Burbot Conservation Aquaculture Progress 2007

A program funded by the Kootenai Tribe of Idaho

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Administered by the Kootenai Tribe of Idaho through a contract from the Bonneville Power Administration as part of the Northwest Power and Conservation Council’s Fish and Wildlife Program (Project 198806400, Contract No. 00020490).
Abstract - Development of burbot conservation aquaculture has been ongoing at the University of Idaho since 2004. Cooperative efforts between regional US, provincial, and tribal entities have made this project a success. In 2004, project efforts confirmed that burbot could be produced in a hatchery. During 2005 and 2006, specific experiments concluded that: 1) captive burbot spawn with or without the use of exogenous hormone, 2) despite gender segregation, females in a recirculation system may volitionally release their eggs, 3) conical egg incubators with volumes ≤ 2 L produce higher egg survival than cylindrical 6 L McDonald type jars, 4) larval burbot fed live prey (marine rotifers and Artemia) for prolonged periods (at least 52 days) survive significantly better than those fed commercial marine cod diets for the same time period, and 5) cryopreservation methods for burbot semen were successfully adapted for North American stocks based on existing technology.

During 2007, all burbot project activity at the University of Idaho was relocated to a different facility on campus where an existing rearing system was modified to accommodate burbot. An incubation and larval rearing system and a marine rotifer system were constructed. The 2007 production goal was 5,000 commercial feed-transitioned burbot. At the onset of the 2007 spawning season 43 broodstock were being held (25 females and 18 males). Nineteen of the 25 females ovulated, and 18 of 18 males produced milt. Approximately 3.2 million eggs were collected and fertilized. Spawning and egg incubation began February 13th and ended April 18th. Embryo and larval losses occurred due to various reasons. Some larvae escaped rearing tanks despite 500 micron mesh screening and intensive feeding methods resulted in a gill disease outbreak. Overall, 850 commercial feed transitioned burbot were produced and used for disease susceptibility investigations, including IHNV, IPNV, and F. psychrophilum. Although we did not meet our 2007 production goal, the 2007 activities resulted in the highest survival of commercial feed transitioned burbot juveniles to date (compared to 73, 0, and 3 artificial feed-transitioned individuals remaining during 2006, 2005, and 2004 respectively). Aquaculture incubation and rearing system design changes and feeding strategy modifications are expected to further improve production during 2008.

Additional related research supported in 2007 included: 1) biological pattern recognition studies (BioPar, Anchorage, AK), 2) sample collection and provision for investigating a novel fungus infecting burbot in the Columbia River Basin, 3) preliminary investigation into burbot egg fungal control methods using formalin, and 4) donation of ~100,000 eyed burbot embryos for a preliminary study of extensive rearing with the Idaho Department of Fish and Game.
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Introduction

Burbot (*Lota lota*) are the only freshwater member of cod family, *Gadidae*, and exist in lakes and rivers of the northern hemisphere (McPhail and Paragamian 2000, Van Houdt et al. 2003). In Idaho, USA, burbot are native only to the Kootenai River and are in the last stages of severe decline due to anthropogenic habitat alterations (Paragamian 2000, Paragamian et al. 2000). The population has been referred to as functionally extinct. With little hope for recovery through natural production for this population following nearly a century of habitat loss and alteration and hydro development (Anders et al. 2002), a conservation aquaculture program is being developed as part of a multidisciplinary, international conservation strategy for Kootenai River burbot (KVRI 2005).

Development of conservation aquaculture techniques for burbot began in 2004, following cooperative efforts among the University of Idaho, British Columbia Ministry of Environment, Kootenai Tribe of Idaho, Idaho Department of Fish and Game, and the Kootenai Valley Resource Initiative’s burbot conservation aquaculture subcommittee, to evaluate whether burbot could be produced in a hatchery setting. Experiments during 2004, 2005 and 2006 confirmed that: 1) captive burbot spawn with or without the use of exogenous hormone, 2) despite gender segregation, females in a recirculation system may volitionally release their eggs, 3) conical egg incubators with volumes $\leq$ 2 L produce higher egg survival than cylindrical 6 L McDonald type jars, 4) larval burbot fed live prey (marine rotifers and *Artemia*) for prolonged periods (at least 52 days) survive significantly better than those fed commercial marine cod diets for the same time period, and 5) cryopreservation methods for burbot semen were successfully adapted for North American stocks based on existing technology.

All University of Idaho burbot related activity was re-located to a different wet lab facility on campus during 2007. The current facility is located at the Aquaculture Research Institute’s main office building on the University of Idaho campus. Formerly used for poultry research, it was converted to a fish rearing facility around 1990. Many aquaculture projects have been carried out at this facility in the past, however, typical water temperatures (Figure 1) were more suited for rearing warm-water or cool-water fish species (i.e. walleye, bluegill, crappie, bass, and tilapia have been raised in this laboratory). Therefore, a water chilling unit was installed for burbot rearing and spawning in this system, and a combination incubation and larval feeding system was constructed. A recirculating rotifer production system was also constructed to meet the initial feeding demands of larval burbot.
2007 Project Goals and Objectives

2007 Goal:

Produce 5,000 artificial feed transitioned burbot for use in developing disease diagnostics and to further develop and refine burbot aquaculture techniques and apparatus.

2007 Objectives:

1. Transition to a new facility, build and observe functionality of rearing systems for broodfish, egg incubation and juvenile feeding, and live prey production.
2. Further develop standard operating procedures for burbot rearing.
3. Support related research.

Methods

New laboratories.-Two rooms were designated for burbot aquaculture development: 1) a main laboratory, and 2) a live prey culture laboratory. The main laboratory is approximately 140 m². The water source is treated domestic city water, supplied to the building through a 3.2 cm inflow line. Incoming water is high in chlorine and has no dissolved oxygen. The incoming chlorine is neutralized with activated carbon, sodium thiosulfate, and aeration upon entry into culture tanks. Laboratory lighting consists of incandescent and fluorescent lights (52-94 lux) electronically controlled with timers.

