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[Article]

Evaluation of Egg Incubation Methods and Larval Feeding Regimes for North American Burbot

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Abstract.—Incubation methods and larval feeding regimes were investigated for North American burbot *Lota lota maculosa* over 2 years. Three upwelling incubators were tested: 6.0-L McDonald-type jars, 2.0-L pelagic egg jars, and 1.2-L Imhoff cones. Larvae were allocated to five feeding regimes in year 1 (trial 1) and three feeding regimes in year 2 (trial 2). In trial 1, a live diet (marine rotifers *Brachionus plicatilis* and brine shrimp *Artemia* spp.) was administered from 11 d posthatch (dph) until introduction of a commercial diet at 21, 31, or 41 dph; the fourth treatment applied the commercial diet exclusively starting at 11 dph, and the fifth treatment used only the live diet. Trial 2 examined (1) exclusive use of live feed beginning at 16 dph; (2) use of live feed at 16–50 dph, which was combined with commercial feed at 31–50 dph, and use of only the commercial diet at 51–76 dph; and (3) use of the live diet at 16–50 dph, the addition of frozen brine shrimp at 31–50 dph, and use of the commercial diet at 51–76 dph. Approximate stocking densities for feeding trials were 25 larvae/L in trial 1 and 250 larvae/L in trial 2. Survival and total lengths (TLs) were measured at 52 dph in trial 1 and at 76 dph in trial 2. Incubation trials showed that Imhoff cones or pelagic egg jars significantly improved embryo survival relative to McDonald jars. Larvae fed a live diet for an extended time had significantly higher survival and TLs in both trials. Introduction of a commercial diet at 31 or 41 dph after live-diet feeding was successful. This study provides a basis for further development of burbot aquaculture.

The burbot *Lota lota* is the only true freshwater species of the cod family Gadidae and has a circumpolar distribution above 40°N (Van Houdt et al. 2003). Despite its broad geographical range, concerns about declining wild stocks in North America and Europe have prompted interest in developing conservation aquaculture techniques (Wolnicki et al. 2001; Harzevili et al. 2003; Van Houdt et al. 2003). There are many uncertainties that must be addressed to develop culture techniques for this species. Egg incubation and larval feeding strategies are two critical aspects requiring development. Early life stage rearing techniques have been attempted for European burbot *L. lota lota*, but no reports on the production of North American burbot *L. lota maculosa* are available. Culture development is challenging because of the minute size and delicate nature of embryos and larvae (Steiner et al. 1996; Wolnicki et al. 2001; Harzevili et al. 2003). Furthermore, use of commercial diets produces low levels of success (Wolnicki et al. 2001). The objectives of this study were to (1) compare survival of burbot eggs and embryos in three different incubators and (2) evaluate survival and growth of larvae fed marine organisms and a commercial diet formulated for Atlantic cod *Gadus morhua*.

Burbot eggs are small, delicate, semibuoyant, and translucent; range in diameter from 0.5 to 1.7 mm; and require low water temperatures during incubation (Prince and Halkett 1906; McPhail and Paragamian 2000; Taylor and McPhail 2000). Water temperatures near or below 7°C appear necessary for incubation and hatching (Lawler 1963; Meshkov 1967; Sorokin 1971; Jäger et al. 1981; Ryder and Pesendorfer 1992; Taylor and McPhail 2000). Egg incubation has been attempted in "trout hatchery trays" with muslin cloth screening (Bjorn 1939), 5.7-L hatching jars (McCrimmon 1959), and aerated, chilled 2.0-L jars (Taylor and McPhail 2000). However, these reports were focused on investigating early life history characteristics and not on development of culture techniques.

Burbot embryos are also small and delicate; total length (TL) at hatch is 3.0–4.0 mm, and the fish exhibit

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no mouth or alimentary tract for a week or more posthatch (McPhail and Paragamian 2000; Harzevili et al. 2003, 2004). Past reports of exogenous feeding indicate that larvae begin to feed after 5–10 d (Ghan and Sprules 1993; Taylor and McPhail 2000; Wolnicki et al. 2001, 2002). Limited information exists on nutritional needs of larvae, and the use of commercial diets has produced poor results (Wolnicki et al. 2001, 2002; Harzevili et al. 2003, 2004). Administering a commercial diet to larvae would be advantageous because it would reduce labor, space, and the risk of pathogen transmission via live feed (Planas and Cunha 1999; Baskerville-Bridges and Kling 2000; Blair et al. 2003).

