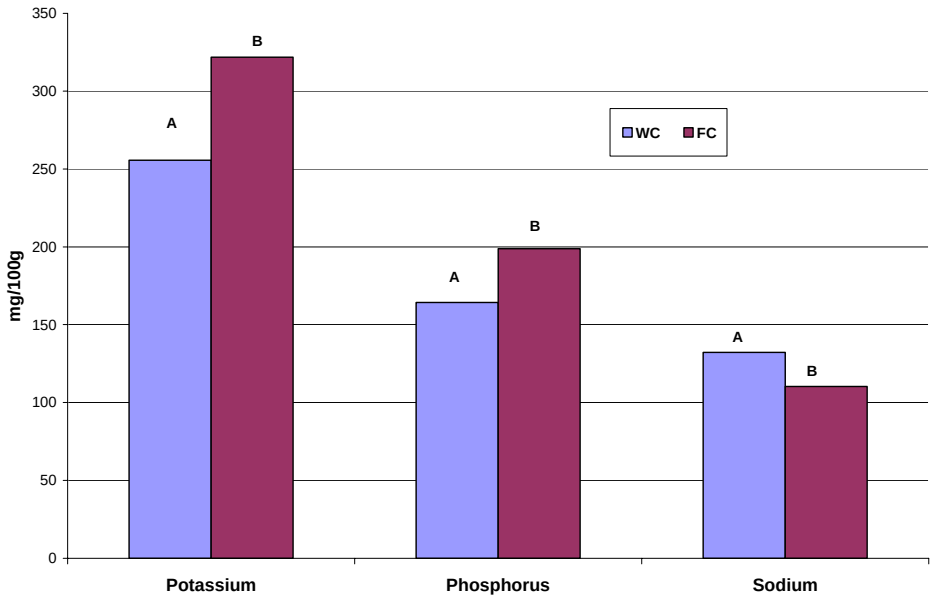
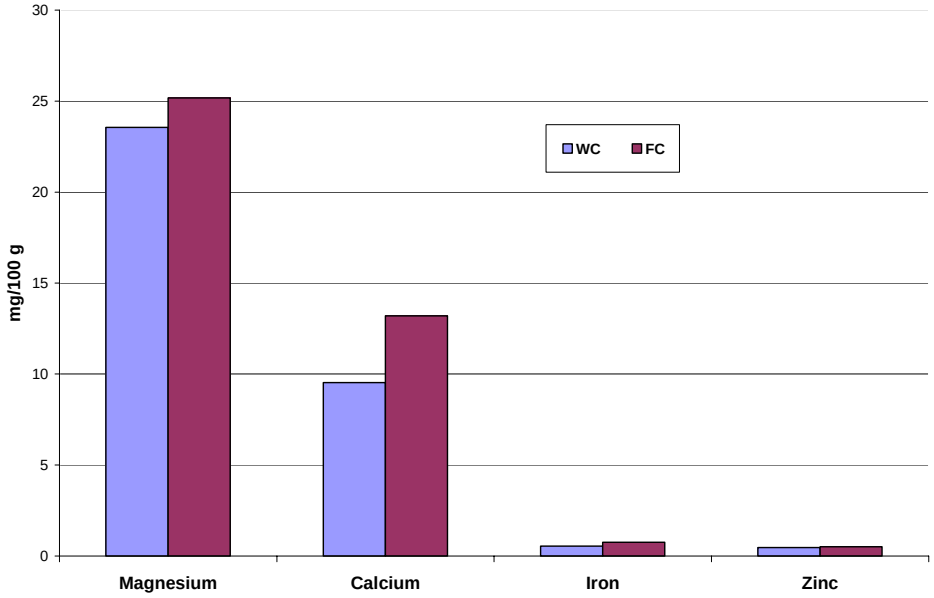


Figure 4.2. Selected mineral contents in farmed and wild Atlantic cod fillets



All values are on a wet weight basis. Each value is the average of three composite samples (3 fish/sample), each analyzed in duplicate. Different superscripts indicate significant ($P < 0.05$) differences between treatments based on analysis of variance.

There were significant differences in potassium, phosphorus, and sodium concentrations between fillets from the different treatments (Figure 4.1). The farmed cod had significantly higher potassium and phosphorus, and significantly less sodium in the fillets compared to the wild fish. No significant differences were observed in magnesium, calcium, iron, or zinc contents between fillets from different treatments (Figure 4.2).

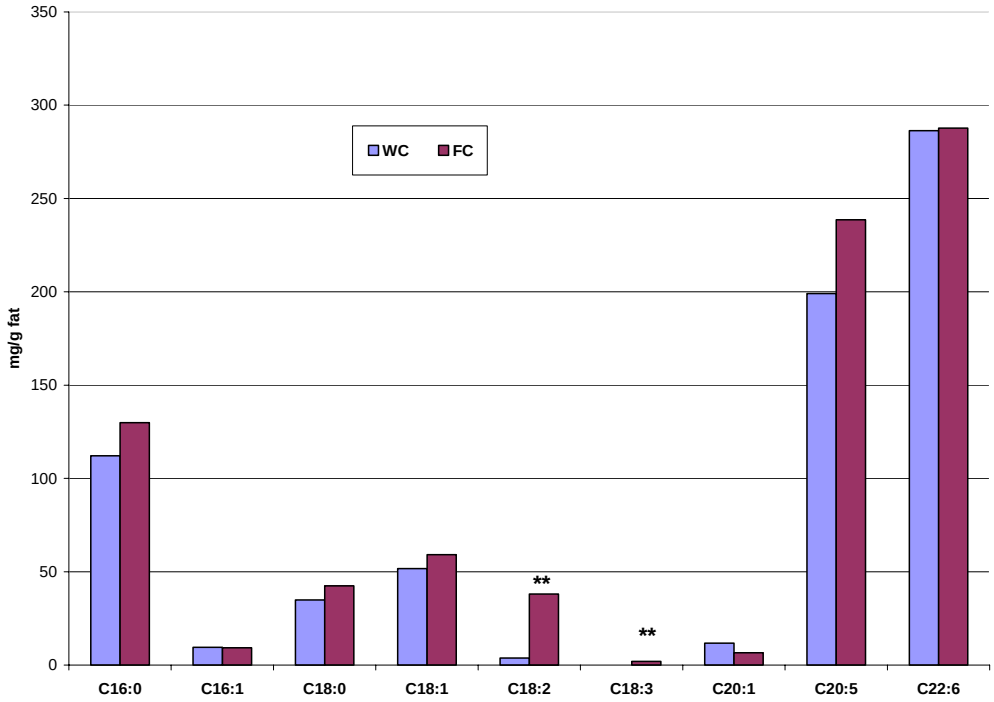
Fatty acid composition

Crude lipid for fatty acid analyses was extracted by homogenizing 15g ground fillet sample in chloroform:methanol (1:1), using a modified Bligh and Dyer (1959) method. The extracted lipid was transesterified for fatty acid analysis by adding 5.5 ml of boron trifluoride and 0.5 ml dimethoxypropane per 100 mg of fat, then heating at 60°C for 10 min (Morrison & Smith, 1964). After the addition of 2 ml hexane, the fatty acid methyl esters in the upper layer were separated and quantified by GC-MS (gas chromatography-mass spectrometry). Commercially obtained fatty acid methyl ester mixtures were used as standards for calculating the concentrations of individual fatty acid in the extracted fat samples. All samples were extracted and analyzed in duplicate.

DHA (C22:6ω3) and EPA (C20:5ω3) were the most predominant fatty acids found in both the farmed and wild fillet samples (Figure 4.3). Although the farmed cod had a slightly higher EPA content than the wild cod, the difference was not significant. With regard to the other omega-3 fatty acid analyzed, linolenic acid (C18:3), it was

detected only in the farmed raised fish. A significant difference was also observed in the linoleic acid (C18:2 ω6) content between the treatments. The farmed samples had much higher levels of linoleic acid, likely contributed by the whole wheat and soybean meal components of the commercial diet. The levels of the other fatty acids detected were similar to fatty acid composition values reported in the USDA food composition tables.

