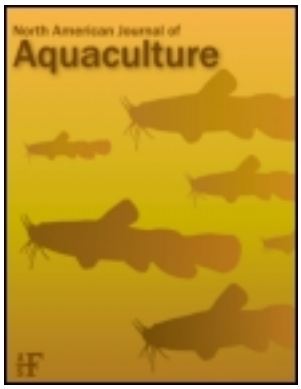


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# Performance and Macronutrient Composition of Age-0 Burbot Fed Four Diet Treatments

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COMMUNICATION

# Performance and Macronutrient Composition of Age-0 Burbot Fed Four Diet Treatments

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*Abstract*

**As there are few larval-diet rearing methods for burbot *Lota lota*, it is important to develop these methods for ongoing conservation and future commercial aquaculture efforts. The performance and macronutrient composition of age-0 burbot were compared after fish were fed four different diets for 8 weeks. Diets included three commercial larval-rearing diets and *Daphnia magna*. Performance metrics involved mean length and weight, survival, and cannibalism. The macronutrient composition (dry matter) of fish and diets was measured as the percentages of moisture, lipid, protein, and ash, along with energy content. Significant differences in mean length and weight occurred, although survival and cannibalism were not different among treatments at the end of the experiment. Mean weight and length were significantly higher with diets 1 and 2. Fish fed diet 2 had the greatest mean survival (32%), followed by those fed the *D. magna* diet (30%), diet 3 (27%), and diet 1 (25%). Fish fed diet 1 experienced the greatest amount of cannibalism (48%), followed by those fed the *D. magna* diet (43%), diet 3 (43%), and diet 2 (26%). The macronutrient compositions of whole-body burbot were 81.83–86.91% for moisture, 4.42–20.49% for lipid, 63.53–75.65% for protein, and 8.41–14.25% for ash, and energy content was 4,818–5,915 kcal/g. Diets 1 and 2 provided the best performance among the all diets and may be used in ongoing and future burbot aquaculture efforts. This study demonstrates that burbot can be reared with multiple commercial larval diets and provides the first reported macronutrient compositions and relative cannibalism of age-0 burbot. This study will help advance the ongoing Kootenai Tribal burbot conservation aquaculture program and may have important applications in other conservation and future commercial production efforts.**

Burbot *Lota lota* are a monotypic freshwater species within the cod family (Gadidae) with a Holoarctic distribution (Scott and Crossman 1973; Howes 1991). Burbot in the Kootenai River, Idaho, and Kootenay Lake, British Columbia, require conservation aquaculture rehabilitation (KVRI 2005; Ireland and Perry 2008). With the exception of a several earlier efforts related to burbot aquaculture (e.g., Bjorn 1939; McCrimmon 1959; Kouřil et al. 1985; Steiner et al. 1996; Lahnsteiner et al. 1997, 2002; Adámek 2000; Taylor and McPhail 2000; Wolnicki et al. 2001, 2002; Harzevili et al. 2003, 2004), burbot have

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largely remained an uncultured species. Beginning in 2003, an experimental research program initiated at the University of Idaho's Aquaculture Research Institute (UIARI) focused on assessing the feasibility of developing burbot culture as a population restoration measure. Spawning, sperm motility inhibition, and semen cryopreservation methods were developed first (Zuccarelli et al. 2007; Jensen et al. 2008a, 2008b), followed by incubation methods and larval and juvenile rearing strategies (Jensen et al. 2008c; Polinski et al. 2010c), and then burbot disease susceptibility was characterized (Polinski et al. 2010a, 2010b). Collectively, these efforts enabled the first releases of cultured burbot of ages 0, 1, 2, and 3 years for conservation purposes in British Columbia and Idaho in 2009. Despite recent developments in burbot aquaculture, uncertainties still exist. The need to transition larval burbot to commercial feeds remains critical to their successful aquaculture production.

Published information regarding burbot rearing with commercial larval diets is limited. Previous studies by Jensen et al. (2008c) and Żarski et al. (2009) successfully reared burbot with commercial larval diets; however, survival was low (0–7.5%), indicating the need for improved methods. Burbot are challenging to culture and rear on commercial larval diets owing to their small size (3–4 mm at hatch) and low thermal demands, which results in prolonged development of the mouth, functional alimentary tract, and swim bladder (McPhail and Paragamian 2000; Taylor and McPhail 2000). Subsequently, they require very small, nutritious live prey for prolonged periods of time (Harzevili et al. 2003, 2004; Jensen et al. 2008c) before they can be successfully transferred to and reared on commercial diets and grown to a large enough size to be tagged for release. Additionally, conservation aquaculture reintroduction programs benefit by producing larger fingerlings for release, which increases the probability of their survival in the wild (King 2004). Moreover, efficient rearing protocols reduce time, labor, live-prey production costs, and reduced pathogen transmission risks associated with culturing live prey in the hatchery (Planas and Cunha 1999; Baskerville-Bridges and Kling 2000; Blair et al. 2003).

We undertook this study to improve culture methods and clarify the rearing bottlenecks that currently limit burbot production. Its primary objectives were to (1) compare performance based on mean weight and length, survival, and cannibalism of age-0 burbot fed three commercial larval diets and one live-prey treatment after 8 weeks and (2) document for the first time the macronutrient composition (percent moisture, lipid, protein, and ash and energy content) of age-0 burbot and their diets for use in future nutritional-related studies.