Burbot larvae require small (<400 μm) live prey items (Steiner et al. 1996; Harzevili et al. 2003, 2004) and prefer rotifers and copepod nauplii in the wild (Ghan and Sprules 1993). In a controlled setting, Harzevili et al. (2003) demonstrated algae as a beneficial first food item for burbot larvae. In that study, larvae were fed green algae *Chlorella* spp. for 3 d, rotifers *Brachionus calyciflorus* for 7 d, and nauplii of brine shrimp *Artemia* spp. for 24 d; survival to 35 d posthatch (dph) was acceptable (Harzevili et al. 2003). The present study expands on this previous work by evaluating survival of North American burbot fed different combinations of marine organisms and Atlantic cod diets for longer time intervals (52 dph in year 1 and 76 dph in year 2). Marine prey organisms were used exclusively for these trials because of their ease of maintenance (Lim et al. 2003).

Methods

Broodstock Source and Rearing Conditions

Adult burbot that provided gametes for these trials were captured in baited cod traps on two separate occasions from Duncan and Arrow Lakes reservoirs in British Columbia (Neufeld and Spence 2004). After capture, Duncan Reservoir adults ($n = 20$; 13 females, 7 males) were transported to the Kootenai Tribal Fish Hatchery (Bonners Ferry, Idaho) and acclimated to captivity for approximately 2 months in a 5,400-L tank receiving filtered Kootenai River water. Duncan Reservoir fish were then transported to the Aquaculture Research Institute (ARI; University of Idaho, Moscow) and maintained in 1,200-L tanks in a closed recirculation water system. Arrow Lakes Reservoir fish ($n = 22$; 15 females, 7 males) were captured in November 2005 (approximately 2 years after Duncan Reservoir adult capture), transported directly to ARI, and stocked into 1,200-L tanks in a closed recirculation water system (total volume ≈ 10,000 L). Adult stocking density averaged 10 kg/m^3 per tank. Adults ranged in mass from 1.5 to 2.4 kg and in TL from 60 to 78 cm. To facilitate gamete development, adults were exposed to photoperiod and temperatures modeled after the Kootenai River in Bonners Ferry. Rearing system water temperatures ranged from 2°C to 12°C. Adult burbot were given an ad libitum ration of live rainbow trout *Oncorhynchus mykiss* weighing an average 5–50 g.

Duncan Reservoir adults were held in captivity for approximately 17 months before trials were conducted in year 1 and for 29 months before trials were conducted in year 2. Arrow Lakes Reservoir adults were held in captivity for approximately 5 months before year-2 trials. Water quality of the adult rearing system had an average dissolved oxygen level of 10.0 mg/L, toxic ammonia levels averaging 0.003 mg/L, nitrite levels averaging 0.153 mg/L, and pH averaging 8.0.

Incubation Trials

Gamete collection and fertilization.—Gamete collection occurred during the month of March for both years. Adult fish were examined for ripeness two or three times per week. Before handling, adults were anesthetized with a 200 mg/L solution (0.51 μmol; 3 ± 1°C) of unbuffered tricaine methanesulfonate (MS-222; Argent Laboratories, Inc., Redmond, Washington). Eggs and milt were collected in 500-mL Whirl-Pak bags (Nasco International, Inc., Fort Atkinson, Wisconsin) with care to prevent urine or mucus contamination. Individual gamete collections were kept separate, stored at 4°C for less than 1 h, and then combined for fertilization.

When all gametes had been collected, each female's ova with ovarian fluid were divided into two separate bags and fresh milt was added to each bag. Chilled water (3 ± 1°C) was then added for activation. At least two males were used to fertilize the eggs of each female. Excess milt was rinsed 1 min postactivation, and a minimum of 90 min was allowed for eggs to water harden before enumeration.

