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Article in *Journal of Applied Ichthyology* · September 2011

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Evaluation of methods for the collection and fertilization of burbot eggs from a wild stock for conservation aquaculture operations

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Summary

In the Kootenay River, British Columbia, Canada, burbot (*Lota lota maculosa*) numbers have diminished to near extirpation due to factors including physical changes to habitat and overfishing. Habitat restoration is currently underway but short-term recovery measures include the release of hatchery-reared burbot. Moyie Lake has been identified as a suitable brood source for a conservation aquaculture program but uncertainties remain regarding current population size, the feasibility of capturing broodstock and the ability to collect eggs from wild spawners. Specific objectives of our study were to: (i) develop a length at age key to provide a non-destructive means of population age structure identification, (ii) determine the location and general habitat characteristics of burbot spawning locations on Moyie Lake, (iii) provide a marked sample for future population estimation, and (iv) investigate the feasibility of collecting gametes for use in a conservation aquaculture program. A total of 181, 554, and 370 burbot were captured in 2009, 2010 and 2011, respectively. No significant relationship was established between length and age for burbot on Moyie Lake. Spawning burbot were observed over a number of different habitats, but high use areas consisted of steep banks dominated by a mix of gravel/boulder/cobble substrates. Mature burbot were reliably collected each year, and eggs from females were fertilized and transported to the hatchery. Egg survival was highly variable (range 0–98%) and resulted in an estimated 353 429, 3 032 143, and 3 970 283 eggs for use in the aquaculture program in 2009, 2010 and 2011, respectively. Results of this study demonstrate that gametes can be collected from adult burbot during spawning and eggs can be successfully fertilized in the field. Further methodological refinement aimed at improving egg fertilization and subsequent survival to the hatchery will be important as recovery moves forward.

Introduction

In the Kootenay River, British Columbia (BC), Canada (spelled Kootenai in Idaho and Montana, USA), burbot (*Lota lota maculosa*) numbers have diminished to near extirpation (Paragamian et al., 2008) due to factors including physical changes to the river and overfishing (Paragamian et al., 2000; Kootenai Valley Resource Initiative (KVRI) Burbot Committee (2005); Stapanian et al., 2010). Both the lake and river supported large sport and commercial harvests of burbot as late as the 1970s, until severe declines resulted in the eventual

closure of these fisheries (Paragamian et al., 2000; Ahrens and Korman, 2002). Recent capture and underwater video work suggests the historic population on Kootenay Lake at Balfour, BC has been functionally extirpated (Baxter et al., 2002a,b; Neufeld and Spence, 2004; Neufeld, 2005) and the most recent River population estimate in BC and Idaho was 50 burbot in 2004 (including tributary populations). At an estimated annual decline in population abundance of 14%, extirpation will likely occur in the next decade (Paragamian et al., 2008). Declines such as these in burbot populations are common and resulting conservation efforts are underway worldwide (Arndt and Hutchinson, 2000; Harzevili et al., 2003; Dillen et al., 2008; Ireland and Perry, 2008; Jensen et al., 2008a; Vught et al., 2008; Worthington et al., 2010). On the Kootenay River, a multilateral conservation agreement has been signed to ensure this issue is addressed (Kootenai Valley Resource Initiative (KVRI) Burbot Committee (2005) and a recovery planning document now guides burbot restoration in Idaho and BC (Neufeld et al., 2011b).

Habitat restoration has been identified as a critical issue in many burbot recovery programs (Paragamian and Wakkinen, 2008; Stapanian et al., 2010 and references within). Habitat improvements are currently being implemented but short-term recovery measures include the release of hatchery reared burbot to supplement the wild stock (Kootenai Valley Resource Initiative (KVRI) Burbot Committee (2005) as the release of young burbot has been shown to be effective in achieving year class survival (Dillen et al., 2008). The population size of the Kootenay burbot population is considered by managers to be too small to recover on its own, and also insufficient to provide suitable numbers as broodstock for conservation aquaculture operations. It is therefore anticipated that fish culture using a brood source from a Canadian waterbody will be key to restoring burbot in the recovery area (Kootenai Valley Resource Initiative (KVRI) Burbot Committee (2005), Neufeld et al., 2011b). Given the poor status of the Kootenay burbot population, it was important to identify a genetically similar stock and one of sufficient viability that brood fish or their gametes could be collected without compromising the donor population. In early attempts at culture method development, burbot from Columbia and Moyie Lake as well as Duncan and Arrow Lakes Reservoirs were provided to the Kootenai Tribe of Idaho (KTOI) and the University of Idaho's Aquaculture Research Institute (UI-ARI; Baxter et al., 2002a,b; Neufeld and Spence, 2004; Neufeld, 2005; Jensen et al., 2008a). Burbot studies on Trout and Columbia lakes in BC had indicated that the populations