## METHODS

*Adult husbandry and gamete collection.*—The burbot used in this study were $F_1$ progeny of captive broodstock (average mass, 2.35 kg; range, 1.35–3.75 kg; average length, 67.5 cm; range, 56–76 cm) originating from Moyie Lake, British Columbia, that were captured in cod traps (Neufeld and Spence 2004). After capture, broodstock were held for 15–27 months before spawning for this trial. These fish were caught in November of 2006 and 2007. Spawning took place in February–March 2009. All broodstock were kept in a recycling system in a hatchery setting. Broodstock were fed live rainbow trout *Oncorhynchus mykiss* (10–50 g) purchased as eyed eggs from Troutlodge, Sumner, Washington. The trout were added to adult rearing tanks on demand (when no more trout were left in the tank) at two trout per adult burbot. Trout eggs were disinfected, hatched, and reared at the UIARI, and fed commercial trout diets (Rangen, Buhl, Idaho. Female burbot broodstock were implanted with Ovaplant (Syndel laboratories, Qualicum Beach, British Columbia) during late January, and were checked daily for ripe eggs as the spawning season approached. Eggs and milt were carefully collected into separate, dry, sterile plastic bags (Whirlpak, Fort Atkinson, Wisconsin). Care was taken to prevent water contamination when collecting gametes, which were stored at 4°C for up to 2 h before activation. Gametes were combined (two males per female; approximately 2 mL of milt per 250,000 eggs), activated with approximately 500 mL of 4°C rearing system water, and manually stirred for 1 min. Fertilized eggs were rinsed of milt after 1 min and allowed to water-harden for 1 h. During water hardening fertilized eggs were treated with 25–50 mg/L buffered iodophore (Ovidine, Western Chemical, Ferndale, Washington) for 30 min before incubation.

*Egg incubation and early rearing.*—Eggs were incubated in flow-through, upwelling, 2-L conical incubators with 500–1,000 mL/min inflow, as described by Jensen et al. (2008c). Water temperature was maintained at 3–5°C. At these temperatures incubation lasted 35–50 d, which included 8–15-d hatching durations. Posthatch (after the first observed hatched larvae) water temperatures were increased gradually to 10°C over a 10-d period by approximately 1°C every 2 d. Live prey were provided at 15 d post hatch (dph) when a swim bladder and complete alimentary tract (functional mouth and anus present) could be observed, indicating that larvae were able to consume and digest live prey. Before the rearing experiment, larval feeding began with rotifers fed three times per day by hand. In addition to hand feedings, rotifers were fed by an automated injection system at night from 15 to 80 dph. From 81 to 100 dph, *Artemia* nauplii were co-fed with rotifers. Feeding only *Artemia* nauplii occurred from 101 to 120 dph and was followed by feeding enriched instar 2–3 *Artemia* metanauplii from 121 to 149 dph (total 134 d of live-prey feeding before the rearing experiment). The amounts of rotifer and *Artemia* that were fed targeted a density of 10 organisms/mL per feeding. Cofeeding of prey included half the target feeding density for each prey item (e.g., 5 rotifers/mL + 5 *Artemia*/mL = 10 organisms/mL per feeding).

*Live prey.*—Live prey included rotifers *Brachionus plicatilis* (Reed Mariculture, Campbell, California), brine shrimp *Artemia* spp. (source: Great Salt Lake, 80%; Salt Creek, Salt Lake City, Utah), and cladocerans *Daphnia magna* (Florida Aqua Farms, Dade City, Florida). Rotifers were produced in 700-L closed recycling systems and fed *Nannochloropsis* spp. algae

paste. *Artemia* cysts were decapsulated and hatched in 18.9-L plastic containers. After 24 h *Artemia* were fed to burbot larvae or were transferred to 40-L, 0.5-m circular tanks for an additional 16 h and fed *Nannochloropsis* spp. algae paste as a preenrichment and food source. *Daphnia magna* were cultured in 1.6-m circular fiberglass tanks (1,100 L water volume) outside under local natural lighting and weather conditions. Each *D. magna* culture tank was supplied with sodium bicarbonate (1 g/L as baking soda), added as a carbonate buffering source, and fed 10 g of spray dried *Spirulina* (Algae feast, Aquatic Ecosystems, Apopka, Florida) weekly. *Daphnia magna* tanks were not aerated, although they were manually stirred to suspend sediments when fed. Nutritional enrichment of rotifers and *Artemia* included *Nannochloropsis* spp. algae paste (1 mL/0.5 L) and *D. magna* were enriched with spray-dried *Spirulina* (1 g/10 L) for 1 h before being fed to the burbot.

*Rearing diets.*—Diet 1 had a particle size of 400–630 μm, diet 2 a particle size of 360–910 μm, and diet 3 a particle size of 400–800 μm (Table 1). Diets 2 and 3 included two different feed types and particulate size ranges. The different feed types were mixed together at a ratio of 1:1.

*Rearing experiment.*—Burbot larvae (approximately 149 dph; $0.016 \pm 0.011$ g wet weight [mean $\pm$ SD]; $13.6 \pm 2.63$ mm total length [TL]) were pooled into a raceway-style trough (3 m long, 0.6 m wide, 0.2 m deep) supplied with 10°C water at 4.4 L/min. The pooled fish were held and not fed for 24 h before initial sampling and stocking rearing experiment tanks. Each rearing experiment tank was stocked with 200 fish from the mixed pool in a predetermined random order. Each of the four diet treatments was randomly assigned to 3 of the 12 rearing tanks before stocking ($n = 3$). The rearing experiment was performed in a flow-through system consisting of twelve 1-m-diameter (250 L) insulated black circular tanks with the bottoms painted gray for contrast to clean more efficiently (Moore 1996). The water volume of each tank was 100 L and inflow was 2 L/min per tank. Each tank was equipped with an air diffuser for surface tension agitation, aeration, and water circulation. Each tank outflow was screened with 500-μm mesh to prevent larval fish from escaping. Water temperature in the rearing tanks was increased from 10°C gradually to 16°C over 1 week following stocking. Light intensity over the experimental tanks was measured at 1,900 1×. Each day before feeding, waste was removed via siphoning and mortalities were counted and recorded. Cannibals were not removed; cannibals were recorded as fish that had another fish in the mouth. Each tank was inventoried at 2-week intervals (for more information see sampling and data collection section) and the experiment was carried out for 8 weeks.