Figure 4.3. Selected fatty acid content in farmed and wild Atlantic cod fillets



All values are on a wet weight basis. Each value is the average of three composite samples (3 fish/sample), each analyzed in duplicate. ** indicates significant (P > 0.05) differences between treatments based on analysis of variance.

Physical analyses

Texture evaluation

Fillet firmness was evaluated by a shear test using the TA-XT2i texture analyzer (Technology Technologies) following a method described by Li *et al*. (2005). A 4 x 4 x 2 cm block of the post-rigor cod fillet was taken from the dorsal region just posterior to the operculum. Each block was kept under refrigerated conditions until testing to ensure a constant temperature across samples. Blocks were placed skin side down and a 60° knife edge blade (not sharpened) with a slotted blade insert pressed downward at a constant speed (1mm/s) through the fillet to determine maximum shear force. Measurements were taken from nine fillets per treatment. The maximum shear force, measured in newtons (N), was the highest peak of the curve, representing the maximum resistance of the sample to shearing.

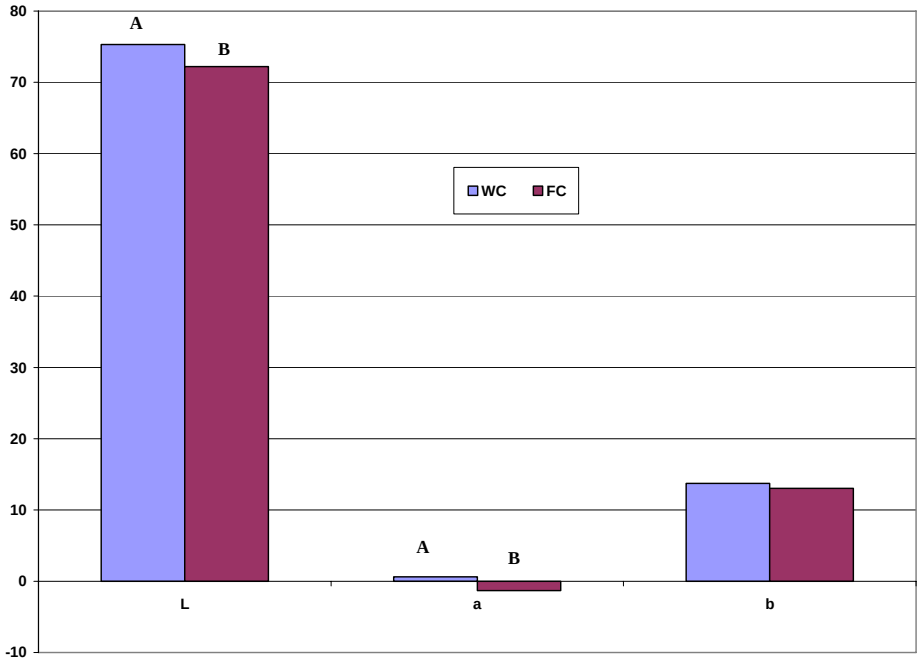
The wild cod fillets exhibited higher shear force than the farmed cod fillets, with average values of 41.5 newtons and 34.9 newtons, respectively. Thus, the wild cod fillets were firmer, although the difference between treatments was not statistically significant (P >0.05). Texture is a very important quality attribute in fish fillets, and farm raised fish fillets are generally considered to be less firm than fillets from wild fish, due primarily to a lack of exercise. However, a recent paper by Bjornevik *et al*. (2003) reported no correlation between exercise regime and fillet texture in farmed Atlantic cod in a 9-month feeding trial.

Colorimetric analysis

Instrumental color was evaluated using a HunterLab ColorScan XE colorimeter. The same sample that was used for texture evaluation (4 x 4 x 2 cm block excised from the anterior dorsal region) was subsequently ground in a food processor until well homogenized, and then used for color analyses. *L* (white intensity), *a* (red intensity) and *b* (yellow intensity) values (CIE, 1976) were recorded for ground samples from 9 individual fish per treatment. The values recorded for each sample were an average of three readings, each taken at a 45 degree angle turn.

Significant differences were observed in the "L" and "a", but not in the "b" values between treatments (Figure 4.4). The wild cod fillets were significantly lighter colored, as demonstrated by their average higher "L" value. The wild cod fillets also had a significantly higher "a" value, indicating the wild fillets were more red, and less green, than the farmed cod fillets. However, the "a" values were so close to zero that any red/green chromaticity was negligible in fillets from either treatment.

Figure 4.4. Instrumental color analysis of farmed and wild Atlantic cod fillets



Consumer acceptability testing

Fillets from the farmed and wild cod were subjected to sensory analyses by a panel of fifty untrained students, staff, and faculty at the University of Maine who customarily eat seafood products. The consumers were asked eight questions about their seafood purchasing and consumption habits. Next, the panelists were served raw cod fillets that had been cut into approximately 5 cm x 5 cm x 1.5 cm sections and then asked to evaluate the overall acceptability, aroma, color, and appearance of the coded fillets using a 9-point acceptability scale, in which 1 = dislike intensely and 9 = like intensely (Meilgaard *et al*. 1991). After evaluating the raw samples, panelists evaluated the overall acceptability, aroma, flavor, texture, and appearance of the cooked cod samples using the same scale. The cooked samples were prepared by microwaving for 45 seconds on high, and were then served warm. The fillet samples were served plain, with no spices, tartar sauce, or other condiments to influence the sensory attributes of the fish. Panelists recorded their scores using the SIMS (Sensory Computer Systems, Morristown, NJ) computerized data collection system. The consumer acceptability testing received prior approval from the Human Subjects Committee and was conducted at the Consumer Testing Center at the University of Maine.

Table 4.2. Average consumer testing scores of farmed and wild cod

	Attribute	Farmed Cod	Wild Cod	P value
Raw fillets	Overall acceptability	6.82	7.58	0.0004 **
	Aroma	6.48	6.98	0.0477 **
	Color	6.32	7.62	0.0001 **
	Appearance	6.32	7.62	0.0001 **
Cooked fillets	Overall acceptability	6.76	6.78	0.93
	Appearance	6.78	7.00	0.36
	Aroma	6.34	6.32	0.93
	Flavor	6.54	6.62	0.75
	Texture	6.70	7.08	0.17

Each value is the average of 50 assessments. Scores are based on a 9-point hedonic scale in which 1 = dislike intensely, 5 = neither like nor dislike, and 9 = like intensely.

With regard to the raw fillet samples, the wild cod received significantly higher acceptability scores than the farm raised cod for all attributes tested (Table 4.2). Wild cod received average scores ranging from 6.98 (aroma) to 7.62 (color/appearance), while the raw farmed cod fillets received scores ranging form 6.32 (color/appearance) to 6.82 (overall acceptability). The biggest difference between treatments was in color and appearance scores. The wild cod fillets were noticeably whiter in color than the farmed fillets, which agreed with the higher "L" values received by the wild cod fillets. Both the color/appearance acceptability scores and the comments by the panelists indicated that they preferred the whiter colored wild fillets.

After cooking, the color differences between treatments disappeared, and there were no significant differences in appearance scores between the farmed and wild cod, or in any of the other rated attributes. Consumer comments indicated that the farmed cod fillets were more tender than the wild cod fillets, however that did not significantly affect the texture acceptability scores, since some consumers prefer firm fish while others prefer more tender fish. The average overall acceptability values of the cooked fillets was ~ 6.8, slightly under the "like moderately" rating. Higher acceptability ratings would likely have been obtained by batter & frying the fillets, which panelists reported as the preferred preparation method for cod served in a restaurant.