in these water bodies were most likely not large enough to support any take for broodstock purposes (Baxter et al., 2002a,b; Neufeld and Spence, 2004; Prince, 2007). Although burbot numbers on Arrow Lakes Reservoir and Duncan Reservoir appeared to be reasonably healthy (Neufeld, 2005; Arndt and Baxter, 2006), samples from these populations suggested that genetic divergence from Kootenay burbot was large enough that other stocks should be considered for conservation aquaculture purposes (Powell et al., 2008). Genetic analyses, the location of Moyie Lake within the Kootenay River drainage and recent behaviour studies from experimental conservation aquaculture releases indicated that Moyie Lake was a suitable brood source for re-introduction into the Kootenay/ai River (Powell et al., 2008; Neufeld et al., 2011a).

Because of the need for the long term use of Moyie Lake burbot as a broodstock source for recovery efforts, there was a need to evaluate population size and other biological indicators, the feasibility of capturing broodstock and the ability to collect eggs from wild spawners. This study followed many of the recommendations of previous sampling efforts (Prince, 2007; Neufeld, 2008, 2010; Neufeld and Spence, 2009) and was designed to address specific objectives including, (i) to develop

a length at age key to provide a non-destructive means of population age structure identification, (ii) to determine the location and general habitat characteristics of burbot spawning locations on Moyie Lake, (iii) to provide a marked sample for future population estimation, and (iv) to investigate the feasibility of collecting gametes for use in conservation aquaculture programs.

Methods

Length, weight and age correlations

Otolith samples collected during previous burbot monitoring projects and creel surveys on Moyie Lake were aged with the 'break and burn' technique (Christensen, 1964) as part of a larger, multi-waterbody burbot otolith aging project (Cope, 2010). Aging data for Moyie Lake was combined with related biological data for individuals and then we fit a simple linear regression to evaluate the potential to use length and age as a non-destructive method of age assignment from existing length samples. We fitted a regression to both a pooled sample, as well as a sample stratified by sex. To evaluate the potential to use length data to predict weight, we also fit a log transformed linear regression to length and weight.