Egg enumeration and stocking.—For enumeration, the eggs were gently poured into a 500-mL graduated cylinder and allowed to settle for approximately 2 min before a total settled volume reading was recorded. Once settled, 5 mL of eggs plus water were removed from the approximate center of the settled eggs and ejected into a chilled (3 ± 1°C) petri dish. Three subsamples of eggs were then removed from the initial sample with a 1-mL tuberculin syringe. The eggs in the tuberculin syringe were allowed to settle by positioning the syringe vertically with the tip pointing up for approximately 2 min. Graduations on the syringe barrel were used to measure subsample settled volumes. Each egg subsample was then manually counted, and the number of eggs/mL was estimated.

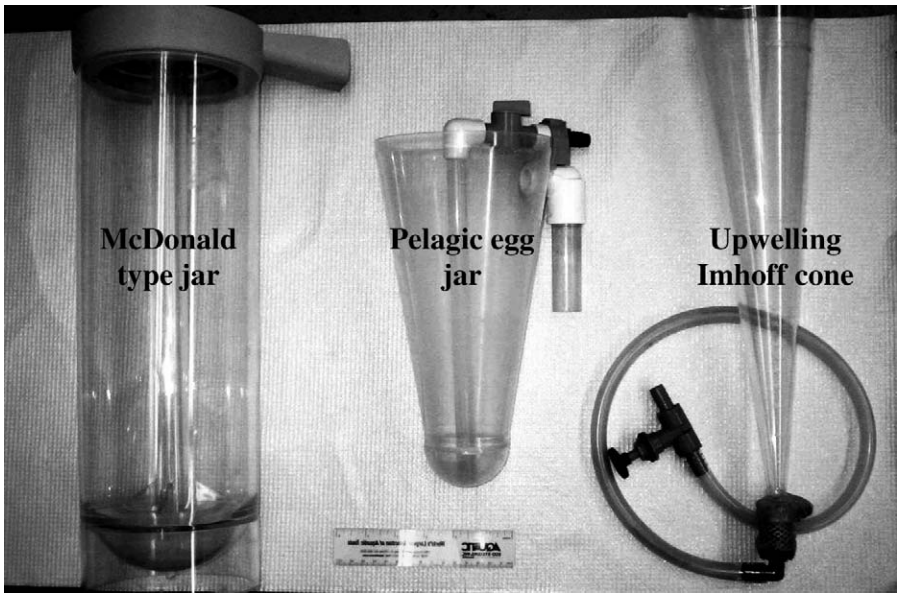

FIGURE 1.—Photograph of three incubator types used in a comparison of burbot egg and embryo survival.

Eggs were incubated in McDonald-type hatching jars, mini egg-hatching jars, or Imhoff cones (Aquatic Eco-Systems, Inc., Apopka, Florida). These incubator vessels are different by volume and shape (Figure 1) and were chosen based on commercial availability. The Imhoff cone was used as a substitute for an upwelling plastic water bottle, which has been reported to produce survival rates of at least 80% for European burbot eggs (A. Shiri Harzevili, Ministry of the Flemish Community, personal communication).

Eggs were equally divided and stocked into incubators. Incubation water temperatures averaged 5.5°C and ranged from 2.8°C to 9.0°C during year 1. During year 2, water temperatures averaged 4.8°C and ranged from 3.0°C to 7.0°C. Water flows to all incubators were set after stocking to keep the eggs continuously moving slowly and to reduce sedimentation. Incubator flows were measured for three of the four replicated incubation trials. Based on these measurements, water flows for McDonald-type jars averaged 312 mL/min and ranged from 178 to 400 mL/min. For pelagic egg-hatching jars, flow averaged 326 mL/min and ranged from 254 to 372 mL/min. For Imhoff cones, the average flow was 185 mL/min and the range was 146 to 222 mL/min. Among incubator styles, water flows varied in incubation trials relative to the egg mass because larger egg volumes required higher flow rates. To prevent fungal manifestations, a solution of formalin at approximately 1,000 mg/L was delivered to incubator inflow lines for 20 min. Treatment frequency was five times per week in year 1 and two times per week in year 2. Formalin treatments were administered from the time of stocking through the end points of trials during both years. Effluent from all incubators was directed through a 40-L aquarium customized with 500-μm mesh screening to monitor egg escapement.