When asked whether they would purchase farm-raised cod, 62% of the consumers said "yes", 34% said "maybe", and the remaining 4% said "no". With regard to consumption frequency, 8% said they consumed cod weekly, 35% consumed cod monthly, 56% selected "yearly", and 2% said they never consume cod. The panelists were also asked which factors were important when shopping for fish, and were allowed to select more than one answer. The number one answer was appearance (25 responses), followed by type of fish (24 responses), price (18 responses), aroma (14 responses), and size (4 responses). Since product appearance is so important for consumers making fish purchasing decisions, the higher overall acceptability scores received by the raw wild cod are reasonable.

4. C. Conclusions

The overall quality of the farm raised Atlantic cod was good, and comparable to the quality of the wild caught product. Positive attributes of the farm raised cod included its lack of parasitic worms and excellent nutritional profile. It had significantly higher protein, potassium, and phosphorus than the wild caught fish, less sodium, and similar levels of omega-3 fatty acids. The farmed cod fillets were significantly darker than the wild fillets, which was noted by the consumer panelists, and resulted in a lower acceptability score for color and appearance of the raw product. Although the raw farmed cod fillets exhibited slightly lower maximum shear values, the difference was not significant and did not affect consumer acceptability scores for cooked fillet texture. Consumers enjoyed the cooked fillets from the

farmed cod and wild cod treatments equally. Given that consumers consider fish appearance vital for acceptability, future Atlantic cod rearing studies should emphasize dietary, rearing, or processing methods that may increase the whiteness or "L" color values of the resultant fillets.

5. Cod Egg Disinfection Trials

5. A. Introduction

Surface disinfection of eggs is a routine practice in commercial hatcheries to prevent both the horizontal (hatchery to nursery) and vertical (broodfish to larvae) transmission of pathogens. Moreover, an overgrowth of bacteria on the surface of the egg chorion can reduce oxygen exchange and can result in asphyxiation of the embryo. Surface disinfection of eggs will not prevent transovarian transmission of pathogens, but can prevent transmission of pathogens during spawning. Cod are allowed to spawn naturally in tanks so eggs are exposed to pathogens residing in the culture water prior to collection. Hansen and Olafsen (1989) observed adherent bacterial flora on cod and halibut eggs dominated by *Flavobacterium sp., Pseudomonas sp., Aeromonas sp. And Alteromonas sp.*. Johnson *et al*. (1971) observed *L. mucor*, which is not intrisically pathogenic, on the chorion of developing cod eggs and when the eggs appeared "overgrown" with the bacteria they sank and the embryos died. Pathogenic bacteria such as *Vibrio anguillarum, Pseudomonas anguilliseptica, Flexibacter maritimus,a nd Pasteurella piscicida* are also known to adversely affect survival and normal development during the embryonic and larval phases (Colwell and Grimes, 1984; Skiftesvik and Bergh, 1993; Munro *et al*., 1995).

Viral pathogens are best eliminated from the hatchery by selecting virus-free broodstock but treatment of eggs is often necessary as a secondary precaution when detection techniques of the virus in the broodfish is not 100% reliable and when you are using wild-caught broodfish. Viruses from the family Nodaviridae have been difficult to detect in broodfish without lethal sampling and go unobserved in broodfish, only to be transmitted to the eggs resulting in very high mortality of larvae.

The effectiveness of different surface disinfectants in eliminating bacterial and viral pathogens vary. Moreover, embryos of fish species differ in their sensitivity to different disinfectants. The ideal egg disinfectant would be one that could be applied to newly fertilized eggs with 100% effectiveness in destroying bacterial, viral and fungal microorganisms without adversely affecting hatchability and is safe to use. Glutaraldehyde has the capability to kill all forms of microbial life including bacteria, fungus, viruses, fungal and bacterial spores (Borick, 1968). Treatment of fertilized cod eggs with 400 ppm glutaraldehyde has been reported to be an effective egg disinfectant while providing for high embryonic survival and good hatching success. However, glutaraldehyde is highly toxic to humans and its use should be discouraged. A disinfectant containing a mixture of peroxyacetic acid, hydrogen peroxide and acetic acid has recently found widespread use in European and Canadian marine hatcheries. It is also an excellent fungicide, retarding the growth of mold and fungus on the eggs. Ozone is a powerful oxidant that when introduced into seawater produces an array of powerful oxidants such as bromine, bromamine, hypobromous acid and hypobromide. Ozone at 0.1 mg/l for 2.5 min was required to inactivate striped jack nervous necrosis virus, a

nodavirus. Newly fertilized Striped jack eggs infected with SJNNV were successfully disinfected by washing the eggs in 0.2 mg/l for 1 to 5 min; however, treatment at 0.5 for 3 min or higher resulted in reduced hatch rates and decrease larval survival (Arimoto *et al*., 1996) Grotmol and Totland (2000) exposed Atlantic halibut eggs at 4 d prior to hatching to nodavirus and then to ozonated sea-water using different ozone concentrations (0.3 to 10 mg/l) and exposure times (0.5 to 10 min). None of the larvae from virus-exposed eggs washed with ozonated sea-water developed viral encephalopathy and retinopathy, which was detected in all dead larvae from eggs exposed to nodavirus but not washed with ozonated sea-water. However, with high total exposure (>4 mg/l for 1 min or higher total ozone exposures) a pronounced negative effect on hatching was observed. Disinfection of Atlantic cod, turbot and Atlantic halibut with ozonated seawater (4mg/l for 1 min or higher) two days prior to hatching showed interspecies differences in tolerance with turbot eggs displaying higher tolerance than cod and halibut eggs. The groups of eggs of all three species exposed to 2 mg ozone/l for 2 min or less showed normal hatching (Grotmol *et al*., 2003). To our knowledge, the effects of disinfection with ozonated seawater on newly fertilized cod eggs has not been evaluated

In this project we evaluated the use of ozonated seawater and compared it with the use of glutaraldehyde and peroxy-acetic acid treatment on fresh spawn of Atlantic cod broodfish.

5. B. Materials and Methods

Egg disinfection.

Eggs were collected from a tank of spawning Atlantic cod held at the CCAR, and shipped in a cooler by vehicle to the Orono campus of the University of Maine. When the eggs arrived they were in early cell division stage. The eggs were maintained at 8^{o}C and all disinfection processes were done in a walk-in cooler maintained at said temperature. Three samples of approximately 50 eggs per disinfectant treatment were taken from the egg batch. Each sample was disinfected using freshly prepared disinfectant. The disinfectant treatments were: 1) Perosan^{TM} at 3.5 ml/l (180 ppm peroxyacetic acid and 780 ppm hydrogen peroxide) for 1 min; 2) glutaraldehyde at 400 ppm for 10 min; and (3) ozone at 800-900 mV for 30 sec; (4); ozone at 800-900 mV for 3 min and (5) no disinfection. After the disinfection treatment, up to 48 eggs from each sample were placed in microtitre plates (Corning 96 flat bottomed) with one egg per individual well along with 40 µl of filtered seawater. Only fertilized eggs were used. The wells were sealed to prevent evaporation and incubated at 8^{o}C. On day 13 the eggs were examined and placed into one of two categories: 1) viable larvae or 2) unhatched egg and deformed larvae.

To determine the effectiveness of the disinfectant treatments, similarly disinfected eggs (25) were aseptically transferred into sterile test tubes (3 replicates/treatment) containing 15 ml of sterile tryptic soy broth plus sodium chloride. Three test tubes without eggs were also incubated to verify the aseptic procedure The test tubes were sealed and incubated at 10^{o}C for 15 days. The clarity of the tryptic soy broth was evaluated daily. Any turbidity in the broth was evidence of microbial growth and declared not to be sterile. .

Description of ozone disinfection apparatus.