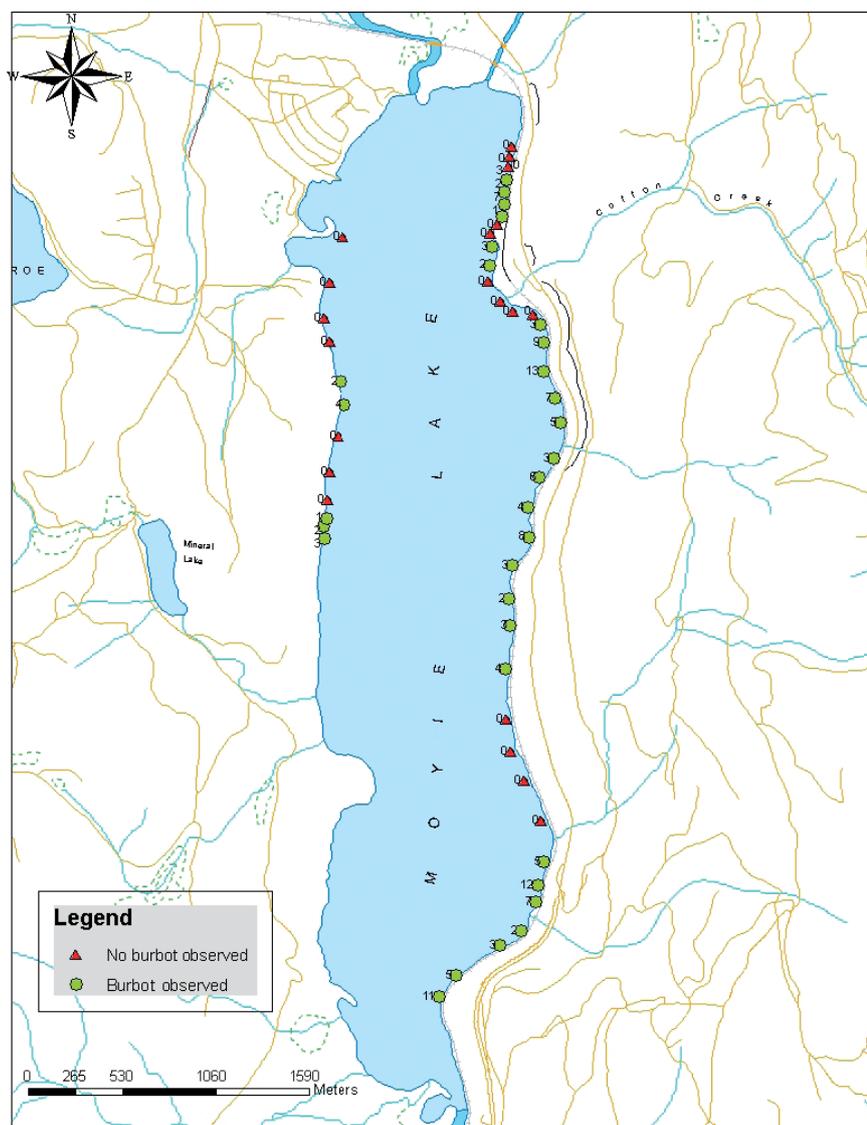


Fig. 1. Location of spawning survey 2009 observations on the north basin of Moyie Lake, identification of which sites we observed burbot

Spawning location survey

Much of the shoreline located in the north basin of Moyie Lake (Fig. 1) was surveyed in the winter of 2009 to identify burbot spawning locations and to characterize the habitat associated with observed spawning concentrations. The survey was conducted by drilling ice holes every 100–250 m in water that ranged from 1.5 to 8 m in depth; typical of the depths at which burbot are found spawning (Clemens, 1951; McCrimmon, 1959; Johnson, 1981; Boag, 1989; McPhail and Paragamian, 2000). At each location, an underwater camera (Cabela's Advanced Anglers Underwater Camera) was lowered to identify the presence or absence of burbot and to document substrate types. Observed burbot were considered to represent fish in spawning condition, because sampling of burbot in these shallow water locations in previous studies has indicated most are in spawning condition and appearance of burbot at these sites corresponds to the spawning period only (Neufeld and Spence, 2009). Substrates were visually assessed on a general scale appropriate with the quality of image available using recreational grade cameras. Substrate classes identified included fines (sand-silt), fines and cobble mix, fluvial (round) rocks and fines, cobble/boulder/gravel, or cliff face. Observations were undertaken for approximately 2 min at each of the locations so that comparisons between the numbers of burbot observed could be compared.

Adult capture and sampling

Adult burbot were captured for gamete collections in 2009, 2010 and 2011. Angling was the sole capture technique used in this study to collect burbot for conservation aquaculture gamete collection and tagging. Angling effort was concentrated along the east shore of the north basin of Moyie Lake (Fig. 1) as this area had previously been identified as a location with high angling capture efficiency, and spawner surveys in this study confirmed significant spawner abundance. Angling was conducted from February 9–17, 2009, February 8–18, 2010, and February 7–18, 2011. Anglers used a variety of barbless hook sizes, baited primarily with kokanee (*Oncorhynchus nerka*) carcasses. Captured burbot were placed in large holding tanks (156 L marine coolers) filled with lake water and oxygenated with a diffusing stone and either pressurized oxygen (2009 and 2010) or by battery powered aerators (2011). Holding tanks were insulated from the surface of the ice to prevent freezing and the water was changed hourly. When ambient air temperatures were below freezing, fish sampling occurred in a tent to minimize fish damage associated with freezing tissue. In 2009 and part of 2010, burbot were allowed to recover in holding tanks prior to release after gamete collections. Since the use of anaesthetic, discussed below, ceased, this was not continued and fish were released immediately after processing.