Incubation experimental design.—A completely randomized block design was used for incubator trials. Incubators were randomly positioned within each trial before egg stocking. Three different incubator styles were tested in each trial. Within each trial, duplicate incubators of each type were tested; four trials were conducted in each year.

Embryo survival estimation.—To estimate embryo survival at the end of the incubation trials, the process described above for initial enumeration was repeated for each incubator at approximately 100 Celsius degree-days. Embryo survival was estimated by manually counting live and dead eggs in each of three tuberculin syringe subsamples and then averaged to estimate mean embryo survival for each incubator. Eggs were considered live if they exhibited a transparent yolk and cytoplasm cleavage. Conversely, eggs were considered dead if they had an opaque yolk, no apparent cytoplasm cleavage, or both.

Larval Feeding Trials

Live diets and commercial diet.—Prey items in this study included a paste of green algae *Nannochloropsis*

TABLE 1.—Nutritional analysis of the commercial Atlantic cod diet (Lansy CW; 200–400 μm) fed to burbot larvae during two laboratory feeding trials.

Component	Concentration or description
Moisture (%)	7
Crude protein (%)	58
Crude lipids (%)	15
Crude ash (%)	12
Crude fiber (%)	1
Phosphorus (%)	1.70
Vitamin A (IU/kg)	30,000
Vitamin D3 (IU/kg)	2,500
Vitamin E (mg/kg)	700
Vitamin C (mg/kg)	2,000
Total omega-3 highly unsaturated fatty acids (mg/g dry weight)	30
Docosohexanoic acid: eicosapentanoic acid ratio	2
Cupric chelate of amino acid hydrate, 10% Cu (mg/kg)	5
Antioxidants	Butylated hydroxytoluene, ethoxyquine, propylgallate

TABLE 2.—Nutritional analysis of the commercial Atlantic cod diet fed to postweaning-stage and nursery-stage (Epac CW; 400–600 μm) burbot larvae during two laboratory feeding trials.

Component	Concentration or description
Moisture (%)	7
Crude protein (%)	57
Crude lipids (%)	14
Crude ash (%)	12
Crude fiber (%)	1
Phosphorus (%)	1.20
Vitamin A (IU/kg)	30,000
Vitamin D3 (IU/kg)	2,500
Vitamin E (mg/kg)	400
Vitamin C (mg/kg)	1,500
Total omega-3 highly unsaturated fatty acids (mg/g dry weight)	30
Docosohexanoic acid: eicosapentanoic acid ratio	1.7
Cupric chelate of amino acid hydrate, 10% Cu (mg/kg)	5
Antioxidants	Butylated hydroxytoluene, ethoxyquine, propylgallate

spp. (Reed Mariculture, Campbell, California), rotifers *B. plicatilis* (Scientific Hatcheries, Huntington Beach, California), and brine shrimp (90+ Great Salt Lake strain; Aquatic Ecosystems). Nutritional enrichment of live prey items consisted of adding Rotirich (Florida Aqua Farms, Inc., Dade City) at a concentration of 1:1,000 along with algae paste at a concentration of 1:1,000. Nutritional enrichments were added to rotifer and brine shrimp cultures approximately 2 and 12 h, respectively, before the cultures were administered to burbot larvae. The rotifer *B. plicatilis* was chosen because it is easier to maintain than the freshwater rotifer *B. calyciflorus* (Lim et al. 2003). Rotifer and brine shrimp cultures were maintained at 5‰. The commercially prepared diets (Lansy CW: 200–400 μm, Epac CW: 400–600 μm; INVE AMERICAS, Inc., Salt Lake City, Utah) used in this study were formulated for cofeeding, weaning, and nursing of coldwater fish species, such as Atlantic cod larvae. Feed particle size ranged from 200 to 600 μm (for nutrition analysis, see Tables 1, 2).