The live prey culture laboratory is approximately 13 m² and uses treated domestic city water. Incoming water is filtered through a 375 L per d reverse osmosis unit, consisting of a 24.5 cm 1-5 micron particulate filter, a 24.5 cm activated carbon filter, 24.5 cm deionization resin, and a 24.5 cm reverse osmosis membrane.

Broodfish system (main laboratory).-This aquaculture system was modified by installing a 3 hp inline water chilling unit to maintain critical spawning temperatures (2-4°C). The system consists of 9 circular fiberglass tanks (three 1.8 m circulars, 2,500 L maximum capacity and six 1.5 m circular fiberglass tanks, 1300 L maximum capacity) and has a 4200 L settling basin. This system has a total capacity of 23,000 L. Treated city water entering this system is pre-filtered through activated carbon. Water within the recycling system is further treated with two sand filters, one activated carbon filter, UV, sodium thiosulfate is added (100-1000 g, based on weekly water quality analysis to neutralize chlorine before use), and is aerated upon entry into culture tanks. Water is recycled up to 340 L per min. To prevent egg loss due to volitional spawning, the adult holding tanks effluent passes through 500 micron mesh screening.

Incubation and larval rearing system (main laboratory).-This system was designed to incubate burbot eggs, collect hatching embryos, and start developing larvae on feed. Treated city water entering this system is pre-filtered through activated carbon, and sodium thiosulfate is added (5-50 g) based on weekly water quality analysis, to neutralize chlorine before use. Water is chilled by recycling incoming water between a chilling sump and an insulated head box using three 1 hp impeller style chillers and one 1/3 hp in-line chiller. Chilled water exits the head box and passes through the incubators and is not
reused, thus providing developing embryos and first feeding larvae a flow through environment. This system consists of twelve 0.9 m circular insulated black plastic tanks, each with a 225 L maximum capacity. Two 2.5 cm inflow lines supplied water to 12 tanks. Thus, 6 tanks were fed by a single 2.5 cm line. Each 2.5 cm line has twelve 1.25 cm needle-valves with 2 valves per tank (Figure 2).

To incubate eggs, conical incubators 1 or 2 L in volume (2 or 3 per tank) were suspended above each 225 L rearing tank. Water up-welled through the egg masses and then overflowed into the rearing tanks, thus eliminating handling of embryos at this sensitive stage of development (Figure 3). Post-hatch, based on past observations, it was known that the very small larvae (3-5 mm) have a tendency to escape. Outflows were therefore screened with 500 micron mesh to prevent larvae escape. Additionally, effluent from all 12 tanks was passed through a separate 225 L tank screened with 500 micron mesh to temporarily catch and hold escaping larvae for recapture. For commercial feed transition, automated feeders were suspended above the rearing tanks. Based on the low light conditions of the main laboratory (54-94 lux), and positive results with cod and European burbot culture efforts, incandescant lights were added above the incubation/rearing tanks to increase light intensity to approximately 1900 lux. An automated local 24 h photoperiod regime provided additional lighting during the larval feeding stages.

**Live prey culture.**—A third system was constructed for intensive rearing of L-type marine rotifiers (for description of L-type rotifers please see larvae feeding section of this report) in a separate room from the main burbot rearing laboratory. This system consisted of two conical bottom 250 L black plastic tanks (one for rotifer rearing and the other for biofiltration). This system was equipped with a protein skimmer, an additional upwelling sand filter for biofiltration, and an automated algal feeding system (Figure 4). Additionally, three 35 L Rotifer aquaria were maintained as a back-up in case the system crashed. *Artemia* were batch cultured in clear 19 L water containers supplied with full spectrum fluorescent lighting (approximately 1900 lux). Batch cultures were agitated with forced air. Water temperature was maintained near 28˚C for hatching cysts.

Live prey culture methods typically followed recommendations of The Plankton Culture Manual, 5th edition (Hoff and Snell 2001). In addition, Ken Massee and Tom Wade of NOAA Fisheries (Manchester, WA) were consulted and provided invaluable insight for maximizing production in sub-optimal temperature and salinity conditions for specific rotifer species (e.g. temperature below optimal 15-18˚C, low salinity < 4 ppt). Rotifers are produced in sub-optimal conditions to acclimate them towards a freshwater existence so they live longer when feeding to larval burbot. Additionally, enrichment reconstitutes (Roti-rich®, Florida Aqua Farms Inc., Dade City, FL) were added to cultures at supplier recommended times and rates to boost the nutritional value of feeding treatments.

**Broodstock and gametes.**—Thirty adult burbot were captured from Moyie, BC, during November 2006 in cooperation with the British Columbia Ministry of Environment and the Idaho Department of Fish and Game and were transported to the Aquaculture Research Institute cold water laboratory on the University of Idaho campus in Moscow, where they were equally divided among six 1,000 L tanks in an isolated recycling water system (~10,000 L). While Moyie adults were acclimating, mortalities and moribund fish were removed and disposed of or submitted to the Washington Animal Disease and
Diagnostics Laboratory for necropsy, histopathology, bacteriology, virology, or donated for an ongoing investigation of a novel internal fungal disease of burbot (Contact Dr. Tim Walsh for further information at WalshT@si.edu).

Burbot broodstock were fed live rainbow trout (5-60 g) ad libitum or on demand. Specific pathogen free certified eyed rainbow trout eggs were purchased from Troutlodge (Sumner, WA), and raised as feed for the broodstock. Feeding live rainbow trout required additional resources and the Aquaculture Research Institute provided in-kind support by rearing them at their coldwater laboratory. One month after the new adult system was online (December 2006), remnant burbot from past experiments (Duncan Reservoir and Arrow Lakes Reservoir, BC, populations consisting of 9 males, and 13 females, average mass 1.9 kg), were stocked to seed biofiltration and to ensure the system would support Moyie Lake burbot. Duncan and Arrow lakes burbot were moved to the new facility in January 2007. Moyie burbot (population 21 (gender unknown), mean mass 2.1 Kg) were transferred to the new laboratory during February 2007. Moyie adults were examined via ultrasound to determine their gender February 7, 2007.