Most captured burbot were measured for both total length (cm) and weight (kg). The sex and maturity of captured individuals was assessed by gently applying pressure to the abdomen of the fish. If an individual burbot expressed gametes (eggs or milt) readily they were considered to be ripe. If only a few eggs could be expressed from a female and many of these were bound together in small amounts of membrane, the fish was referred to as green and if no gametes could be expressed, the individual was marked as unknown for both sex and maturity. Occasionally, female burbot collected had clearly spawned prior to capture and although eggs could be easily expressed, they had few eggs remaining in the body cavity.

These were considered to be spent females. Most ripe females captured, and a random subset of males were used to collect and fertilize eggs. Once sampled and before release, burbot were marked individually with a numbered Floy tag which was inserted through the anterior portion of the dorsal musculature and fin rays. In 2011, a select number of individuals were also given a PIT (Passive Integrated Transponder) tag to assist in future estimates of tag loss, which was injected in the cheek musculature.

In 2011, holding tubes were constructed as a trial to hold green females overnight and allow time for final egg maturation. Holding tubes were constructed of 2 m long, 15 cm diameter schedule 40 PVC, with up to 20, 3 cm holes drilled in the exterior surface to allow water to circulate through the tube. End and threaded top caps were installed, a tether rope was attached and then they were lowered through 20 cm ice holes to the lake bottom, at a depth of approximately 2 m. Females were placed in these tubes for a period not exceeding 48 h.

Gamete collection and fertilization

In 2009, burbot selected for gamete collection were anesthetized prior to handling, following methods described in Neufeld and Spence (2009). Initial trials in 2010, collected gametes from adults without the use of anaesthetic to reduce possible stress associated with anaesthetic, increased handling and total time in holding tanks prior to release. These early trials indicated gametes could be easily expressed from burbot that were not anesthetized, and therefore the use of anaesthetic was discontinued for the remainder of the 2010 study period and in 2011.

During gamete collection, moisture was first removed around the urogenital opening with paper towel to avoid water contact with eggs or milt. In 2009 and 2010, eggs from one female and milt from 3 to 5 males were then manually expressed into a bowl by applying pressure to the abdomen of the fish. However, in 2009, on one occasion, we also collected sperm several hours prior to use (in 120 ml whirl-pak bags). In 2011, all milt was collected from males prior to fertilizations, and stored in 120 ml whirl-pak bags between 2 and 4°C while efforts to collect females were underway each day. Sperm motility was assessed with the use of a battery powered microscope (Celestron model 44106). After no less than 5 min following collection and immediately prior to use, <1 ml of milt was placed on a slide, water activated and immediately observed to assess the percentage of motility. Males with good motility (roughly 75% or more) were used to fertilize eggs, while the others were discarded.

Once milt and eggs were combined, gametes were then water activated, and after approximately 2 min, rinsed with fresh water. In 2009, fertilized eggs were held in water and not handled for 60 min to allow water hardening to take place and disinfection of eggs with an Ovadine™ solution (Western Chemical Inc.) of 100 ppm was not completed until arrival at the UI-ARI facility. In 2010 and 2011, eggs were water hardened for 30 min, disinfected using an Ovadine solution of 25 ppm for a 30 min exposure time, and finally the Ovadine solution was drained and replaced with fresh water for an additional 30 min to allow eggs to fully water harden before transport. In 2009, lake water was used for all fertilizations and water hardening, while water used in 2010 and 2011 was obtained from a well at the Kootenay Trout Hatchery in Wardner, BC (following recommendations in Neufeld and

Spence, 2009) to reduce the risk of transferring biological material and parasites with egg shipments, and to allow a reduced disinfection dose. Water temperature was monitored hourly with a handheld thermometer to ensure that water being used for fertilizations was constant with that of Moyie Lake. In 2011, water containers were placed under the ice to keep temperatures constant with that of the lake.