The ozone disinfection apparatus consisted of two 30 gal plastic totes connected together with PVC pipes to allow the water to circulate. A small pump was attached to the bottom chamber and water (32 ppm seawater, pH 8, 10^{o}C) was pumped to the upper chamber. An AquaZone Ozone generator (200mg/hr) attached to a tank of pure oxygen was used to convert the oxygen to ozone and the ozone was injected into the water via venturi injection. The ozone levels were checked using both a test kit and an ORP meter. The amount of ozone in the water was determined by measuring the amount of ozone in the water using a Lamotte Smart Colorimeter^{TM} and reading the corresponding mV with a Pinpoint ORP meter. In our trials, 1 mg/l ozone corresponded to around 800-900 mV ORP. ORP measures the oxidizing activity of the water and not specifically ozone concentration. When ozone is introduced into the water it reacts with a wide array of compounds to produce new oxidants such as bromine, bromamine and hypobromide and thus oxidation-reduction potential offers "real time" monitoring of water disinfection potential and may be more reflective of the oxidizing environment than ozone concentration itself. ORP monitoring provides a rapid and single-value assessment of the disinfection potential of the water. ORP readings are not a linear function of ozone concentrations in water but varies with organic load, pH and temperature.

5. C. Results

Eggs treated with 800-900 mV ORP ozonated water for 30 sec were not disinfected as evidenced by turbidity of the tryptic soy broth after five days incubation. Eggs treated for 1 min and for 3 min resulted in no turbidity after 15 days of incubation. Both the glutaraldehyde and perosan treatment also resulted in no observable turbidity. Overall hatch rate for the cod eggs was 88.1%. A Chi square test of independence indicated that there was no effect of disinfectant treatment on hatchability.

5. D. Conclusions

Disinfection of cod eggs with ozonated water (~1 mg/l for 3 min) maintaining an ORP reading of 800-900 mV gave comparable results to disinfection with glutaraldehyde (400 ppm for 10 min) and Perosan (3.5 ml/l for 1 min). No bacterial growth was observed on the eggs. While we did not test for the presence of nodavirus, according to Arimoto *et al*. (1996) SJNNV, a nodavirus, was destroyed on infected striped jack eggs with as little as 0.2 mg/l for 1 min. Similar results were found by Grotmol and Totland (2000) disinfecting Atlantic halibut eggs exposed to nodavirus. Ozone concentrations as low as 0.3mg/l for 0.5 min. were found to be an effective egg disinfectant. Treatment of newly fertilized cod eggs with ozonated water maintained at an ORP reading between 800-900 mV is a recommended cod hatchery procedure to minimize the transfer of pathogen.

6. Development of Quarantine System and Best Management Practices

6. A. Introduction

Alternative marine species under development in Maine include cod and halibut. Founding broodstock populations for these species come from the wild and although work on these species began a few years ago, and some F1 juveniles are reaching maturity, we are still basically working with wild parents. Breeding programs are being planned for these species and will likely happen in collaboration with the USDA Agricultural Research Service, National Cold Water Marine Aquaculture Center. Until we have established sufficient family lines of domesticated fish, we will continue to require new wild fish for these programs for many years to come.

Having established acclimated stocks for initial development of rearing technologies for halibut, cod or other potential species, it will be important to protect them from pathogens that may be introduced by newly acquired wild fish. This can only be done by isolating these fish in quarantine systems. As yet, there is neither a designated quarantine facility in the region or protocol for holding fish in quarantine. This part of the project, addresses this issue. A new purpose built quarantine system was constructed at the CCAR and was completed in 2005. Best management practices were then developed in close consultation with fish health professionals in the region, including the State veterinarians, members of the New England Fish Health Technical Committee and Extension veterinarians.

6. B. System Description

The quarantine system was designed by Nick Brown and Peter Harvey at the CCAR. The facility is attached to Building 1 but has its own entry doors. This means that although not physically isolated, it is operationally isolated from other fish holding areas. There is no possibility of contaminated water flowing from the quarantine system to other fish holding facilities on site. There will be no traffic from the quarantine system to other parts of the facility. Figure 6.1 shows a close up of the quarantine system with general layout, tanks and equipment. Figure 6.2 shows the quarantine system in relation to building 1 and indicates traffic flows within the facility.

The facility consists of 4 holding tanks and a water treatment system, completely isolated from the other facilities on site. The system is recirculating to reduce the amount of effluent that will need to be treated and will have a makeup equivalent to between 5 and 10% system volume per day. The total system volume is 75 m^3 (20,000 gallons) and will have a maximum makeup flow of 5.2 L/min (1.4 gpm). Each of the four holding tanks is 12 ft diameter, 6 ft deep and will be suitable for most adult marine fish under consideration.

The tanks each have two drains. Most water leaves the tanks via a side drain while the remaining flow (around 10%) will take most of the larger solids, uneaten feed and larger particulate waste through the center bottom drain. Each drain will pass to a drum filter. Solid backwash from these two filters will be taken to the settlement tank which is in the treatment room. Solids will be stored here until removal by waste trucks to a municipal waste treatment facility.

Water is pumped from the sump through a foam fractionater Water is pumped from the sump to a header tank via a U.V. sterilizer and a side loop passing through a bead filter for biofiltration and further solids removal and then through a degassing tower back to the sump. Back wash and foam condensate from these units drains to the settlement tank.

Overflow from the sump passes to a separate pump sump from where it is pumped up to an ozone contactor. All water leaving the system is ozonated to a residual concentration of 1.0 mg/l for a minimum contact time of 2 min. This results in a CT (Concentration x Time) of 2. According to Liltved *et al*., (1995), a CT of 0.6 (0.15 to 0.2 mg/l ozone for 3 min) is sufficient to inactivate 99.9% of IPNV, *Vibrio anguillarum* and *Aeromonas salmonicida*. Colberg and Lingg (1978) reported that at levels of between 0.1 mg/l and 1.0 mg/l for 1 min (CT of 0.1 to 1.0), *A. salmonicida*, *A. liquefaciens, Psedomonas fluorescens and Yersinia ruckeri* were all 99% killed. The concentration of ozone in mg/L is difficult to monitor and more commonly the oxidation-reduction potential (ORP) is measured continuously on a probe. The relationship between residual ORP in the system and actual ozone concentration (mg/L) will be determined using a Hach Spectrophotometer based indigo test. Such a relationship was developed for a sea water recirculation system similar to this one by Tango and Gagnon (2003) where ORP (mV) = 1336.3 x (concentration ozone in mg/L) + 71.8. If a similar relationship is found in our system, the ORP will likely be set at around a level of 1400 mV or above for complete disinfection at 1.0 mg/lL.

There are two probes to monitor ORP and one backs up the other. If either probe reads an ORP below the critical level, a solenoid valve will shut incoming flow to the system and shut off the sump pump that delivers water to the ozone contactor. The system will remain running on zero makeup. Any water overflowing the settlement tank generated by backwashing from drum filters and the bead filter will be trapped in the pump sump. There will be more than 24 hours capacity to hold overflow water. At the same time that the makeup flow is shut off, the system will alarm out. Outside normal working hours, a dial out system will alert on call staff members and the system malfunctions would be rectified within 1 hour. There is a back up ozonation system ready to replace the working unit should it need to be sent out for repair.

Water quality measurements are made on a regular basis (temperature and dissolved oxygen twice daily, pH, ammonia, CO_2, nitrite and nitrate weekly). Makeup flows and feeding rates are adjusted to ensure good water quality at all times.

6. C. Protocols

Hygiene

Hygiene barriers at entry points consist of

- Changing area
- Storage area for outdoor clothing
- Footbaths (containing Virkon S at 1000 ppm, changed weekly)
- Hand wash sink and alcohol hand spray station

- A seating area with cleanable boot (for employees) or disposable bootee (for visitors) storage area
- A dry floor in a heated room
- A bin for disposable outer clothing

Equipment designated for the facility will only be used in the facility. This includes nets, totes, cleaning equipment, hoses etc. Vehicles should not have access to fish holding facilities. Fish tanks and other equipment associated with fish transport should be disinfected with chlorine @ 200 ppm or a quaternary amine compound such as Roccal @ 800 –1000 ppm or Virkon-S at 1000 ppm (All disinfectants will be used as directed on the label). Where applicable, wall and floor surfaces will be durable, waterproof and cleanable with high-pressure water/steam and/or sanitizing agents. Floors will be smooth but non-slip and will be self-draining to prevent pooling of water.