Following gender determination, hormone (sGnRHa) implants, designed for advanced maturation and spawning synchronization of salmonids (Oviplant 75 µg, Syndel International, Vancouver, BC), were given to females to foreshorten the spawning period. Hormone implants occurred on February 15, 2007. Implants were inserted in dorsal white muscle about 1cm lateral between the dorsal fins. The injection site was carefully wiped dry with a soft towel and a 1% iodine solution was applied to the injection site surface as a measure to prevent internal transmission of external pathogens. A fabricated passively integrated transponder (PIT) tag syringe was used to inject the implants. The antibiotic Neosporin® was applied to the wound following all hormone implant injections.

Catheter sampling occurred on two different occasions to examine egg development. The catheter consisted of a small piece of clear tubing (approximately 3 mm O.D.) attached to a 1 mm syringe. The basic procedure included: 1) disinfecting tubing with 1% Iodine solution, 2) wiping the urogenital area dry, 3) inserting the tubing and, 4) gently using the plunger to pull a sample into the tubing. Samples were examined, under 3X magnification, by looking for a coalesced oil globule within the egg (Figure 6) that indicated when a female was near ovulation (Rob Holm, USFWS Garrison Dam National Fish Hatchery, personnel communication 2007).

Manual gamete collection occurred twice per week or when a volitional spawning event was observed. When volitional spawning events were observed, all females were removed, anesthetized, and weighed to determine which fish had spawned. Each time volitional spawning occurred, eggs were salvaged from the bottom of the tank siphoning. All females that had not spawned prior to the day of handling and males selected for use on a given day were handled each gamete collection day. When a ripe adult became available, the area around the urogenital opening was wiped dry with soft towels and the fish was carefully hand stripped to collect gametes. Gametes were stripped into 500 ml sterile plastic bags. Ova were split between two bags and individual milt collections were kept in separate bags. All gametes were stored in a refrigerator (4°C) until being mixed for fertilization. One female Arrow Lakes adult was sacrificed in an attempt to lethally collect ova. This attempt was made because it was getting late in the spawning season.
This fish was lethally anesthetized, ovaries were removed, ovaries split lengthwise, and carefully stripped into sterile plastic bags and manually fertilized.

Fertilization followed standard dry method procedures (Piper et al. 1982). Briefly, eggs were split between two bags, one male’s milt was added to each bag, mixed gently, and activated with chilled rearing system water. Following sperm activation (at least 1 minute), egg masses were rinsed several times. Following rinse, eggs were transferred to a refrigerator (4°C) for water hardening. Water hardening was allowed for at least 90 min. Eggs were not disinfected during water hardening or before being transferred to the incubation units.

Sub-samples of eggs were pulled from each egg mass and enumerated. A 3-5 ml sample of eggs was drawn into a 20 ml syringe equipped with a piece of tubing long enough to reach the bottom of the egg mass. As the sample was drawn into the tubing, the tubing was pulled up through the egg mass to provide a representative sample. Then 3 or 4 sub-samples were drawn up into 1 mm syringes and settled volumes were measured using the graduations on the syringe barrels. Once a volume measurement was taken, the eggs were ejected onto a counting wheel and counted. Sub-sample counts were averaged and multiplied by the total settled volume to estimate the number of eggs in a given egg mass.

Additionally, sub-samples of milt were transported to the U of I Biological Science Department for establishing a germ plasm repository in the cryopreservation unit purchased for KTOI. Steve Patton provided support for sample processing and semen was stored in Dr. Joe Cloud’s laboratory (Table 3).

_Egg incubation._—Incubation water flows were maintained from 420 to 1,380 ml/min (higher compared to past incubation trials of 50-300 ml/min due to more and larger egg masses collected (150-500 ml eggs). Water flows were adjusted periodically so incubating egg masses continuously moved (rolled) yet eggs were not leaving incubators. Incubators included 2 L commercial pelagic egg hatching jars, called mini-egg hatching jars, and 1 L customized upwelling Imhoff cones (Figure 5). Incubator effluent was not filtered or screened and water flows were adjusted if eggs were observed leaving. Regarding egg mass settling and periodic adhesiveness, those egg masses were gently stirred to break up the clusters.

Egg fertilization success was estimated 48 h post stocking. Sub-samples from each egg mass were examined to determine if fertilization was successful. Using 3X magnification, eggs were examined for a uniform blastomere (cluster of cells atop the vitelline membrane or yolk) indicating successful fertilization (Figure 6). Percent fertilization for each incubator egg mass was estimated by counting 3 or 4 subsamples from each incubator and counting good eggs (live; i.e. blastomere apparent) and bad eggs in each sub-sample (dead; i.e. cloudy in appearance, irregular in shape and size relative to the others in sample, no blastomere; Figure 6).

To treat for fungus, formalin (37% active formaldehyde) was added to the inflow lines (flow rate 50 ml/min) at 1000 ppm for 20 min daily. Formalin was introduced via drip over a series of incubators until a hatch was observed. Formalin treatment was terminated for each hatching jar when hatch was observed.
Embryo and larval rearing.-Water temperature was electronically controlled to maintain water temperatures near 3°C. Hatched embryos were allowed to swim out and collect into rearing tanks. This method was employed based on recommendations by European burbot culturists and past experiences where high mortality (~80%) followed handling during this developmental period. When an egg mass completely hatched, the larvae were left undisturbed for 10 d, with the exception of periodic individual examinations of development to determine when to begin adding live prey. Morphological attributes such as yolk absorption and mouth and gastrointestinal tract development were monitored with each larva examined. When a complete alimentary tract was apparent (mouth to anus; Figure 7) feeding live prey to the culture tank began.