*Feeding.*—All feeding in the experimental tanks began with enriched *Artemia*, which were hand-fed to the fish twice per day for 14 d at a density of 2 *Artemia*/mL. From 15–28 d, enriched *Artemia* were co-fed with treatment diets that included 1 enriched *Artemia*/mL and 1 g of rearing diet. Rearing diets were fed by hand first and live prey were added 1 h later. When a diet of *Artemia* and *D. magna* were co-fed, feed consisted of 1 enriched *Artemia*/mL and approximately 3,500 *D.*

TABLE 1. Manufacturers' information for the commercial diets used for age-0 burbot. Blank cells indicate that no information was available.

| Diet characteristic | Diet 1 | Diet 2 | Diet 3 |
|---|---|---|---|
| Size range (μm) | 400–630 | 360–910 | 400–800 |
| Moisture (%) | | 6.3–6.6 | 7 |
| Ash (%) | 9.6 | 16 | 12 |
| Fiber (%) | 0.5 | 3 | 1 |
| Fat (%) | 15 | 10 | 14 |
| Protein (%) | 60 | 50 | 57 |
| Ca (%) | 0.5 | 2.3 | |
| P (%) | 1.5 | 1.5 | 1.2 |
| Na (%) | 1.1 | | |
| Vitamins | | | |
| A (IU/kg) | 15,000 | | 30,000 |
| $D_3$ (IU/kg) | 2,400 | | 2,500 |
| E (mg/kg) | 300 | | 400 |
| C (mg/kg) | 1,000 | | 1,500 |
| Cupric chelate of amino acid hydrate 10% Cu (mg/kg) | | | 5 |
| Omega-3 highly unsaturated fatty acids (mg/g dry weight) | | | 30 |
| Docosahexaenoic acid/eicosapentaenoic acid | | | 1.7 |
| Antioxidants | Butylhydroxytoluene, ethoxyquine | | Butylhydroxytoluene, ethoxyquine, propylgallate |

*magna* no larger than 1,000 μm in size (1,000 μm USA Standard testing sieve, Fisher Scientific, Pittsburgh, Pennsylvania). The fish were fed twice daily on an approximate dry matter basis, based on Ward and Robinson (2005) who reported *D. magna* averaged 0.17 mg dry weight. Cofeeding of *Artemia* and rearing diets was also based on approximate dry matter (e.g., 5 *Artemia*/mL per feeding ×100 L water volume ×2 μg *Artemia* nauplii dry matter [Hoff and Snell 2001] ≈ 1 g of diet). After *Artemia* feeding ended, all cofeeding ceased. From 29–56 d, approximately 2-g rearing diets were fed daily to fish in the treatment tanks receiving commercial diets, and approximately 7,000 *D. magna* were fed twice daily to fish in the *D. magna* treatment tanks. Premeasured rearing diets were fed at a rate of 1 g/12 h with automatic feeders (Fish Mate, F14 aquarium fish feeder, Petco Animal Supplies, San Diego. California). All feeds were fed on an approximate dry weight basis with larval-rearing diets weighed to the nearest 0.1 g. Live feeds were fed based on a volumetric basis, after making counts of three groups of known volume, averaging the counts, and using the average to determine the final volume of live diets to feed.

*Sampling and data collection.*—To determine initial mean wet weights and lengths, 30 larval fish were collected from the mixed pool before stocking the experimental tanks. These larvae were weighed to the nearest 0.001 g wet weight individually with a Mettler AE 163 microscale (American Instrument Exchange, Haverhill, Massachusetts) and measured to the nearest 0.01 mm with the ImageJ version 1.43 software package (Abramoff et al. 2004) where a ±1-mm scale was used in each image, precalibrated before measurements. These fish were not returned to the rearing tank mixed pool of fish.

Survival and cannibalism were calculated for each tank based on inventory counts. Each tank of fish was inventoried by counting all of the fish to determine survival and uncounted mortality (cannibalism). Cannibalism = number of fish at $t_1$ − number of fish at $t_2$ − mortality counts between $t_1$ and $t_2$, where $t$ = time point. Total mortality since the previous inventory was added to the inventory count and the missing uncounted fish were considered lost to cannibalism. Each tank of fish was counted one at a time and pooled into a 18.9-L container from which they were further sampled to assess growth-related metrics. After the initial individual weights and lengths were obtained, group weights and individual lengths were determined from a 30-fish sample from each tank during each inventory. A group weight was recorded for each experimental tank and a digital image was taken of each 30-fish group in a shallow pan (approximately 0.5 cm water depth) and each individual fish measured to the nearest 0.01 mm with the ImageJ 1.43 software (Abramoff et al. 2004) for length. All fish sampled for measurements during inventories were returned to the tank they came from. All tanks were inventoried after 2, 4, 6, and 8 weeks poststocking.