Stocking with Fish

The quarantine system will be stocked with fish on an all-in/all-out basis. It is likely that this would happen up to twice per year, depending on the quarantine period agreed upon. The number of fish stocked into the facility depends on the size and the species. The capacity of the system is 52.5 m^3. At 10 kg/m^3, up to 350 cod at 1.5 kg could be held. It is very unlikely that this number would be collected at one time. More realistic numbers would be 75 cod at 1.5 kg average weight or 40 halibut at 15 kg average weight. Fish entering the system will come in through the side entrance (Normally an Emergency Exit see Figure 6.2) that leads directly to the tank bay. Fish that do not appear healthy will be euthanized. Fish will be treated with a formalin bath at 250 ppm for 30 min prior to stocking into the tanks to removed external parasites.

Transport water should be disinfected before discharge @ 50 ppm chlorine. Chlorinated water should be neutralized after 1 hour with sodium thiosulfate at a rate of 2.85 × the amount of chlorine (g) according to the OIE International Animal Health Code.

Personnel and Visitors

Those individuals authorized to enter the facility during the 90-day quarantine period will be established prior to fish being put into the system. This may include appropriate fish health professional, CCAR director, other CCAR personnel and those specifically assigned to work within the isolation facility.

Prior to being assigned to work within the isolation facility all personnel will be given training on disinfection and biosecurity, including some type of examination or worksheet demonstrating proficiency. Signs will instruct staff entering the facility to comply with biosecurity protocols. Biosecurity training will be reviewed by each person assigned to the facility prior to each 90-day quarantine period. This will include information on those individuals authorized to enter the facility and those individuals to be contacted in the event of facility maintenance, fish morbidity or fish mortality.

All staff and authorized personnel should enter through the designated entrance lobby. Outdoor clothing and footwear should be deposited in the designated area and a clean set of footwear and coveralls should be provided before entering the facility. There

should be no chance of cross-contamination between the "clean" and 'dirty' sides of this entrance.

Footbaths will be provided and stationed at the main entrance and will be inspected daily and maintained weekly to ensure germicidal effectiveness. Staff will be provided with rubber boots and outer clothing and this should be left in the facility. There will be no visitors to the quarantine system. All personnel authorized to enter the facility will be determined prior to fish entering the system for that 90-day quarantine period.

Security

The facility will be locked at all times. Only designated staff will have access to the fish or the systems. Signs above the entrance door will advise of restricted access and will indicate that this is a quarantine facility. A sign-in sheet will list all personnel entering the facility during the 90-day quarantine period. Specific individuals will be assigned to husbandry and maintenance of the facility for each specific 90-day quarantine period. Those individuals assigned to care and observe fish during the quarantine period will review quarantine protocols, disinfection policies, biosecurity protocols, etc prior to accepting responsibility for the fish during the 90-day quarantine period. Personnel should not change during the 90-day quarantine period. Personnel assigned to the isolation facility should have limited exposure to other species at CCAR as directed by the best professional judgment of the CCAR director.

Fish Health Testing

Fish that are introduced to the facility from the wild will not have health testing records for obvious reasons. Fish will be observed daily for obvious clinical signs of disease, lack of appetite, unusual swimming behavior, change of color etc. Mortalities will be removed and recorded daily. An increase in morbidity or mortality exceeding 1% /day will require that the facility be visited by both CCAR director and a fish health professional within 24 hours.

Relatively small numbers of valuable fish will be collected and placed in quarantine at any given time. For this reason, it is not practical to lethally sample a proportion of the population sufficient to satisfy normal fish health testing requirements typical for cultured lots. Fish health testing will be implemented as follows. At capture a blood sample will be taken from each fish. A sub-sample of each blood sample will be submitted to a fish diagnostic laboratory for evaluation. The number of samples submitted for diagnostics will be determined by the best professional judgment of the CCAR director. Diagnostic tests on samples may include viral, bacterial and/or parasitic pathogens. Diagnostic tests performed on blood samples may vary depending upon the species entering in the isolation facility. Unused blood samples will be archived during the 90-day quarantine period. The quarantine period will be a minimum of 90 days. During this time, any mortalities and moribund fish will be necropsied in the facility and appropriate diagnostic samples (eg., kidney, liver, spleen, brain and eye) will be sent to a fish pathology laboratory for testing. This testing will may include cell culture for viral pathogens and microbiological culture for pathogenic bacteria. Appropriate histology samples may also be collected. Mortality remains will be frozen and then taken off site for composting. Any eggs or milt that may be released or collected from any mature fish

that happen to spawn during the quarantine period will also be tested for virology and bacteriology.

At the end of the quarantine period, the CCAR director will determine if morbidity levels, mortality levels, and pathogen levels warrant movement of the fish into the broodstock holding system. Fish that do not meet CCAR standards will be destroyed and will not be returned to the wild under any circumstances. Fish that meet CCAR standards to be included in the broodstock program will then participate in CCAR's broodstock health program. Currently (2006) that program focuses on testing offspring from broodstock rather than direct testing of brood fish.

System Maintenance

Key maintenance tasks and scheduling will include

- Pulling standpipes to flush solids (daily)
- ORP probe calibration (weekly)
- Drum filter screen cleaning (monthly)
- Settlement tank clean out (every 6 months)
- UV sterilizer bulb replacement (every 9,000 hours)

To ensure effective sterilization, effluent water, sample will be plated on a routine basis to confirm 99.9% kill rate of bacterial organisms.

After every batch of fish has passed through the quarantine system, the entire system, including the biofilter will broken down and cleaned with soap and hot water, then flushed with bleach.

The system will be left dry prior to restocking.

In the event of equipment failure or the need for repairs, all tools and equipment entering and then leaving the facility will be disinfected. Personnel assigned to repair equipment during a 90- day quarantine period will be instructed on disinfection and biosecurity.

Audits

The quarantine system and protocol will be developed in conjunction with fish health experts and in consultation with the New England Fish Health Technical Committee. A sub committee will inspect the facility and CCAR will make any necessary modifications to ensure that it meets with their approval.

Following commissioning, it is suggested that a periodic audit be carried out to ensure that standards are maintained or that advances in the industry, where applicable and reasonable, be implemented to maintain the highest standards of biosecurity.

Record keeping

All water quality measurements, stock inventory, mortalities, feed rates and health testing records, and treatment records will be kept at the CCAR for a minimum of 5 years. A record of all individuals trained for work within the facility will be kept.

6. D. Demonstration

Once the quarantine system was completed, it was decided that fish would be selected from the population of cod at the net pen site. These fish were deemed valuable since they were F1 generation and faster growing fish, if selected, would be superior broodstock. At the time, the salmon at that site in adjacent net pens had been culled due to the detection of ISAV. UMaine applied for a transfer permit to bring selected cod from Eastport to Franklin. DMR consulted the Fish Health Technical Committee who decided that even though the quarantine system had been inspected and approved, a full round of fish health testing was needed before making a decision. This was done and the tests were negative for all pathogens of regulatory concern (see Appendix). This finding in itself is of great significance and confirms recently published data on the susceptibility of cod to ISA (Snow and Raynard 2006). However, the Fish Health Technical Committee ruled against the transfer of these fish to Franklin, applying the "precautionary principle". Several attempts were then made to catch wild fish at Eastport in the fall of 2005 but these were not successful. At this time, to test the system, the remaining 12 cod broodstock were transferred to the facility and the survivors were recently donated to Great Bay Aquaculture as a source of eggs for their 2007 spawning run. The system now holds 80 F1 halibut broodstock.