Larval feeding.-Larvae were fed algae (Nannochloropsis spp.), marine rotifers (B. plicatilis, L type, average size 280 microns TL, optimal temperature range 18-25°C, salinity 10-20 ppt, pH 6.5-8.0), Artemia nauplii (Great Salt Lake strain, 90%+ hatch rate guaranteed, typically in the umbrella stage through the second or third instar, average size 489 microns TL), and commercial cod diet (Lansy Cold Water Marine Cod Diet from INVE, Belgium, and EPAC Cold Water Marine Cod diet, particulate size range 100-1200 microns). The feeding order was: 1) algae and rotifers mixed, 2) algae and rotifers mixed and Artemia nauplii, 3) solely Artemia nauplii, 4) Artemia nauplii and commercial diet, and 5) commercial feed only. Larval feeding began as soon as a complete alimentary tract was developed (Figure 7). Feeding larvae at this stage was not based on larvae density but on rearing tank volume. The minimum target feeding rate was 1 live organism per 1 ml tank volume, fed twice per day. However, feed availability was dependent on harvest(s), and prey delivery was ad libitum during hours when hatchery personnel were present. When feeding began, rotifers were harvested in the morning and fed to larvae during late-mornings and evenings. If surplus prey were available, a portion was evenly divided among all the tanks receiving live prey via drip buckets customized with a small plastic valve (Figure 8) or left out to feed the next morning. Larvae were monitored for food in the gut periodically throughout the feeding process (Figure 9) until they were no longer transparent.

Outdoor tank culture.-Although outdoor tank culture was not a priority during 2007, eight circular fiberglass tanks (six 1.5 m circular tanks 1,300 L capacity and two 1.2 m circular tanks 1,000 L capacity) were placed outside the new laboratory. Each tank was filled with approximately 200 L of treated domestic city water and 10 g of sodium thiosulfate to neutralize residual chlorine. Habitat structure was added to each tank including two or four 46 cm lengths of 1.8 m diameter PVC pipe. The 1.5 m circular tanks were not covered which allowed full sunlight penetration. The 1.2 m circular tanks were 75% covered and located in a shaded area to prevent sunlight penetration. All tanks were left stagnant and no commercial fertilizer was added.

Outdoor tanks were stocked with a mixture of eyed embryos and first feeding larvae (4-5mm) from April 10th through April 20th. Only one outdoor tank was stocked per d. The numbers of embryos stocked was not quantified because they consisted of escaped fish and those inadvertently siphoned during daily cleaning of the incubation and larval rearing tanks. Thus, they contained a variety of embryo and larval life stages as well as undesirable sediments. Each tank was inoculated with live zooplankton and crustaceans collected with a fine mesh net from a local pond on April 23, 2007. The exact
zooplankton species collected were not identified or quantified, although *Daphnia* and copepods were observed in the collected samples. Periodic observations were made looking for free swimming larvae and live zooplankton abundance. Aeration was added only when live juveniles were observed.

**Results**

*New laboratory and systems.*-The main laboratory systems were adequate for spawning broodfish, incubating embryos through hatch, and collecting and holding the majority of larvae reared through the onset of commercial feed transition. Thus, the burbot systems maintained water temperatures required for spawning, egg, and embryo survival which was of primary concern. However, residual chlorine was present and many larvae escaped the incubation and larval rearing system tanks during mandatory screen cleaning. Ambient laboratory temperatures averaged 19.9°C during the later part of incubation and during larval feeding which likely affected system water temperatures (Figure 10, 11). The doors to the lab had to be propped open day and night to vent out heat. However, water temperatures following the incubation period were manually increased and remained relatively constant averaging 10.6°C (Figure 10).

The live prey production room was small for current needs and prevented building a second (back-up) rotifer production system. Maintaining live prey cultures at desirable levels and optimal conditions was successful at times although difficult and affected by ambient temperature fluctuations, ciliate infestation, inconsistent prey enrichment quality, water supply and quality issues, (e.g. chlorine, ammonia, and nitrite spikes). Maintaining the rotifer system was a daily chore that took 2-3 h and was relatively expensive to feed the rotifers (Table 1).

*Broodstock and gametes.*-Annual adult (gamete) ripeness, gender determination, and general inventory began on February 7, 2007. At first inventory there were 18 male and 25 female broodfish. Ultrasound gender determination was 100 % successful and was also used to detect irregular gonad development. At the time of gender determination, 8 of 9 Moyie males were producing milt and Duncan and Arrow males were not. Overall, milt production continued through April 3, 2007. The first female spawned on February 13, 2007 and the last female spawned April 3, 2007. There were 18 observed spawning events. The mean number of days to spawn following hormone implantation, by stock, was 8 days for Moyie, 23 days for Duncan, and 37 days for Arrow Reservoir stocks (Table 2). Moreover, the individual stocks spawned at different times: Moyie fish spawned first (February 13, 2007-February 28, 2007), followed by Duncan fish (March 2, 2007-March19, 2007) and Arrow Reservoir fish (March 7, 2007-April 3, 2007) (Table 2).

Volitional spawning was common for all stocks and preceded hormone implantation for the second time during the last four years. Overall, 12 of 18 (67%) observed spawning events were volitional. Behaviors noted were similar to observations by Fabricius (1954) where females used their tail to agitate settling eggs with their tail lifting them in the water column. Despite outflow screens with 500 micron mesh, all eggs from five volitional spawns were lost due to eggs clogging the screen then overflowing. When volitional spawning was observed, we speculated that >80% of the eggs were recovered, but in such cases fecal material was always present, possibly compromising the quality of
the recovered gametes. Six females had a “rest year” (Table 2). Subsamples of milt were collected and cryopreserved from Moyie and Duncan stocks (Table 3).