Macronutrient composition (percent moisture, lipid, protein, and ash and energy as kcal/g) of larval burbot (approximately 150 dph) and feeds were characterized at the beginning of the experiment. Reared burbot (approximately 206 dph) and a group of "pond reared" burbot were characterized at the end of the experiment for observational comparison. Pond-reared burbot were cultured in aerated, 7,000-L fiberglass tanks supplied with *D. magna*. The ponds were not fed or fertilized. Duplicate samples were processed for macronutrient composition from the following groups: (1) a pool of 400 larvae from the initial mixed pool; (2) 10 juveniles at the end of the experiment from each tank, pooled to make a 30-fish sample from each treatment; (3) six juveniles reared in ponds for observational comparison; and (4) live and commercial diets fed during the experiment. Moisture content was estimated with the Association of Official Analytical Chemists (AOAC) method 930.15 (AOAC International, Gaithersburg, Maryland). Lipid content was estimated with the ANKOM XT15 extraction system for rapid determination of oil–fat using high temperature solvent extraction (AOCS official procedure Am 5–04), protein with the LECO TruSpec N (St. Joseph, Michigan), ash by using AOAC method 942.05, and energy with a Parr 6300 calorimeter (Moline, Illinois). Temperature was measured and samples for chlorine, pH, ammonia,

TABLE 2. Wet-weight- and length-related growth metrics (means ± SDs) for age-0 burbot fed different diets. Within rows, values with different letters are significantly different ($P \leq 0.05$).

| | Diet treatments | | | |
|---|---|---|---|---|
| Growth metric | *Daphnia magna* | Diet 1 | Diet 2 | Diet 3 |
| Initial mean weight (g) | 0.016 ± 0.01 | 0.016 ± 0.01 | 0.016 ± 0.01 | 0.016 ± 0.01 |
| Final mean weight (g) | 0.464 ± 0.16 z | 0.921 ± 0.07 y | 0.839 ± 0.07 y | 0.539 ± 0.03 z |
| Weight gain (g) | 0.448 | 0.905 | 0.823 | 0.523 |
| Weight gain (g/d) | 0.008 | 0.016 | 0.015 | 0.009 |
| Weight SGR (%/d)[a] | 6.01 | 7.23 | 7.07 | 6.28 |
| Initial mean length (mm) | 13.6 ± 2.6 | 13.6 ± 2.6 | 13.6 ± 2.6 | 13.6 ± 2.6 |
| Final mean length (mm) | 41.04 ± 6.5 z | 49.38 ± 8.6 y | 46.08 ± 7.3 zy | 42.64 ± 4.9 z |
| Change in length (mm/d) | 0.489 | 0.638 | 0.579 | 0.517 |
| Length SGR (%/d)[a] | 1.96 | 2.29 | 2.17 | 2.03 |

[a]Specific growth rate (SGR;%/d) = $100 \cdot (\log_e X_2 - \log_e X_1)/(t_2 - t_1)$, where $X$ = mean wet weight or length and $t$ = time.

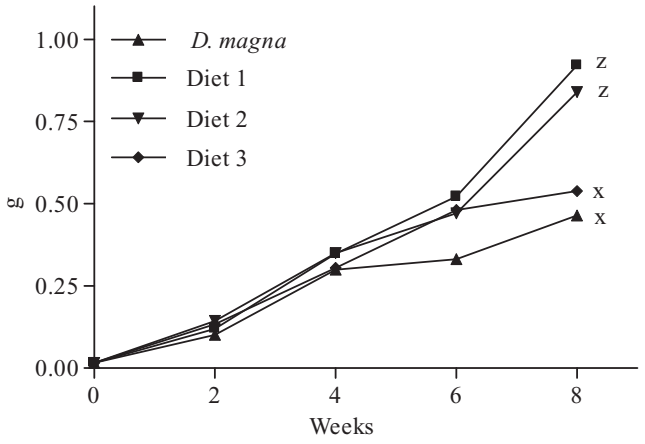


FIGURE 1. Mean wet weight (g) of burbot fed *Daphnia magna* and three different commercial larval diets over time.

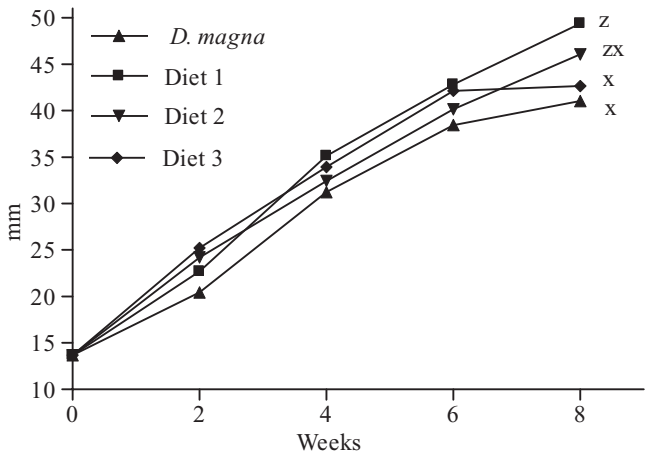


FIGURE 2. Mean length (mm) of burbot fed *Daphnia magna* and three different commercial larval diets over time.



nitrite, alkalinity, salinity, and dissolved oxygen measurements were taken four times during the 8-week experiment. Water samples were collected from experimental tanks with fish.

*Experiment design and statistics.*—Statistical procedures were performed with SAS (Statistical Analysis Software program Proc GLM, version 9, SAS Institute, Cary, North Carolina) software. Normality of the data (Shapiro and Wilk 1965) and homogeneity of variance (Snedecore and Cochran 1993) were tested to ensure that assumptions of one-way analysis of variance (ANOVA) were satisfied. Weight and length data were analyzed with one-way ANOVA as reported by Snedecor and Cochran (1993). Survival and cannibalism data were transformed (arcsine square root) before conducting ANOVA. The significance level was $\alpha \leq 0.05$. When significant effects were found, a post hoc Tukey's multiple comparisons means test was used to compare treatment differences.