In 2009 and 2010 water hardened eggs from each female were transferred into double layer plastic bags and additional fresh water was added. The bags were inflated with oxygen, sealed and placed in transport coolers to maintain ambient lake water temperatures during transport to the University of Idaho's Aquaculture Research Institute (UI-ARI). In 2011, water hardened eggs were transferred to 3.8 L drink coolers (Igloo brand), partially filled with water and then topped up with oxygen and placed in larger coolers for transportation. The period between collection of gametes and arrival at U of I was between 6 and 48 h due to the distance of the facility and other logistical constraints. Once at U of I, volumes and survival of eggs were estimated. Eggs were poured into a 500-ml graduated cylinder and allowed to settle for approximately 2 min. Once settled the total volume was recorded and three subsamples were removed with a 1-ml tuberculin syringe. The eggs from the subsample were allowed to settle for approximately another 2 min and then measured to the nearest 0.1 ml. The number of eggs was counted from each subsample and an estimate of eggs was produced per 1.0 ml. Using this estimate, the total number of eggs per 1.0 ml was multiplied by the original total volume of eggs to estimate the total number of eggs (Jensen et al., 2008a,b,c) taken and fertilized from adult burbot. Fertilization rate was estimated 48 h post activation by subsampling eggs and recording the number of fertilized and unfertilized eggs (see methods in Jensen et al., 2008a,c).

Results

Length, weight and age correlations

A total of 91 otolith samples were aged from the Moyie Lake population. Otolith age ranged from 5 to 16 years and was combined with the available biological data to evaluate linkages between length and age (Fig. 2). Regression lines were fit for a pooled length at age data set, as well as stratified by sex. No significant relationship was established between length and age for burbot on Moyie Lake (Pooled sample $R^2 = 0.09$; Females $R^2 = 0.13$; Males $R^2 = 0.18$). Additional

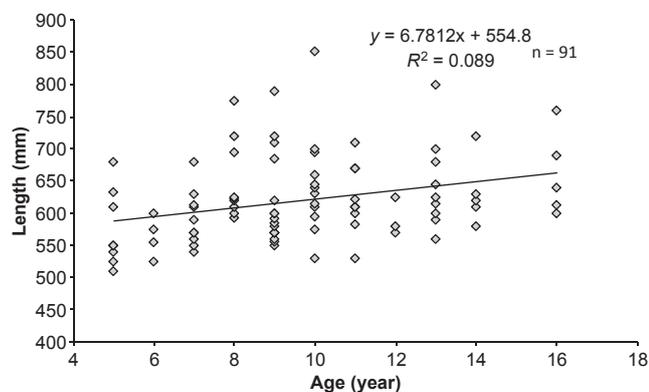


Fig. 2. Relationship between length and age of burbot otoliths collected from the recreational fishery (interpretation provided by Westslope Fisheries Ltd.)

data from other populations in the Kootenay Region (Cope, 2010), suggests that no significant relationship between length and age was evident in any population sampled.

A regression line was also fit for the pooled length and weight sample, to evaluate the potential to estimate weight from length samples. Although not highly significant ($R^2 = 0.74$) there was a relationship length and weight for burbot on Moyie that could be used to estimate weight from length sample in the future.

Spawning location survey

Spawning concentrations of burbot were observed along most of the east shore and some of the west shore of Moyie Lake (Fig. 1). Although time and logistical constraints (thin ice cover) precluded a comprehensive survey of the entire lake, the regular occurrence of spawners in areas surveyed suggested spawning likely also occurs in a portion of areas not surveyed. A total of 39 holes were surveyed on the east shore, with a total of 135 burbot observed within 26 of the holes. On the west shore, 12 holes were surveyed, having a total of 12 observed burbot in 5 of the holes.

Burbot were observed over a number of different habitats, generally classified as areas consisting of steep banks dominated by a mix of gravel/boulder/cobble substrates. No burbot were observed over fines (sand/silt). In the few occasions where higher numbers of spawners were observed at a single site, they were typically localized over cobble/boulder/gravel substrates. The majority of high use area observations occurred near ballast rock which typically ranged in size from 25 to 80 mm diameter, this rock was deposited from an adjacent rail grade. Some larger material was also present at these sites and was typically angular rock from rail corridor blasting (up to 1 m diameter). Observation of 'spawning balls' and high numbers of burbot were often associated with this habitat; likely preferred as spawning substrate (Fig. 3). The substrates observed on the east shore were typically larger in size (80–300 mm) and fluvial rock with little angular substrate present.