The total estimated eggs collected by stock during 2007 were 4,200,000 eggs from Moyie, 828,000 from Duncan, and 828,000 from Arrow Reservoir fish. The number of eggs collected per female by stock (including volitional spawning and egg losses) ranged from 221,100 to 908,000 (mean 468,000) for Moyie, 313,000-476,000 (mean 414,000) for Duncan, and 92,000-380,000 (mean 276,000) for Arrow Reservoir fish. Fertilization estimates, by stock, ranged from 0% to 98% (mean 55%) for Moyie, 93% to 97% (mean 95%) for Duncan, and 18% to 58% (mean 33%) for Arrow Reservoir fish. The estimated number of fertilized eggs collected by stock was 2,181,000 for Moyie, 790,000 for Duncan, and 318,000 for Arrow Reservoir stocks (Table 4).

Egg incubation and larval rearing.-The incubation period began February 13, 2007 and continued through April 18, 2007. Fungus was apparent in all incubators despite fungicide treatments. Fungal manifestation began approximately 10 d post stocking. Living eggs were observed within the fungal mats. Aside from fungus, eggs clustered and adhered to one another with a gelatinous consistency, and several incubating egg masses became intermittently adhesive then de-adhesive during development.

Water temperature ranged from 4 to 6°C depending on the water inflow rate, ambient room temperature, and the number of incubators online. Average water temperature during the incubation period was 4.4°C (Figure 11). The time to first observed hatch ranged from 13 to 40 d post fertilization (mean 33 d post-fertilization). At an average water temperature of 4.4°C, that equated to 57-178 degree C d (mean 143 degree C d) to first hatch. By stock, average degree C d to hatch for Moyie was 178 d, Duncan 134 d, and Arrow Reservoir 57 d. Water temperature likely affected developmental rates (Figure 10).

Larval losses were substantial between 15-30 d post-hatch. High larval densities may have been a primary cause for initial losses, however, removing screens for daily cleaning also allowed escape losses. We did not quantify changes in larval densities to reduce handling stress, however, it was very high. For example, assuming 100% hatch of fertilized eggs (the estimated numbers of fertilized eggs per tank ranged 94,000-516,000 (mean 303,000) and a tank water volume of 150 L, equates to a range of 625-3,400 larvae per L (mean >2,000 larvae per L) in the rearing tanks. Aside from high densities, feeding larval burbot was delicate, labor intensive, and it was difficult to gauge when the larvae were ready to receive live prey.

Larval feeding.-Algae and rotifer feeding began March 21, 2007 and ended June 16, 2007. Feeding rotifers and Artemia together began May 13, 2007 and ended June 16, 2007. Artemia only feeding began June 17, 2007 and ended July 29, 2007. When all larval tanks were feeding solely on Artemia the daily live prey maintenance chores were reduced to 1-2 h. Rotifer and Artemia hatches were inconsistent and subsequent harvest quality and quantity varied (Figures 13, 14).

Commercial diet feeding began August 1, 2007. Feeding larvae and juvenile burbot commercial feed at rates of 20-80 g per d resulted in high waste accumulation and subsequent fungus, gill disease, and chronic mortality. At this point, cleaning took 6-8 hours per d to clean 17 tanks via siphon. The majority of the juveniles exposed to
commercial diet developed gill disease. High feeding rates combined with their bottom
dwelling behavior and high rearing densities exposed them to accumulating waste feed,
feces, and subsequent fungus on the bottom of the tanks (Figure 12). Mortalities during
this period were typically emaciated with flared opercula. There were also many
juveniles with scoliosis and lordosis. Post mortem larvae typically had a white head and
cranial protrusion and black caudal peduncle. Cannibalism was also observed in all tanks
during this period and typically first observed about a week after commercial diet was
introduced. Cannibals were pooled into one 225 L tank then transitioned to commercial
diet (8/12 EPAC CW).

Juveniles approximately 20 mm in length attempted to hide when given any available
structure. Accordingly, habitat structures (small sections of PVC pipe; approximately 2.5
cm diameter and 5 to 10 cm TL) were added to tanks. Juvenile behavior included clinging
to air stones, outflow screening, and tank walls and hiding under habitat structures in
piles or balls and behaved much like a “spawning ball” noted by McPhail and
Paragamian (2000).

Outdoor tank culture.-One 1.2 m circular tank 75% covered and placed in shade to
prevent sunlight penetration produced 14 juveniles. This tank was stocked last of all the
outdoor tanks on April 20, 2007. Harvest occurred during the first week of September, 23
weeks post stocking. It took 3 d to harvest all the individuals from the tank. Harvested
juveniles were transferred to an indoor rearing tank for feed transition. At inventory, 12
individuals remained. We speculate that two of the juveniles were victims of cannibalism
after being transferred into the 225 L indoor culture tank. Harvested juveniles ranged in
size from 6-8 cm TL (mean 6.83 cm) and ranged in weight from 1.1 to 2.1 g (mean 1.44
g). Regarding growth, they were stocked at approximately 4 to 5 mm (TL) averaging 680
mm when harvested, equating to an approximate growth rate of about 30 mm per week.
Following inventory, juveniles were pooled with segregated cannibals, based on size, for
transition to commercial diet.

Support of related research.-Four studies related to this project were supported during
2007, including:

1) Dr. Paul Skvorc (January 19, 2007) photography for biological pattern recognition
(BiOpPar, Anchorage, AK).

2) Dr. Tim Walsh collected samples of tissues for investigating a novel fungus infecting
burbot in the Columbia River Basin.

3) Mark Polinski, (UI) conducted a preliminary investigation into burbot egg incubator
fungus control using formalin, and ran disease susceptibility trials.