These quarantine protocols have been disseminated to fish health professionals within the region including IF&W, DMR, USDA, the Maine Aquaculture Association and other industry stakeholders. They are to be incorporated into the new fish health regulations currently being drawn up by the Maine DMR.

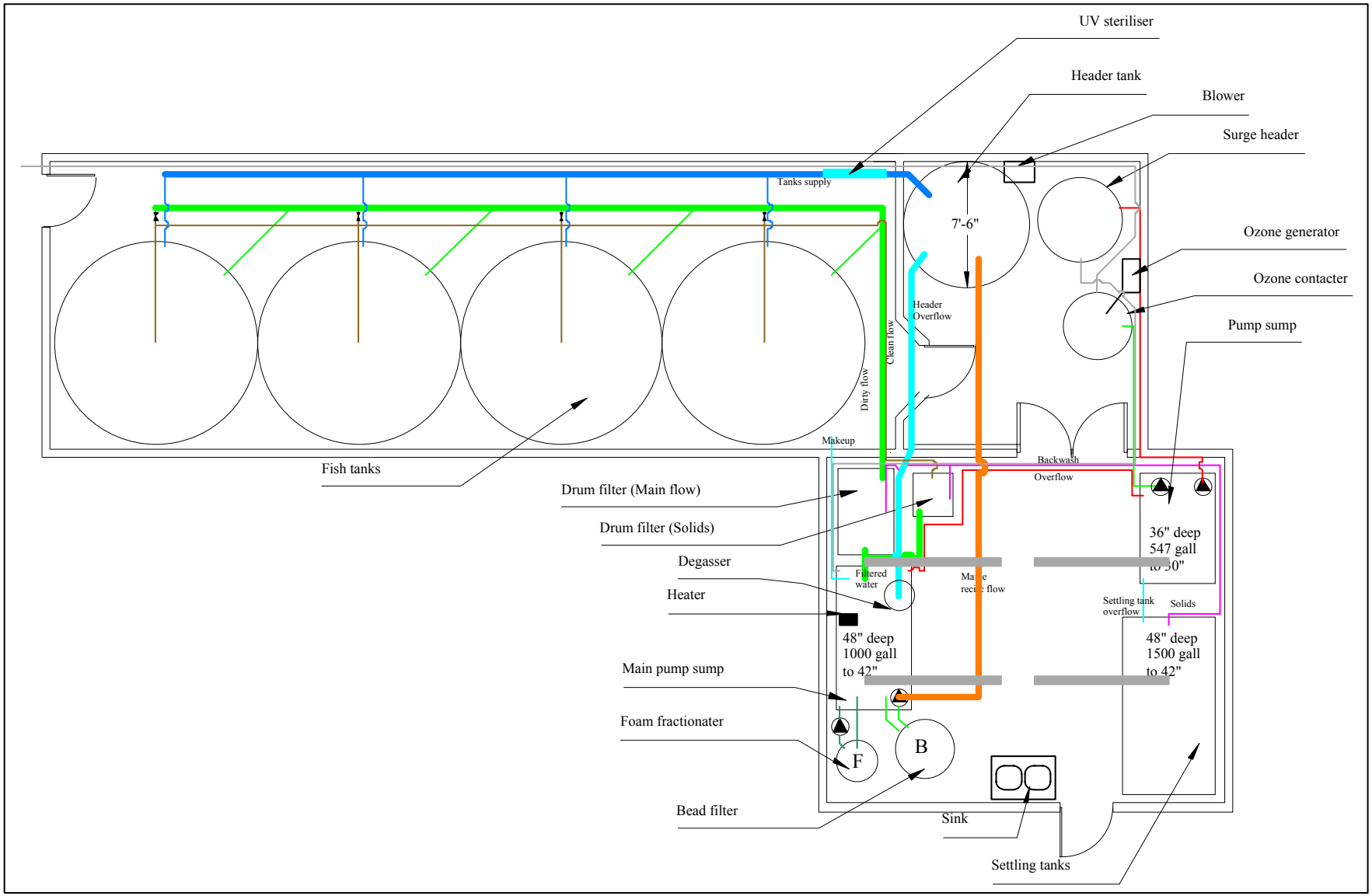


Figure 6.1. Quarantine system schematic.

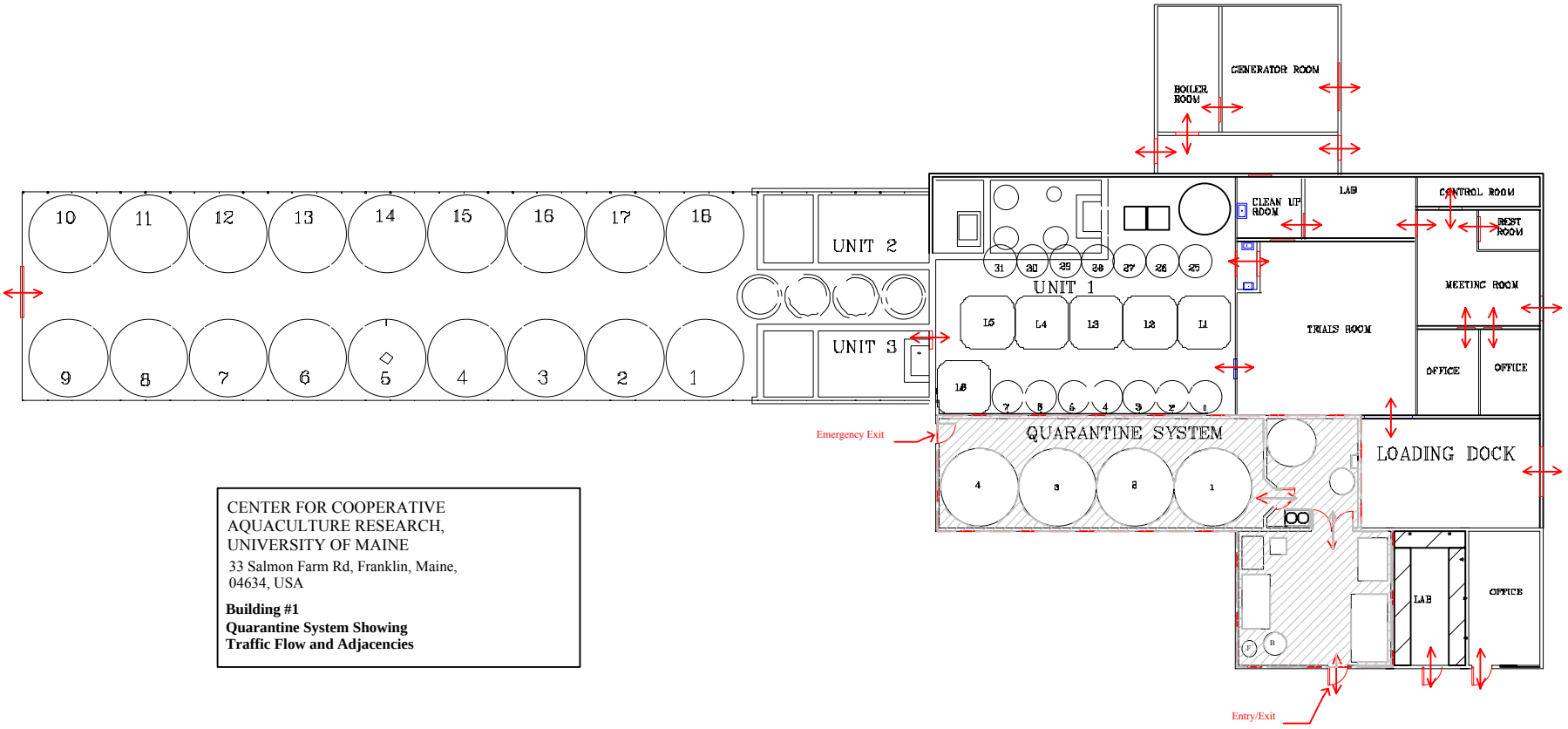


Figure 6.2. Building 1 layout including quarantine system.