## RESULTS

Significantly different mean wet weight and length growth metrics for fish were found among diet treatments (Table 2). The fish fed diet 1 and diet 2 had higher mean wet weights at the end of the experiment compared with the fish fed diet 3 and *D. magna* (Figure 1). At the end of the experiment (Figure 2), fish fed diet 1 were, on average, longer than those fed diet 3 or *D. magna*. However, mean length for fish fed diet 2 did not differ significantly among treatments. The specific growth rates (SGRs) for fish fed diets 1 and 2 were 7.07%/d and 7.23%/d for weight SGR, respectively, and were 2.29%/d and 2.17%/d, respectively, for length SGR. For fish fed diet 3 and *D. magna*, weight SGRs were 6.28%/d and 6.01%/d, respectively, and length SGRs were 2.03%/d and 1.96%/d, respectively (Table 2). Mean survival of fish after 8 weeks for all treatments ranged from 25% to 32% (Figure 3) but was not significantly different among treatments (Table 3). Across all treatment tanks, losses due to cannibalism ranged from 26% to 48% (Figure 4) but were not found to be significantly different among treatments after 8 weeks (Table 3).

Macronutrient analysis of whole-body age-0 burbot yielded the following results: moisture ranged from 81.73% to 86.91%, lipid content ranged from 4.42% to 20.49%, protein content ranged from 63.53% to 75.65%, and ash content ranged from 8.41% to 14.25%. Energy content of whole-body age-0 burbot ranged from 48.18 to 59.15 kcal/g (Table 4). For macronutrient compositions of live and commercial larval diets, respectively, moisture content ranged from 90.85% to 94.50% and from 5.75% to 6.82%, lipid content ranged from 4.30% to 12.38% and from 12.89% to 15.24%, protein content ranged from 45.07% to 63.52% and from 60.09% to 64.02%, and ash content ranged from 8.82% to 21.64% and from 10.46% to 12.93%. Energy content of the live and commercial larval

TABLE 3. Final percent survival, mortality, and cannibalism (means ± SDs) for age-0 burbot fed different diets. Within rows, values with different letters are significantly different ($P \leq 0.05$).

| Survival and mortality | *Daphnia magna* | Diet 1 | Diet 2 | Diet 3 |
|---|---|---|---|---|
| Survival | 30 ± 8.6 z | 25 ± 3.2 z | 32 ± 3.6 z | 27 ± 6.2 z |
| Counted mortality | 27 | 27 | 42 | 30 |
| Cannibalism[a] | 43 ± 9.6 z | 48 ± 5.0 z | 26 ± 11.2 z | 43 ± 16.6 z |
| Total mortality[b] | 70 | 75 | 68 | 73 |

[a]Number of fish at $t_1$ − number of fish at $t_2$ − mortality counts between $t_1$ and $t_2$.
[b]Counted and uncounted (cannibalism) mortality.

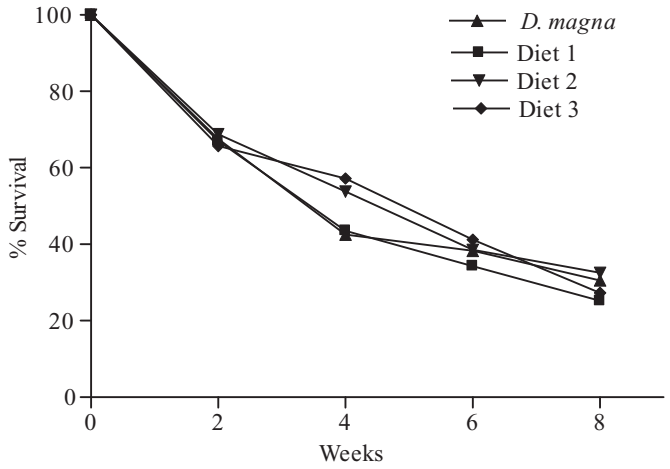


FIGURE 3. Mean survival of burbot fed *Daphnia magna* and three different commercial larval diets over time.

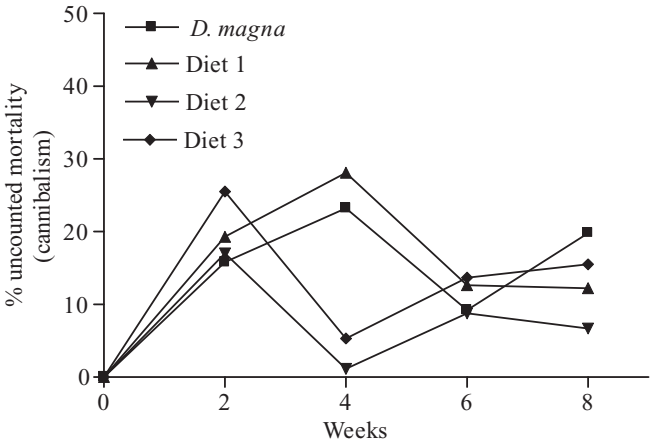


FIGURE 4. Mean unaccounted-for mortality attributed to cannibalism of burbot fed *Daphnia magna* and three different commercial larval diets over time.



diets ranged from 4,183 to 5,313 kcal/g and from 5,215 to 5,427 kcal/g, respectively (Table 5).

During the experiment water temperature ranged from 10.0°C to 18.3°C, chlorine ranged from 0 to 0.02 mg/L, pH ranged from 7.5 to 7.8, ammonia ranged from 0 to 0.005 mg/L, nitrite ranged from 0 to 0.132 mg/L, alkalinity ranged from 171 to 188 mg/L, salinity ranged from 0 to 0.1 g/L, and dissolved oxygen ranged from 5.08 to 6.75 mg/L.