4) Vaughn Paragamian of the Idaho Department of Fish and Game used ~100,000
embryos in a preliminary study of extensive rearing.
Discussion

Project activities during 2007 successfully produced 850 commercial feed-trained juveniles to be used for ongoing research investigating viral and bacterial disease susceptibility, and to further develop and refine burbot aquaculture techniques and apparatus. Although our goal of 5,000 commercial feed trained burbot was not met, 2007 production represented the highest survival of burbot fully training onto commercial feed to date by an order of magnitude. The new facility and systems designed for adult husbandry and spawning, egg incubation and larval rearing were adequate, although improvements are needed. An abbreviated burbot conservation aquaculture annual activity schedule was created for reference (Table 5). Recommended improvements include:

- Minor system designs alterations and assurance of water quality standards

  The small scale recycling water systems being used currently are not recommended as a long term option for successful burbot production. The water source is limited in supply and pressure, high in chlorine, and subject to high water temperatures. Due to the low water temperatures required during spawning there is a prolonged amount of time when the adult system filters cannot be backwashed and the settling basin solids cannot be pumped out without risk of water temperature and chlorine increases. The chlorine target concentration is 0.2 ppm for the university water supply at the furthest point, however it is typically 1 ppm (personnel communication U of I Water Systems Manager, Mike Holthaus). To improve water quality in our facility during 2008, additional aeration will be supplied in tanks and we will improve sodium thiosulfate treatments by incorporating continuous drip treatments (7 ppm / 1 ppm CI-) versus weekly treatments based on water quality analysis.

  Water chilling capabilities of the adult rearing system should be improved; we are currently operating without a back-up chiller. Within the next year it is recommended that we purchase and install an additional chiller to prevent a situation where critical water temperature requirements cannot be met. Furthermore, small scale systems are not recommended for juvenile commercial feed transition for similar reasons (e.g. waste feed and fecal accumulation on the bottom of the tanks, where burbot prefer the bottom; Figure 12), and an increased likelihood that introduced pathogens can thrive. To help improve juvenile rearing in 2008 we have plumbed in four 4 m trough raceway tanks that we believe will improve cleaning efficiency by providing additional surface area while incorporating baffles to force sediments to collect in the troughs’ quiescent areas.

  For future efforts beyond the U of I, if recycling systems continue to be used it is recommended that a quarantine system be built to provide an alternative holding system to facilitate system disinfection. Having an alternate system would also provide initial quarantine and treatment possibilities for external pathogens on incoming wild broodstock. Alternatively, a larger scale system with a different water source, increased chilled water capacity, settling, and filtration capability would improve system function and water quality, fish health, and project success at all captive life stages.

  To improve the egg incubation and larval rearing system we have plumbed in additional inflow lines that will allow for three incubators per rearing tank. This arrangement should produce increased exchange rates to help pull sediments out during juvenile rearing. A
UV sterilizer has also been installed to increase water quality in the incubation and larval rearing system.

To improve live feed production, a second rotifer system will be constructed and an additional water filtration system will be installed. Installing an additional water filtration system will also serve as a precautionary back-up in case of a water shortage or existing system malfunction.

- Improve broodfish survival

Between 2003 and 2006, more than 100 adults have been captured and transported to the ARI. Currently 34 remain from previous years, excluding Moyie adults captured in the fall of 2007. This lower than desired survival rate indicated that adult capture and husbandry should be improved.

Many adult mortalities have had ruptured swim bladders, a diagnostic indication that decompression procedures during trapping may be a primary cause of delayed mortality. In 2007, 19 Moyie reservoir adults were captured in shallower waters than those trapped in previous years (Courtesy to Matt Neufeld and the B.C. MoE) to prevent this trauma. No mortality from this newly captured stock occurred yet, however, this is a premature conclusion because these 19 adults have only been in captivity for approximately two months. Alternatively, it may be a good indication that rearing wild adults in small tanks (approximately 1500 L) in a recycling system is not optimal for long term survival. Improving water quality within the adult rearing system is expected to aid survival by creating a more stable and clean environment.

Involving both the capture in cod traps and general handling, the majority of adult mortalities have had abrasions on their snouts, skin, and fins. These abrasions have led to secondary fungal infections. Burbot appear to have sensitive skin. Thus, handling methods may also have been contributed to mortality. We plan to reduce handling stress in 2008 by only conducting spawning ripeness checks once per week during spawning instead of twice per week to reduce adult’s skin surface abrasion with repeated netting. All nets have also been re-netted with fine mesh knotless bags.

Adult feeding methods may have also contributed to poor adult survival. To date, we have been feeding adult captives live rainbow trout because it is known that wild burbot are piscivores and their diet typically consists of > 80% fish (McPhail and Paragamian 2000). However, the nutritional requirements for adult burbot are currently unknown and should be investigated. Rainbow trout supplies have varied in past years and there have been shortages at times and surpluses at other times. To date, we have been feeding rainbow trout ad libitum or on demand and have not quantified relative rainbow trout biomass consumed. However, we have recorded the numbers of trout fed during past years and plan to characterize future adult feeding frequency by surveying past feeding records to help determine when rainbow trout are needed most to help gauge when we need to purchase eyed eggs.

- Improve spawning methods

Volitional spawning was evident again during 2007, as it has been consistently since the first captive spawning season (2004). Aside from trying to control this behavior in captivity with bi-weekly manual gamete checks and exogenous hormone analogs,
methods (manual or automated) should be developed to capture naturally fertilized gametes. Improved methods are expected to reduce labor and other costs associated with captive spawning. Handling stress would also be reduced, which would likely improve broodstock survival. Developing these methods should allow for future breeding program development (e.g. paired mating strategies).

As an aside, an observational study will be conducted during 2008 to further observe if hormone is needed to induce spawning. We have noted that adults will spawn without hormone and it is known that some adults have rest years (Table 2, Pulliainen and Korhonen 1993). If it is determined that hormone is not necessary it would eliminate the stress associated with handling and injection.

- Improve fungus control

Egg disinfection during water hardening has not been carried out to date and will be employed during 2008 to improve egg quality and reduce the spread of unwanted pathogens as it does with salmonids (Piper et al. 1982).