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8. Overall Conclusions and Dissemination of Project Findings

Current trends suggest that the cod industry in the region will continue to develop in the coming years. At the time of writing, a NOAA funded project with Great Bay Aquaculture is about to get underway at the CCAR. The main industry player in the region, Cooke Aquaculture, has made a major commitment to development of cod farming and has some 200,000 juveniles lease sites in New Brunswick and a Canadian funded cod breeding program is underway there. Meanwhile, the development of the industry in Norway and Scotland continues apace. Product from Europe is already reaching Boston and New York markets, adding to the seafood trade deficit for the US.

This project helped to identify potential bottlenecks as well as to demonstrate some of the potential characteristics of the Atlantic cod that make them suitable for culture in the region. They are straightforward to culture in the hatchery, they transport well, they are suited to the environmental conditions in the region and there seems to be no reason why they cannot be reared in similar net pen based rearing facilities, alongside salmon. Clearly, with hindsight, the fish in this study were not representative of juvenile cod that one would normally stock out to a net pen. The cataract issue affected all aspects of the performance in the growout stage and thus the data is of little use for empirical modeling the economics of cod culture in Maine. As far as cod health is concerned, the project highlighted the fact that cod eyes are susceptible to damage, that cataracts can be non reversible but also that cod are not susceptible to ISAv, and that other major diseases were not seen in this cohort of fish.

The project also demonstrated that farmed cod are comparable to wild cod nutritionally and in terms of consumer acceptance. Farmed cod is already successfully marketed in Europe and in the US.

This project was well publicized and featured on Maine Public Broadcasting Network and in the local press (Ellsworth American and the Bangor Daily news). It was also the subject of an article in UMaine today: http://umainetoday.umaine.edu/issues/v4i4/cod.html and the project is summarized on the CCAR web site: http://www.ccar.um.maine.edu/cod.html

Part of the project has been presented at the Trans-Atlantic Fisheries Technology Conference in Quebec City in October 2006 "Quality evaluation of farm-raised Atlantic cod" by Denise Skonberg, Shari Baxter, and Nick Brown and this work is to be submitted to the Journal of Food Science. There are further plans to publish the quarantine system design and protocols.

William Palmer, the graduate student on the project has already moved out to the commercial sector and is employed as a manager of a new cobia farm in West Virginia. He gained valuable training whilst involved in the project.

The quarantine protocols have been disseminated to fish health professionals within the region including IF&W, DMR, USDA, the Maine Aquaculture Association and other industry stakeholders. They are to be incorporated into the new fish health regulations currently being drawn up by the Maine DMR.

9. Appendix: Health Reports

Health reports

Friday, May 14, 2004 10:38 AM -0500

From: Hugh Ferguson <h.w.ferguson@stir.ac.uk>
Subject: R040120
To: Stephen Eddy
Cc: "Opitz Mike (Opitz, Mike)" <mopitz@umext.maine.edu>
Attachments: Attach0.html 12K

Dear Steve,

Your cod samples were very interesting, and somewhat confusing. Major lesions were found in eyes, gills, and there were a few incidental lesions elsewhere. Eyes were indeed badly affected; some had pronounced cataract, but all had varying degrees of retinal atrophy, most markedly affecting photoreceptors, and those that were centrally located. I had the impression that rods were more affected than cones, a few of which still remained in otherwise totally devoid areas. A couple of fish had obvious retro-retinal gas bubbles (choroidal rete). A couple of eyes were really quite far advanced, with early stages of lysis of lens.

The gill lesions comprised widespread single-cell infection of immediately sub-epithelial cells in lamellae - the affected cells were cytomegalic, with both nucleus and cytoplasm involved. They looked like early lymphocystis, but they also seemed to be too small, so I am not sure. I wondered also about rickettsia/Chlamydia. It would be worthwhile doing some virology/and/or EM just to see what's there. If I have any tissue left in formalin, I'll have a quick look in the TEM.

A few fish had several foci of microsporidia, and all had peritoneal granulomatous inflammation - I assume they've been vaccinated?

So, more questions than answers here. I'm inclined towards the possibility of excess light for the retinal lesions, but combined possibly with some gas bubble disease early on. A regular sampling protocol might help to sort these things out.

Hope that this helps and is interesting.

micro technologies, inc. 18 November 2005

FISH HEALTH REPORT
Accession No. M05102001

Contact/Company: Nick Brown, Ph.D. Center for Cooperative Aquaculture Research 33 South Bay Road Franklin, ME 04634

Samples/Sources: One hundred fifty (150) Atlantic cod (*Gadus morhua*) from Stolt Sea Farm's Johnson Cove marine site were sampled on 20 October 2005 at Micro Technologies. Fish were collected and transported to the laboratory on ice by Dr. Steve Ellis of USDA.

Diagnostic Tests:

Test	No Tested	Pooling	Diagnostic Tool	Tissue	Comment(s)
Bacteriology	150	Individual	50/50 TSA	Kidney	
Viral culture	150	5-fish	CHSE/EPC/SHK SSN-1	Kidney/Spleen & Brain/Eye	Brain/Eye tested only on SSN-1 cells
PCR	60	2-fish	ISAV rt-PCR	Kidney	Only first 60 tested
PCR	15	Individual	VNNV rt-PCR	Brain	
Histology	15	Individual	H&E stain	Kidney/Spleen/Liver/Heart Gill/Brain/Pyloric caeca	
Parasitology	15	Individual	Direct observation	Gill/Skin scrape	

Results:

Necropsy/Parasitology**:** Necropsies were unremarkable except for the presence of cataracts inalmost all of the fish. No external parasites were observed in either the gills or on the skin.

Bacteriology**:** Bacterial growth ranging from few colonies to confluent growth was observed on 93% of the sectors streaked from kidney samples. The predominant colonies were identified as *Photobacterium damsela* and *Vibrio spp.* most similar to *V. logei* and *V. tubiashii*. These organisms were present in moderate to confluent levels in the majority of the sectors, with two bacterial species co-present in most cases. Colonies of *P. damsela* and the two *Vibrio* species were observed in equal frequency on the sectors.

Virology**:** All cell lines were **negative** for any observable cytopathic effect after 28 days of incubation.

PCR**:** All 15 fish tested for VNNV using brain tissue were **negative** using the Gagne/DFO primer set. Kidneys tested from 60 fish (in 2-fish pools) were also determined to be **negative** for the presence of ISAV. However, additional testing was required before this could be concluded due to the observation of a single amplification band in a majority but not all of the samples tested that was similar in size to that expected from ISAV. The testing involved additional amplifications on a portion of the samples using the FA3/RA3 and 1D/PM41 primer sets specific for ISAV genome segment 8, and a third set of primers specific for ISAV segment 6 (Cook 04). When similar suspect bands were also observed with these primers, several of the original amplifications were analyzed by DNA sequencing. Results indicated that the sequences did not

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share any significant homology with any sequence in the databases searched and that the bands were likely a result of non-specific binding by the primers, resulting in spurious bands in some of the samples that were coincidentally of similar size to those expected from ISAV. Although we can conclude from the overall results that the cod presently tested are free of ISAV, this sort of phenomenon has not been observed in routine samples from Atlantic salmon or wild fish sampled to date, except for a single group of pollock, indicating that this may need to be investigated further before the results can be satisfactorily explained.