## DISCUSSION

Diet 1 significantly outperformed diet 3 and *D. magna* treatments in terms of mean wet weight and length, although fish fed diet 1 did not significantly outperform those fed diet 2. Survival results of this study document the highest reported survival rates to date regarding burbot (approximately 150–206 dph) reared on commercial larval diets. The present study also provides the first look into cultured burbot cannibalism and the first macronutrient composition profiles for age-0 burbot at multiple life stages fed different diets and cultured in multiple ways. This information will be valuable as a reference for improving rearing strategies, selecting commercial diet formulations, and providing an empirical foundation for formulating and testing new diets for burbot.

This study demonstrates that cannibalism is a most important mortality factor in burbot rearing during the time period of this study and also provides the first quantitative estimates of burbot cannibalism during a feed transition study when fish are not removed. These levels of cannibalism are near or higher than the survival percentages in this study and clearly suggest that if cannibalism can be reduced production will increase.

With cannibalistic species, the timing of feed transition (when semistarvation may occur), rearing density, and life stage (near metamorphosis from larvae to juvenile, for example) are important factors that must be taken into account (Folkvord 1997). In the present study, commercial diet transition did not begin until after 164 dph, much later in development than in the previous study by Jensen et al. (2008c), which found that prolonged live-prey feeding improved survival and growth. The present study also incorporated 2-week-long cofeeding tactics to reduce semistarvation during feed transition times. Regarding rearing density, the present study intentionally incorporated a low density of 2 larvae/L, because it is known that burbot are highly piscivorous and cannibalistic (Kahilainen and Lehtonen 2003). Although, this low larval stocking density may

TABLE 4. Whole-body (dry-matter) age-0 burbot macronutrient compositions of fish fed different diets and cultured by different methods.

| | | | Diet | | | |
|---|---|---|---|---|---|---|
| Macronutrient | Larval burbot[a] | Pond burbot[b] | *D. magna* | Diet 1 | Diet 2 | Diet 3 |
| Moisture (%) | 86.91 | 81.73 | 84.68 | 81.83 | 84.30 | 84.48 |
| Lipid (%) | 10.10 | 10.22 | 4.42 | 20.49 | 15.90 | 14.06 |
| Protein (%) | 70.12 | 74.06 | 75.65 | 63.53 | 67.46 | 69.14 |
| Ash (%) | 11.43 | 11.85 | 14.25 | 8.41 | 10.67 | 10.19 |
| Energy (kcal/g) | 5,281 | 5,303 | 4,818 | 5,915 | 5,609 | 5,594 |

[a]Initial sample (30 fish pooled).
[b]Semi-intensive pond-reared burbot sample (6 fish pooled).

TABLE 5. Macronutrient compositions of live and commercial larval diets (dry matter) determined for use in age-0 burbot diet treatments.

| | Live diets | | | Commercial larval diets | | |
|---|---|---|---|---|---|---|
| Macronutrient | *Artemia*[a] | *D. magna*[b] | *D. magna*[c] | Diet 1 | Diet 2 | Diet 3 |
| Moisture (%) | 94.50 | 95.80 | 90.85 | 6.82 | 5.75 | 5.14 |
| Lipid (%) | 12.38 | 10.25 | 4.30 | 15.24 | 12.89 | 15.01 |
| Protein (%) | 63.52 | 49.61 | 45.07 | 64.02 | 60.49 | 60.09 |
| Ash (%) | 8.82 | 16.31 | 21.64 | 10.46 | 12.93 | 11.88 |
| Energy (kcal/g) | 5,313 | 4,802 | 4,183 | 5,427 | 5,215 | 5,337 |

[a]*Artemia* (GSL 80%, decapsulated) enriched with *Nannochloropsis* algae, roti rich.
[b]Sieved, ≤1,000 μm mesh, enriched with spray-dried *Spirulina*.
[c]Unsieved, enriched with spray-dried *Spirulina*.



or may not be applicable to large-scale production efforts owing to expense, it is applicable to current experimental scale efforts that are needed for conservation rehabilitation efforts. Indeed, density and behavior studies are needed that involve burbot of the size and age as those used in the present study. Regarding life stage, Curnow et al. (2006) reported that barramundi *Lates calcarifer* cannibalism appeared to increase at a size of 6–8 mm. The present study began with burbot averaging 13.6 ± 2.6 mm. Compared with Atlantic cod *Gadus morhua*, 14 mm is a size nearing metamorphosis when metabolic changes occur, and the size variation could have affected the cannibalistic tendencies of the burbot in our experiment resulting in the smaller individuals becoming prey of the larger individuals (Folkvord 1997; Baskerville-Bridges and Kling 2000). Thus, grading is highly recommended to improve survival and decrease cannibalism for future burbot production efforts. Until density- and behavior-related studies can be conducted with burbot, density dependence as it relates to optimal growth, survival, and production of burbot remains unknown.