Gamete collection and fertilization.—Methods for gamete collection and fertilization used to supply fish for larval feeding trials were identical to those used for incubation trials. Eggs from each female were fertilized with the sperm of two males. Only one female's eggs were used to supply burbot larvae for feeding trials in each year. Therefore, differences in gamete quality, fertilization success, and larval survival between years were likely, given that dams and sires were independently mated in those years.

Embryo enumeration and stocking.—Hatched embryos were collected in 22-L white plastic buckets plumbed with a 500-μm mesh screen that covered the outflow to prevent escape. Once hatching commenced, embryos were allowed to trickle into the collection bucket. To enumerate a bucket of embryos, airflow to the bucket was carefully increased to distribute embryos evenly throughout the water column. A glass beaker (total volume = 19 mL) was inverted and plunged into the center of the bucket. The beaker was then upturned and pulled directly up and out. The larvae were counted in a 10-mL zooplankton counting wheel (WARD's Natural Science, Rochester, New York). This process was repeated four times to obtain an average number of larvae per milliliter. When enumerated, larvae were stocked into 20 separate 10-L aquaria. Approximately 25 larvae/L were stocked in feeding trial 1 and 250 larvae/L were stocked in feeding trial 2. For both trials, larvae were stocked when a functional mouth could be observed. Aquarium and larval collection bucket water temperatures averaged 8°C at stocking and gradually increased to 15°C during the course of the feeding trials.

Larval feeding trial 1.—A completely randomized block design was used for feeding trial 1 to compare mean survival and larval TL among the five different feed treatments (Table 3). Each treatment was replicated four times, and two controls were used. The positive control consisted of administering only nutritionally enriched live prey following methods similar to those outlined by Harzevili et al. (2003). The negative control involved use of only the commercial Atlantic cod diet.

In feeding trial 1, greenwater conditions (10^5 cells/mL) and rotifers were applied as live feed starting at 11 dph (24 h after stocking); one exception was that a

TABLE 3.—Description of burbot feeding trial 1, in which larvae (starting at 11 d posthatch [dph]) were fed a commercial Atlantic cod diet, a live diet (marine rotifers *Brachionus plicatilis* plus either green algae or brine shrimp *Artemia* spp.), or a live diet followed by a commercial diet. Stocking density was approximately 25 larvae/L; four replicate tanks were used per treatment. Within a column, different letters indicate significant differences ($P \leq 0.05$). Survival and TL are presented as means ± SE.

Feeding regime	Greenwater + rotifer (dph)	Rotifer + *Artemia* (dph)	Cod diet (dph)	Survival (%)	TL (mm)
1			11–52	0.0 z	0.0 z
2	11–20		21–52	0.0 z	0.0 z
3	11–20	21–30	31–52	0.4 ± 0.4 z	6.0 ± 0.4 z
4	11–20	21–40	41–52	3.1 ± 2.1 zy	9.3 ± 0.3 y
5	11–20	21–52		6.5 ± 1.0 y	13.7 ± 0.3 x

single treatment was only given the commercial diet. For all treatments receiving live prey, the administration of nutritionally enriched rotifers and brine shrimp began at 11 dph and continued until the commercial diet was introduced. All live prey were added to treatment aquaria with the brackish prey culture water. Thus, prey items were not rinsed before being fed to the larvae. All treatments were fed twice daily by hand. The amount of commercial diet fed did not exceed 1 g/d for each 10-L aquarium and was delivered at a rate of 0.5 g/feeding. The commercial diet treatments involved initially administering 200–400-μm particulates followed by use of 400–600-μm particulates beginning at 31 dph. Burbot larvae were fed live prey at a rate of 1 organism (org)/mL. Feeding trial 1 lasted from 11 to 52 dph (Table 3).

Larval feeding trial 2.—Feeding trial 2 further examined the commercial diet introduction at 31 dph and compared mean survival and TL. Each treatment receiving the commercial diet was replicated six times and the exclusively live diet treatment was replicated eight times (Table 4).