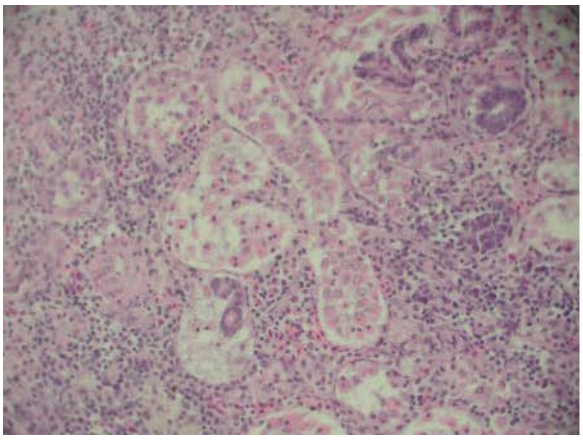
Histology: Many (8 of 15 sampled) of the fish had tubular necrosis of the posterior kidney. Diffuse (15/15) bridging of the secondary gill lamellae was also observed. Occasional nodular inflammation of liver parenchyma (2/15) and spleen (1/15) were also noted. All but one of the fish sampled had mild to moderate hemorrhage present in one or more tissues or organ systems, likely secondary to handling or transport. Photos of representative lesions (3 digital images, total) of conditions mentioned accompany this report. Significance of these findings is unknown. Systemic bacterial infection may explain all lesions, including lamellar bridging.

Sincerely,

William Keleher

William R. Keleher Michele Walsh, DVM Fish Health Inspector Aquatic Animal Veterinarian

Histology Micrographs



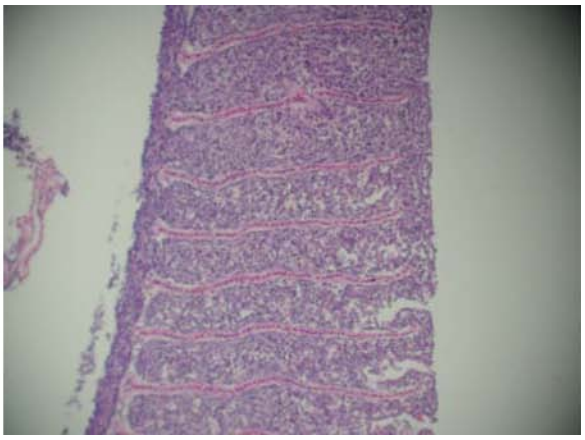


Figure A: Kidney tubular necrosis **Figure B:** Gill lamellar bridging

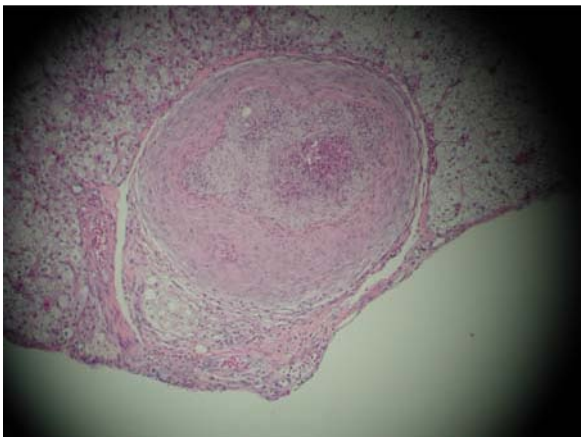


Figure C: Nodular inflammation of liver parenchyma

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Aug. 8, 2003

Fish Health Report
Micro Technologies Accession #M03071003

Contact/Company: Nick Brown, PhD.
Center for Cooperative Aquaculture Research
33 South Bay Rd.
Franklin, ME 04634

Samples/Sources:
60 fish were sampled from the CCAR facility on 7/10/2003 for this screening from a production lot with a population of 15,000 juvenile cod (*Gadus morhua*). Fish were picked up by lab courier, transported on ice and arrived in good condition the same day.

Diagnostic Tests:
All submitted fish were necropsied at Micro Technologies and sampled for bacteriology (TSA 50/50 agar) and virology (using the EPC, CHSE-214, SHK and SSN-1 cell lines). In addition, 6 fish were screened for parasitology (ecto & endoparasites) by skin scrapes and gill clips. 5 fish were submitted for histological screening using a variety of tissues ,including eye and brain.

Results:
No pathogens of regulatory concern were observed through any of the screening tests used.

Necropsy/Parasitology: necropsies were unremarkable; no external or internal parasites were observed from any sample.

Virology: All cell lines used were negative for observable cytopathic effects after a 28 incubation period.

Bacteriology: No bacteria of regulatory concern were isolated from any of the fish tested. An incidental finding from the bacteriology was a moderate prevalence (9 of 60 sectors) of a bacterial species isolated from the kidney inoculae and identified through morphology, biochemical and API20E profiles as being most similar to *Vibrio harveyi*, a typical environmental bacterial species. This species has been observed previously in other farmed fish, including cod.* *V. harveyi* is not usually associated

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with primary pathology or increased mortality in cod, but may be a secondary/opportunistic bacterial species and should be considered in that context. The general population should continue to be monitored for any increased mortality.

Histology: The histology was reviewed from the 5 fish submitted for slide preparation, and was generally within normal limits of interpretation. Gill lamellae were hyperplastic in 2 of 5 samples, with some inflammation evident. No overt disease-related pathology was observed in any of the samples.

If you have any questions, please get in touch anytime. Thanks for using Micro Technologies, Inc. for your fish health needs.

Sincerely,

Deborah A. Bouchard
AFS Fish Health Inspector
Canadian Fish Health Official
USF&W Title 50 Inspector

Peter L. Merrill, DVM
Aquatic Animal Veterinarian

micro
technologies, inc.

04/30/04

Fish Health Report
Micro Technologies Accession #**M04040801**

Contact/Company: Nick Brown, PhD.
Center for Cooperative Aquaculture Research
33 South Bay Rd.
Franklin, ME 04634

Samples/Sources: 60 juvenile Atlantic cod (*Gadus morhua*) were sampled from 2 tanks at CCAR. The 60 fish were randomly selected for fish health inspection purposes.

History: The one year old juvenile cod were assigned lot # M04-016C and consisted of 22,000 fish. Lot M04-016C originated as eggs from broodstock held at CCAR and GBA facilities. The average weight per fish was ~150 grams.

Diagnostic tests included necropsy, bacteriology, virology (SSN-1, CHSE, EPC, and SHK cell lines), PCR for nodavirus, and histology.

Necropsy: A moderate number of cod had very pale gills. No other overt abnormalities were observed during necropsy and skin and gill scrapes from four fish produced no significant findings.

Bacteriology: Culture samples were inoculated aseptically from kidneys onto 50/50 TSA plates. Few-to-moderate colonies of mixed bacterial growth were observed on 25 of 60 kidney sectors. Confluent growth was observed on 3 of 60 sectors, but all three sectors had different isolates identified (*Vibrio cholerae* non-01, *Vibrio spp.low* discrimination, and *Pseudomonas fluorescens*. Other rare isolates keyed out to *Vibrio/Photobacterium* species with low discrimination for species identification.

Virology: No CPE was observed 21 days post-cell culture inoculation on any of the cell lines. Two random cell well pools were selected from the SSN-1 cell line and the supernatant was tested by RT-PCR for Nodavirus. RT-PCR results were negative for Nodavirus.

PCR: Brain tissues and optic nerve tissue collected from 6 fish were tested for Nodavirus by RT-PCR. All six fish tested negative. Positive and negative controls tested appropriately.

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Histology: Large numbers of unidentified parasites in cross-section (likely protozoan in nature) were observed along the gill lamellae of most of the samples used for histology. Some inflammatory damage to lamellae had occurred. Other tissues, including hearts, kidneys, livers, spleens, GI, brain/nerve tissue, and eyes were within the normal limits of interpretation.

Comments: A formalin bath treatment was prescribed as a result of the gill parasites.

If you have any questions, please get in touch anytime. Thanks for using Micro Technologies, Inc. for your fish health needs.

Sincerely,

Peter Merrill, DVM
Aquatic Animal Veterinarian

Deborah A. Bouchard, **President**
AFS Fish Health Inspector/ US Title 50 Inspector/Canadian Local Fish Health Official