Larval burbot lipid content was found to be 10.10%, and they had a high protein level of 70.12%. Based on the observed burbot growth in this study, fish fed diet 1 performed the best, which suggests that the higher levels of lipid and protein in the diet may help improve burbot growth. However, performance of fish fed diet 2 was not significantly different and provides evidence that the observed higher diet lipid content may not be necessary for improving cultured burbot performance. To confirm this observation, additional research into micronutrients is needed and this study did not analyze micronutrient compositions of the fish or diets. By comparison, Atlantic cod larvae (39 dph), another gadiform, that were fed *Artemia* and a control rearing diet, Baskerville-Bridges and Kling (2000) reported lipid levels of 19.8% and 23.9%, respectively. Moreover, the observed lipid content of burbot (approximately 150–206 dph) in the present study was near to the highest levels found by Rosenlund et al. (2004), who reported that lipid levels in much larger (193 g average body weight) Atlantic cod juveniles ranged from 5.5% to 10.3% and protein levels ranged from 57.7% to 67.6%. Clearly life stage and species differences exist between the freshwater burbot and marine Atlantic cod, but in comparison this provides evidence that proximal lipid levels may change as gadiform fishes grow, which needs to be considered in future nutritional studies with burbot. Of interest is that fish fed diet 2 had the lowest lipid content; yet, these fish outperformed fish fed diet 3 based on mean ending wet weight and diet 2-fed fish were not significantly different from those fed diet 1, which had the highest levels of lipid at the end of the trial. Again, comparing burbot with juvenile Atlantic cod, lipid concentration had no significant effect on SGR of the cod (Rosenlund et al. (2004), which may also be the case with burbot; however, further study is needed to confirm this. Taken together, both diet 1 and diet 2 appear to be the most suitable of the four rearing diet treatments tested. However, further nutrient-related studies (similar to Baskerville-Bridges and Kling 2000 and Rosenlund et al. 2004) are needed. These could include palatability and digestibility studies and a more in-depth biochemical analysis of fatty acid and amino acid profiles of feeds and at different life stages (i.e., yolk composition, embryo, adult anatomy), which could improve our understanding of nutrient-related factors that may be affecting burbot growth and survival.

This study contributes to the rapidly emerging body of scientific literature on aquaculture methods, nutritional status, and biology of age-0 burbot and may serve as an empirical basis for ongoing and future burbot aquaculture efforts. The results of this study will help advance the ongoing Kootenai Tribal burbot conservation aquaculture program and may have important applications for other conservation and future commercial production efforts.

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## REFERENCES


Abramoff, M. D., P. J. Magelhaes, and S. J. Ram. 2004. Image processing with ImageJ. Biophotonics International 7:36–42.

Adámek, Z. 2000. The applicability of feed mixtures in burbot, *Lota lota*, farming. Krmiva 2:71–76.

Baskerville-Bridges, B., and L. J. Kling. 2000. Development and evaluation of microparticulate diets for early weaning of Atlantic cod *Gadus morhua* larvae. Aquaculture Nutrition 6:171–182.

Bjorn, E. E. 1939. Preliminary observations and experimental study of the ling, *Lota maculosa* (LeSueur), in Wyoming. Transactions of the American Fisheries Society 69:192–196.

Blair, T., J. Castell, S. Neil, L. D'Abramo, C. Cahu, P. Harmon, and K. Ogunmoye. 2003. Evaluation of microdiets versus live feeds on growth, survival and fatty acid composition of larval haddock (*Melanogrammus aeglefinus*). Aquaculture 225:451–461.

Curnow, J., J. King, G. Partridge, and S. Kolkovski. 2006. Effects of two commercial microdiets on growth and survival of barramundi (*Lates calcarifer* Bloch) larvae with various early weaning protocols. Aquaculture Nutrition 12:247–255.

Folkvord, A. 1997. Ontogeny of cannibalism in larval and juvenile fishes with special emphasis on Atlantic cod. Pages 251–278 *in* R. C. Chambers and E. A. Trippel, editors. Early life history and recruitment in fish populations. Chapman and Hall, London.

Harzevili, A. S., D. De Charleroy, J. Auwerx, J. Van Slycken, P. Dhert, and P. Sorgeloos. 2003. Larval rearing of burbot (*Lota lota*) using *Brachionus calyciflorus* rotifer as starter food. Journal of Applied Ichthyology 19:84–87.

Harzevili, A. S., I. Dooremont, I. Vught, J. Auwerx, P. Quataert, and D. De Charleroy. 2004. First feeding of burbot, *Lota lota*, larvae under different temperatures and light conditions. Aquaculture Research 35:49–55.

Hoff, J. H., and T. W. Snell. 2001. Artemia culture. Pages 95–106 *in* J. Neslen, editor. Plankton culture manual, 5th edition. Florida Aqua Farms, Dade City.

Howes, G. J. 1991. Biogeography of gadoid fishes. Journal of Biogeography 18:595–622.

Ireland, S. C., and P. N. Perry. 2008. Burbot restoration in the Kootenai River basin: using agency, tribal, and community collaboration to develop and implement a conservation strategy. Pages 251–256 *in* V. L. Paragamian and D. H. Bennett, editors. Burbot: ecology, management, and culture. American Fisheries Society, Symposium 59, Bethesda, Maryland.

Jensen, N. R., S. R. Williams, S. C. Ireland, J. T. Siple, M. D. Neufeld, and K. D. Cain. 2008a. Preliminary captive burbot spawning observations. Pages 155–165 *in* V. L. Paragamian and D. H. Bennett, editors. Burbot: ecology, management, and culture. American Fisheries Society, Symposium 59, Bethesda, Maryland.

Jensen, N. R., M. D. Zuccarelli, S. J. Patton, S. R. Williams, S. C. Ireland, and K. D. Cain. 2008b. Cryopreservation and methanol effects on burbot sperm motility and egg fertilization. North American Journal of Aquaculture 70:38–42.

Jensen, N. R., S. C. Ireland, J. T. Siple, S. R. Williams, and K. D. Cain. 2008c. Evaluation of egg incubation methods and larval feeding regimes for North American burbot. North American Journal of Aquaculture 70:162–170.

Kahilainen, K., and H. Lehtonen. 2003. Piscivory and prey selection of four predator species in a whitefish dominated subarctic lake. Journal of Fish Biology 63:659–672.

King, K. 2004. Growth, survival, and body composition of juvenile Atlantic sturgeon fed five commercial diets under hatchery conditions. North American Journal of Aquaculture 66:53–60.