Feeding trial 2 was extended to 76 dph based on results of feeding trial 1, which suggested that the duration of the feeding regimes were not long enough to permit observation of a true treatment effect. Additional larvae were also used because of their availability. A similar experiment (where Atlantic cod larvae were fed a microparticulate diet until 71 dph; Baskerville-Bridges and Kling 2000) provided further support for extending the feeding trial. Before larvae were stocked in feeding trial 2, a mixture of reconstituted algae paste (10^5 cells/mL) and enriched rotifers (1 org/mL) was added to collection buckets at 11–15 dph. The fish were then enumerated and stocked into treatment aquaria. For this trial, application of feed to the treatment aquaria commenced at 16 dph and larvae were fed the previous mixture plus brine shrimp (1 org/mL) until 30 dph for treatments 1 and 2. Treatment 3 received this mixture through 50 dph. All live prey were added to aquaria with brackish prey culture water at 3–5‰ because Jäger et al. (1981) reported that early life stages can tolerate salinities up to 12‰. All treatments were fed twice daily by hand. The amount of commercial diet did not exceed 1 g/d for each aquarium and was delivered at a rate of 0.5 g/feeding. For instances where commercial diet was fed in combination with live prey, half rations were fed together. When fed, commercial diets were scattered on the water surface and live diets were poured in. Commercial diet introduction began in combination with brine shrimp (live or frozen, depending on treatment) at 31–50 dph. Use of the 200–600-μm

TABLE 4.—Description of burbot feeding trial 2, in which larvae (starting at 16 d posthatch [dph]) were fed a live diet only (green algae, marine rotifers *Brachionus plicatilis*, and brine shrimp *Artemia* spp.), a live diet followed by a commercial Atlantic cod diet in combination with live brine shrimp, or a live diet followed by a commercial diet in combination with frozen brine shrimp. Stocking density was approximately 250 larvae/L; eight replicate tanks were used for feeding regime 1, and six replicate tanks were used for regimes 2 and 3. Within a column, different letters indicate significant differences ($P \leq 0.05$). Survival and TL are presented as means ± SE.

Feeding regime	Greenwater + rotifer + live *Artemia* (dph)	Live *Artemia* (dph)	Live *Artemia* + cod diet (dph)	Frozen *Artemia* + cod diet (dph)	Cod diet (dph)	Survival (%)	TL (mm)
1	16–50	51–76				0.6 ± 0.1 z	26.0 ± 0.4 z
2	16–50		31–50		51–76	0.2 ± 0.1 y	15.6 ± 0.7 y
3	16–50			31–50	51–76	0.0 x	0.0 x

commercial diet began at 51 dph. The entire duration of feeding trial 2 lasted 76 d (Table 4).

Larval harvest.—Larvae were harvested with a soft-mesh aquarium net (Aquatic Ecosystems) at 52 dph for feeding trial 1 and 76 dph for feeding trial 2;fish were counted and measured to the nearest 0.1 mm TL. Average survival for each treatment was calculated by dividing the number of larvae counted by the approximate stocking number. Average larval TL was calculated by averaging all aquaria within feeding regime treatments.

Statistical Analysis

Incubation experiments.—After tests to confirm no interaction between trials, percent survival data were arcsine, square-root, angular transformed before analysis to meet the normality assumption (Snedecor and Cochran 1980). One-way analysis of variance (ANOVA) was used to compare transformed mean survival rates among incubator types and blocks. A Tukey post hoc analysis was used to determine significant differences between treatments ($P \leq 0.05$). Data were analyzed with Statistical Analysis System (SAS) software.

Larval feeding experiments.—After testing to confirm no interaction among treatments, percent survival data were arcsine, square-root, angular transformed before analysis to meet the normality assumption (Snedecor and Cochran 1980). A one-way ANOVA was used to compare mean survival among treatments and blocks in year 1. A Tukey post hoc analysis was used to assess mean survival between treatments with a significance level of 0.05. Length data were not subject to angular transformation. Due to unequal sample sizes, average TLs among treatments were analyzed with a type 3 sum of squares and an adjusted Tukey–Kramer analysis. In year 2, mean survival and length were also analyzed. However, due to unequal sample sizes, the transformed survival data and the TL data were analyzed with a one-way ANOVA with the type 3 sum of squares and were subjected to an adjusted Tukey–Kramer analysis. Data were analyzed with SAS software.