Higher flows were used to incubate eggs during 2007 than during previous years. It remains unknown if developing burbot eggs are resistant to agitation during incubation. Hagen (1952) noted that mechanical agitation during incubation decreased siltation and subsequent fungus resulting in higher quality eggs. Based on the pelagic nature of burbot eggs it seems plausible that they would be resistant to a degree of movement during incubation but whether they have a “green stage” or critical period is unknown. However, based on an image of abnormally developing embryos taken during 2007 (Figure 15) it may be that the increased water flows affected embryo development. By increasing the number of incubators we will be able to stock smaller egg masses per jar, which will require less flow and agitation.

Egg incubation and larval rearing was affected by fungus in 2007 and in previous years despite 1000 ppm formalin treatments. In 2008, we plan to increase formalin dosage to 1500 ppm during incubation.

- Improve feeding protocols

We did not use cold storage for live prey harvests in 2007 which likely contributed to high ammonia levels due to increased metabolic activity of live prey harvests and bacterial additions to larval tanks. In 2008, cold-storage of live prey cultures will be incorporated and prey item additions to larval tanks will only occur once per d. Feeding fine particulate diets (100-600 micron particulate) caused fungus to manifest even in a flow through environment. Fungus contributed to severe gill disease and subsequent chronic mortality for young-of-year burbot in 2007. Reducing commercial feed feeding rates and increasing cleaning efficiency by plumbing in raceway trough rearing tanks in 2008 will improve fungus control.

- Stabilize live diet supply

There was a shortage of rotifers in 2007 during a critical time when larvae were feeding exclusively on rotifers. To prevent this from happening again during 2008, and to improve stability of prey supply, an additional rotifer culture system will be constructed. Live prey feeding costs were examined during 2007 and feeding rotifers algae was relatively expensive (Table 1). However, outsourcing algae is the best option since space
and labor limitations exist and will continue in 2008. Nonetheless, more cost-effective products should be investigated. Also, *Artemia* naupli are important in order to keep the larvae healthy before transitioning to commercial diets. In 2008, *Artemia* will be decapsulated during 2008, not done previously, to improve their nutritional value.

- Improve young-of-year burbot rearing efficiency

Selected families will be transferred to the University of Idaho’s College of Natural Resources wet lab in Moscow and reared in trough style raceway tanks. This lab will provide a clean water source, a flow through environment, and upgraded water chilling capabilities.

Juvenile size variability likely contributed to increased cannibalism in 2007. Differences in size between cannibals and feed-transitioned juveniles were obvious. Cannibalism was apparent in all rearing tanks during feed transitions. Grading methods must be developed to circumvent cannibalism and maximize size uniformity. Again, trough style raceway tanks will be used to aid cleaning efficiency.

- Improve feeding strategies

Feeding of juveniles at optimal times and rates with preferred food items of the right size and nutritional quality while maintaining an optimal rearing environment remains the foremost bottleneck currently limiting burbot production potential. Juvenile nutritional requirements remain unknown. Commercial and natural feed trials are warranted. The incubation/Larval feeding system currently in place was constructed with this in mind. Assuming spawning success and improved larvae and juvenile health, we will begin evaluating alternative feeds.

Outdoor tank culture methods will be further investigated during 2008. Outdoor tank harvest consisted of 14 individuals during 2007 (6-8 cm TL). This demonstrated that it is possible to culture juvenile burbot in this way. However, because burbot juveniles >10 cm (TL) are desirable for tagging purposes, it is recommended that tanks not be harvested until the end of September or early October.

To expand outdoor tank extensive rearing, six 7500 L fiberglass tanks were excavated into the ground outside the adult rearing laboratory. There is currently a search being conducted to employ a MS graduate student (contact Dr. Ken Cain for further details). Aside from graduate research potential in this area, we will utilize these tanks during the coming year to stock replicate groups of first feeding and post first feeding larvae to determine when the best time may be to stock larval burbot into an outdoor extensive rearing environment.

- General project improvements

Additional labor will be required to maintain an additional rotifer system in 2008. Overall, recommended personnel would include: a full time manager, a full time technician, and during the months February-August hire at least one half-time laborer. In 2007, one full time technician and one half-time technician represented a minimum personnel and did not sufficiently meet staff requirements for spawning, live prey production, and general cleaning and maintenance. In addition, we consistently used a number of student volunteers in order to meet these demands.
Implementing the above recommendations is expected to increase the success of the project during 2008 and beyond while building upon the progress made and the things learned since 2004.
Table 2. 2007 spawning behavior by stock and capture year.

<table>
<thead>
<tr>
<th>Stock and capture year</th>
<th>Gender</th>
<th>Population at first inventory</th>
<th>Spawning dates</th>
<th>Number spawned</th>
<th>Artificial spawned</th>
<th>Volitional spawned</th>
<th>Rest year</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moyie 2006 M</td>
<td>M</td>
<td>9</td>
<td>2/7-3/19</td>
<td>9</td>
<td>9</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Moyie 2006 F</td>
<td>F</td>
<td>12</td>
<td>2/23-2/28</td>
<td>10</td>
<td>2</td>
<td>8</td>
<td>2</td>
</tr>
<tr>
<td>Arrow 2005 M</td>
<td>M</td>
<td>5</td>
<td>3/26-4/3</td>
<td>5</td>
<td>5</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Arrow 2005 F</td>
<td>F</td>
<td>9</td>
<td>3/7-4/3</td>
<td>5</td>
<td>1</td>
<td>4</td>
<td>4</td>
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<tr>
<td>Arrow 2004 M</td>
<td>M</td>
<td>0</td>
<td>NA</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Arrow 2004 F</td>
<td>F</td>
<td>0</td>
<td>NA</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Duncan 2003 M</td>
<td>M</td>
<td>4</td>
<td>2/15-3/29</td>
<td>4</td>
<td>4</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Duncan 2003 F</td>
<td>F</td>
<td>4</td>
<td>3/2-3/19</td>
<td>4</td>
<td>1</td>
<td>3</td>
<td>0</td>
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</tbody>
</table>
Table 3. 2007 semen cryopreservation inventory (Courtesy to Steve Patton; UI Biological Sciences Department, Dr. Joe Cloud laboratory).