Kouřil, J., O. Linhart, K. Dubský, and P. Kvansnička. 1985. The fertility of female and male burbot (*Lota lota*) reproduced by stripping. Prace Vyzk Us tav Rybarsky Hydrobiol Vodnany 14:75–79.

KVRI (Kootenai Valley Resource Initiative). 2005. Burbot conservation committee. Kootenai River/Kootenay Lake conservation strategy. Prepared by the Kootenai Tribe of Idaho and S. P. Cramer and Associates, Bonners Ferry, Idaho.

Lahnsteiner, F., B. Berger, R. Patzner, and T. Weismann. 1997. Semen motility and seminal fluid composition in the burbot (*Lota lota*). Journal of Applied Ichthyology 13:113–119.

Lahnsteiner, F., N. Mansour, and T. Weismann. 2002. The cryopreservation of spermatozoa of the burbot, *Lota lota* (Gadidae, Teleostei). Cryobiology 45:195–203.

McCrimmon, H. R. 1959. Observations on spawning of burbot in Lake Simcoe, Ontario. Journal of Wildlife Management 23:447–449.

McPhail, J. D., and V. L. Paragamian. 2000. Burbot biology and life history. Pages 11–23 *in* V. L. Paragamian and D. W. Willis, editors. Burbot: biology, ecology, and management. American Fisheries Society, Fisheries Management Section, Publication Number 1, Spokane, Washington.

Moore, A. A. 1996. Intensive culture of walleye fry on formulated feed. Pages 195–198 *in* R. C. Summerfelt, editor. Walleye culture manual. North Central Regional Aquaculture Center, Culture Series 101, Iowa State University, Ames.

Neufeld, M. D., and C. R. Spence. 2004. Evaluation of a simple decompression procedure to reduce decompression trauma in trap-caught burbot. Transactions of the American Fisheries Society 133:1260–1263.

Planas, M., and I. Cunha. 1999. Larviculture of marine fish: problems and perspectives. Aquaculture 177:171–190.

Polinski, M. P., J. D. Drennan, W. N. Batts, S. C. Ireland, and K. D. Cain. 2010a. Establishment of a cell line from burbot *Lota lota* with characterization of susceptibility to IHNV, IPNV and VHSV. Diseases of Aquatic Organisms 90:15–23.

Polinski, M. P., T. R. Fehringer, K. A. Johnson, K. R. Snekvik, S. E. LaPatra, B. R. LaFrentz, S. C. Ireland, and K. D. Cain. 2010b. Characterization of susceptibility and carrier status of burbot to IHNV, IPNV, *Flavobacterium psychrophilum*, *Aeromonas salmonicida*, and *Renibacterium salmoninarum*. Journal of Fish Diseases 33:559–570.

Polinski, M. P., N. R. Jensen, K. D. Cain, K. A. Johnson, and S. C. Ireland. 2010c. Assessment of formalin and hydrogen peroxide use during egg incubation of North American burbot. North American Journal of Aquaculture 72:111–117.

Rosenlund, G., Ø. Karlsen, K. Tveit, A. Mangor-Jensen, and G. I. Hemre. 2004. Effect of feed composition and feeding frequency on growth, feed utilization and nutrient retention in juvenile Atlantic cod, *Gadus morhua* L. Aquaculture Nutrition 10:371–378.

Scott, W., and E. J. Crossman. 1973. Freshwater fishes of Canada. Fisheries Research Board of Canada Bulletin 173.

Shapiro, S. S., and M. B. Wilk. 1965. An analysis of variance test for normality (complete samples). Biometrika 52:591–611.

Snedecor, G. W., and W. G. Cochran. 1993. Levene's test for homogeneity of variance. In Statistical methods, 8th edition. Iowa State University Press, Ames.

Steiner, V., N. Schotzko, M. Kletzel, and E. Kainz. 1996. Ein Beitrag zur wirtschaftlichen Aufzucht kleiner, sensibler Fischlarven am Beispiel der Aalrutte (*Lota lota* L.). [A contribution to the economical raising of small, sensitive fish larvae such as those of burbot (*Lota lota* L.).] Österreichs Fischerei 49:160–172. (In German with English summary).

Taylor, J. L., and J. D. McPhail. 2000. Temperature, development, and behavior in the early life history of burbot from Columbia Lake, British Columbia. Pages 30–37 *in* V. L. Paragamian and D. W. Willis, editors. Burbot: biology, ecology, and management. American Fisheries Society, Fisheries Management Section, Publication 1, Spokane, Washington.

Ward, T. J., and W. E. Robinson. 2005. Evolution of cadmium resistance in *Daphnia magna*. Environmental Toxicology 24:2341–2349.

Wolnicki, J., R. Kamiński, and L. Myszkowski. 2002. Temperature-influenced growth and survival of burbot *Lota lota* (L.) larvae fed live food under controlled conditions. Archives of Polish Fisheries 10(Fascicle 1):109–113.

Wolnicki, J., L. Myszkowski, and R. Kamiński. 2001. The influence of water temperature on the growth, survival, condition factor and biological quality of juvenile burbot, *Lota lota* (L.). Archives of Polish Fisheries 9(Fascicle 1):79–86.

Żarski, D., W. Sasinowski, D. Kucharczyk, M. Kwiatkowski, S. Krejszeff, and K. Targońska. 2009. Mass initial rearing of burbot *Lota lota* (L.) larvae under controlled conditions. Polish Journal of Natural Sciences 24:76–84.

Zuccarelli, M. D., N. Jensen, and R. L. Ingermann. 2007. Inhibitory effect of osmotic concentration, potassium and pH on motility of the sperm of the North American burbot *Lota lota maculosa*. Journal of Fish Biology 70:178–189.