Results

Incubation Trials

In year 1, incubation involved an average of 66,000 eggs/incubator (range = 44,000–88,000 eggs/incubator) across all trials. Egg density averaged 1,050 eggs/mL and ranged from 800 to 1,300 eggs/mL. The McDonald-type jar, pelagic egg-hatching jar, and Imhoff cone had mean survival rates of 18, 51, and 55%, respectively. Mean embryo survival rates in the Imhoff cones and pelagic egg-hatching jars were

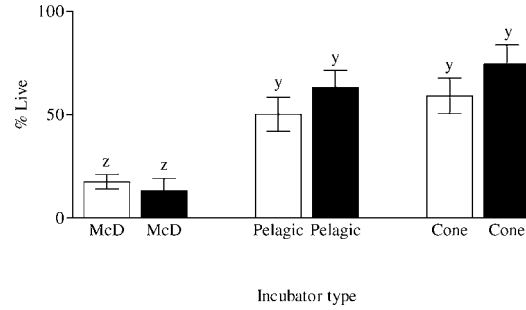

FIGURE 2.—Percentage (mean ± SE) of live burbot embryos before hatch (18–22 d postfertilization; approximately 100 Celsius degree-days) produced by three incubator types: McDonald-type jar (McD), pelagic egg-hatching jar (Pelagic), and Imhoff cone (Cone). Trials were conducted in two years (white = year 1; black = year 2). Different letters indicate significant differences ($P \leq 0.05$).

significantly higher than those in the McDonald-type jars (Figure 2). In year 2, incubators averaged 70,250 eggs/incubator and ranged from 33,500 to 107,000 eggs/incubator across all trials. Egg density averaged 935 eggs/mL and ranged from 220 to 1,650 eggs/mL. The McDonald-type jar, pelagic egg-hatching jar, and Imhoff cone had mean survival rates of 13, 63, and 75%, respectively. Fungus inhibited accurate final egg mass measurements of two trials in year 2. Despite fungus, the Imhoff cone and the pelagic egg-hatching jar had significantly higher egg survival rates than the McDonald-type jar (Figure 2).

Larval Feeding Trial 1

Larval survival across all treatments in year 1 averaged 2% and ranged from 0% to 9.2%. The introduction of a commercial Atlantic cod diet initially (11 dph) or at 21 dph resulted in 0% survival. Larvae given live prey from 11 to 30 dph with subsequent commercial diet introduction at 31 dph had an average survival rate of 0.4% and ranged from 0.0% to 1.6%. Survival of larvae fed live prey from 11 to 40 dph with subsequent commercial diet introduction at 41 dph averaged 3.1% and ranged from 0.4% to 9.2%. Survival of larvae that were exclusively fed live prey from 11 to 52 dph averaged 6.5% and ranged from 4.4% to 9.2%. Exclusive use of live prey throughout the experiment resulted in significantly better survival than the use of live prey from 11 to 30 dph and commercial diet introduction at 31 dph ($P = 0.005$). However, the administration of live prey at 11–40 dph and subsequent commercial diet introduction at 41 dph did not result in significantly different survival relative to the exclusively live prey treatment ($P = 0.172$; Table 3).

Larval lengths across all treatments in year 1

averaged 11.9 mm TL and ranged from 5 to 17 mm TL. Larvae that were given live prey from 11 to 30 dph averaged 6.0 mm TL and ranged from 5 to 7 mm TL. Larvae that were fed live prey from 11 to 40 dph exhibited an average TL of 9.3 mm and ranged from 6 to 12 mm TL. Larvae that received live prey from 11 to 52 dph averaged 13.7 mm TL and ranged from 9 to 17 mm TL. Average length was significantly greater for larvae fed only live prey than for larvae fed live prey from 11 to 30 dph ($P = 0.01$) or from 11 to 40 dph ($P = 0.02$; Table 3).