2007 burbot semen cryopreservation summary

<table>
<thead>
<tr>
<th>Stock</th>
<th>KTH ID #</th>
<th>Motility range (%)</th>
<th>.5 ml straws</th>
<th>stored</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moyie Reservoir stock</td>
<td>3444A</td>
<td>60-90</td>
<td>70</td>
<td>KTH MVE</td>
</tr>
<tr>
<td></td>
<td>7363A</td>
<td>50-90</td>
<td>110</td>
<td>KTH MVE, A1</td>
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<tr>
<td></td>
<td>4567A</td>
<td>50-90</td>
<td>130</td>
<td>KTH MVE, A1</td>
</tr>
<tr>
<td></td>
<td>9734A</td>
<td>20</td>
<td>0</td>
<td>KTH MVE</td>
</tr>
<tr>
<td></td>
<td>3337A</td>
<td>70</td>
<td>20</td>
<td>KTH MVE</td>
</tr>
<tr>
<td></td>
<td>8553A</td>
<td>60-90</td>
<td>70</td>
<td>KTH MVE</td>
</tr>
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<td></td>
<td>4664A</td>
<td>70-90</td>
<td>115</td>
<td>KTH MVE, A1</td>
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<td></td>
<td>1513A</td>
<td>60-70</td>
<td>40</td>
<td>KTH MVE</td>
</tr>
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<td></td>
<td>1211A</td>
<td>70</td>
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<td>KTH MVE</td>
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<td>Duncan Reservoir Stock</td>
<td>6003</td>
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<td></td>
<td>3F52</td>
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<td>20</td>
<td>KTH MVE</td>
</tr>
<tr>
<td></td>
<td>531B</td>
<td>90</td>
<td>10</td>
<td>KTH MVE</td>
</tr>
<tr>
<td>Arrow Reservoir stock</td>
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</tr>
<tr>
<td></td>
<td></td>
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</tr>
</tbody>
</table>
| There was no semen cryopreserved for Arrow Reservoir stock in 2007

Table 4. 2007 egg collection summary.

Estimated egg collection by stock and number of females spawning

<table>
<thead>
<tr>
<th>Stock</th>
<th>Number of females spawning</th>
<th>Total eggs collected</th>
<th>Average eggs per female</th>
<th>Average % fertilization</th>
<th>Estimated fertilized eggs collected</th>
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</thead>
<tbody>
<tr>
<td>Moyie</td>
<td>10</td>
<td>4,200,000</td>
<td>468,000</td>
<td>55%</td>
<td>2,181,000</td>
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<tr>
<td>Duncan</td>
<td>4</td>
<td>828,000</td>
<td>414,000</td>
<td>95%</td>
<td>790,000</td>
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<tr>
<td>Arrow</td>
<td>5</td>
<td>828,000</td>
<td>276,000</td>
<td>33%</td>
<td>318,000</td>
</tr>
</tbody>
</table>

*Five spawns were lost to volitional spawning. This table only reflects eggs collected.
Table 5. Abbreviated burbot conservation aquaculture annual activity schedule.

<table>
<thead>
<tr>
<th>Activity</th>
<th>Jan</th>
<th>Feb</th>
<th>Mar</th>
<th>Apr</th>
<th>May</th>
<th>Jun</th>
<th>Jul</th>
<th>Aug</th>
<th>Sep</th>
<th>Oct</th>
<th>Nov</th>
<th>Dec</th>
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</thead>
<tbody>
<tr>
<td>Wild adult capture</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Gamete collection</td>
<td>x</td>
<td>x</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Larval rearing</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>x</td>
<td>x</td>
<td>x</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Juvenile rearing</td>
<td></td>
<td></td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td></td>
<td></td>
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<tr>
<td>Adult rearing</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
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<tr>
<td>Forage fish rearing*</td>
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<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
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</tr>
<tr>
<td>Rotifer production</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td></td>
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<td></td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>* Rainbow trout</td>
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<td></td>
<td></td>
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</tbody>
</table>

* Rainbow trout
Figure 1. 2003 water temperature data in the Aquaculture Research Institute’s main office laboratory on University of Idaho campus.
Figure 2. Incubation and larval rearing system (Main laboratory).
Figure 3. Typical egg incubator set up. Water up-wells from the bottom of the cones and overflows into the culture tank transferring newly hatched fry to their rearing environment with no handling.
Figure 4. Rotifer production system 1.
Figure 5. Incubators used for burbot eggs. Left to right: 1L Customized Imhoff cone, 2L mini-egg hatching jar, and McDonald type jar. Note: in 2007 McDonald type jars were not used.
Figure 6. Burbot eggs. Lower left egg showing blastomere (live egg) and lower right egg showing cloudy yolk (unfertilized or dead egg).
Figure 7. Burbot larvae (4-5mm) showing alimentary tract development.
Figure 8. Drip bucket set over top of larval rearing tank to feed live prey.
Figure 9. Prey in gut of larval burbot (4-5mm TL).
Figure 10. Ambient temperature of main laboratory and water temperature of incubation and larval rearing system 2007.
Figure 11.- Water temperature of incubation and larval rearing system during the 2007 incubation period.
Figure 12.-Juvenile burbot rearing tank with waste feed and fungus on bottom.
Figure 13.-Live prey production graph 1. R/ml represents Rotifer harvest per 1 ml and A/ml represents Artemia harvest per 1 ml during the live prey feeding period. Note the Rotifer crash prior to 5/22/2007.

Figure 14.-Live prey production graph 2. R/ml represents Rotifers harvested and A/ml represents Artemia harvested during the live prey feeding period.
Figure 15.-Live burbot embryo deformities observed and collected during 2007.
References