Larval Feeding Trial 2

Larval survival across all treatments averaged 0.3% and ranged from 0.0% to 1.2%. Commercial diet in combination with live brine shrimp introduced at 31 dph resulted in an average survival of 0.2% and ranged from 0.1% to 0.4%. The use of frozen brine shrimp in combination with the commercial diet was not successful and resulted in 0% survival. Survival of larvae that were fed live prey at 11–76 dph averaged 0.6% and ranged from 0.2% to 1.2%. Use of live prey produced significantly greater survival than did combined use of the commercial diet and live brine shrimp starting at 31 dph ($P = 0.013$; Table 4).

Larval lengths across all treatments averaged 24.1 mm TL and ranged from 11 to 37 mm TL. Use of the commercial diet plus live brine shrimp at 31 dph resulted in an average length of 15.6 mm TL and ranged from 11 to 24 mm TL. Larvae that were fed live prey at 11–76 dph averaged 26 mm TL and ranged from 18 to 37 mm TL. Average larval lengths were significantly greater in treatments that exclusively used live prey relative to treatments that used the commercial diet in combination with live brine shrimp ($P < 0.01$; Table 4).

Discussion

This study evaluated North American burbot embryo and larval survival among different incubator styles and feeding regimes. Incubation of eggs in Imhoff cones and pelagic egg-hatching jars produced significantly higher embryo survival compared with the McDonald-type jars. The semibuoyant nature of these eggs and their tendency to become adhesive probably affected results. In support of this, McDonald-type jars have been used with semibuoyant eggs of grass carp *Ctenopharyngodon idella* and low success was reported (Stanley and Jones 1976). Those authors reported that high flows led to egg escapement and low flows led to contamination. Similar results were observed in this study, where low flows caused sedimentation and fungus; however, flows were low enough to prevent egg escapement. Egg mass adhesiveness was also observed between 10 and 14 d poststocking during both years, which is consistent with past observations by Bjorn (1939) that egg adhesiveness occurred at 12 d poststocking. Past research in Canada suggested that burbot egg stocking density should not exceed 300,000 eggs/L in McDonald-type incubators, because the required flow caused eggs to escape (McCrimmon 1959). In the present study, the volumes of eggs stocked were well under this threshold. For our purpose, a conservation aquaculture program will probably include small groups of ordered mating; however, it remains unknown what the optimum number of eggs and flow rates should be for the Imhoff cone or pelagic egg jar.

Differences between incubator trials were probably a result of the different shapes and volumes of the incubators (Figure 1). Despite the McDonald-type jar's wide use for a variety of fish eggs, its cylindrical shape and larger volume probably affected sedimentation because of slower turnover rates. The Imhoff cone was shown to produce good results and, depending on needs, may be more appropriate than the pelagic egg-hatching jar for large-scale use.

For feeding trials, high larval losses occurred after stocking into experimental aquaria during both years. Although impossible to directly measure owing to size and rapid decomposition of larvae, it was estimated that approximately 80% mortality occurred within 24 h poststocking. Larvae are extremely delicate at this stage, and our observations are consistent with those of Wolnicki et al. (2001), who also noted high mortality after stocking. Wolnicki et al. (2001) additionally reported that dead larvae rapidly dissolved, making it difficult to quantify mortality. To reduce mortality in future efforts, egg incubation over rearing tanks may reduce handling stress but will make exact enumeration difficult. In this study, the handling stress associated with enumeration was believed to affect larval survival. It is also probable that the individual females used and male sperm quality contributed to larval loss. However, actions were not taken to assess adult gamete quality before use.

Despite high initial larval losses, significant differences between feeding regimes resulted, and larval survival was higher when the fish were fed live prey items beyond 30 dph. This study's findings further support the idea that burbot larvae have a high level of live-prey dependence (Watanabe et al. 1983; Harzevili et al. 2003) and that the early use of commercial diets produces low levels of success.

In summary, this study demonstrated that upwelling incubators, such as Imhoff cones or pelagic egg-hatching jars, are appropriate for burbot eggs, and future larval rearing efforts should use live prey items

for at least 5 weeks after mouth and alimentary tract development. This study also demonstrated that burbot larvae are capable of transitioning to a commercial diet and represents the first report of burbot larvae successfully transitioning to such a diet.

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