Adult capture

A total of 181, 554, and 370 burbot were captured in 2009, 2010 and 2011, respectively. Catch per unit effort was 2.4 burbot per rod hour in 2009, but was not recorded in 2010 or 2011 because of logistical constraints. Of the total individual burbot captured within each year, 141, 514, and 346 were tagged with numbered Floy tags in 2009, 2010 and 2011, respectively. In 2011, 155 Floy tagged fish were also tagged



Fig. 3. Spawning ball of burbot observed over rail grade ballast and blast material deposited during construction and rail maintenance using an underwater camera setup

with PIT tags to evaluate tag loss rates in future sampling programs. A total of 28 (5.2%), 11 (2%), and 24 (6.5%) burbot captured in 2009, 2010, and 2011, respectively were recaptures from tagging efforts in previous years. In 2009 the mean total length (TL) of captured burbot was 585 mm (range of 380–1020 mm, standard error, SE = 7.1) and the mean weight was 1.45 kg (range 0.35–3.1 kg, SE = 39.4). In 2010 the mean total length was 559 mm (range 365–940 mm, SE = 3.5) and the mean weight was 1.38 kg (range of 0.4–8.0 kg, SE = 0.03). In 2011 the mean total length was 555 mm (range 340–830 mm, SE = 3.8) and the mean weight was 1.4 kg (range 0.3–5.0 kg, SE = 0.03).

Sex and maturity were assessed for the majority of captured burbot each year. In 2009, 103 (57%) were flowing males, 28 (15%) were green females, 16 (9%) were ripe females, and 33 (18%) were unknown. A single female in 2009 had previously spawned prior to capture and was identified as spent. In 2010, 384 (69%) were flowing males, 93 (17%) were green females, 26 (5%) were ripe females, 43 (8%) were of unknown sex and 8 (1%) were spent females. In 2011, 246 (66%) were flowing males, 43 (12%) were green females, 20 (5%) were ripe females, 54 (15%) were of unknown sex and 7 (2%) were spent females. In 2011, a total of six green females were placed into PVC holding tubes, five of which ripened and were used for fertilizations the following day.

Gamete collection and fertilization

In 2009, 7 females and 23 males were used to provide an estimated total of 2 990 615 fertilized eggs. In 2010, a total 24 females were used to provide an estimated total of 7 724 888 fertilized eggs. In 2011 a total of 19 females and 33 males were used to provide an estimated total of 3 278 635 fertilized eggs. Males used for fertilizations were not recorded in 2010. In 2009, egg survival to 48 h post fertilization ranged from 0 to 40% with a mean of 12% ($n = 5$). In 2010, egg survival ranged from 0 to 92%, with a mean of 48% ($n = 18$). In 2011, egg survival ranged from 10 to 98 and with a mean of 73% ($n = 27$). This resulted in an estimated 353 429; 3 032 143; and 3 970 283 live eggs for use in the aquaculture program in 2009, 2010 and 2011, respectively.

In 2009, the mean TL of burbot used for fertilizations was 570 mm (range of 380–740 mm) and the mean weight was 1.26 kg (range of 0.35–2.4 kg). In 2010, the mean TL was 608 mm (range of 425–860 mm) and the mean weight was 1.62 kg (range of 0.65–5.5 kg). In 2011, the mean TL was 564 mm (range of 340–830 mm, SE = 11.3) and the mean weight was 1.37 kg (range of 0.30–3.30 kg, SE = 0.07).

Discussion

Age structure analysis of Moyie Lake burbot indicated that length did not vary with age consistently enough to provide a usable link between length and age. Stratification by sex did little to improve the correlation, and the slight slope of the regression line suggests that larger sample sizes would likely not strengthen this analysis. A larger study of burbot length at age (Cope, 2010) also found that other populations in southern BC also did not show significant links between length and age. Variability in growth rates by individual appear to exhibit enough variability that this method of age structure analysis is not possible for Moyie Lake or other waterbodies in the southern region of BC. The length/weight relationship for burbot used in fertilizations between 2009 and 2011 sampling

years, although not extremely strong (Fig. 4; $R^2 = 0.74$) should provide a reasonable tool for estimating weight from length samples collected in the future.

Locations and habitat type used by spawning burbot were primarily observed in concert with ballast and blasted rock deposited by the adjacent rail line during construction and maintenance. The high use of ballast rock that was not historically available prior to rail construction highlights the potential for spawning site habitat restoration elsewhere. Given the high current use in these areas on Moyie Lake, it is likely that restoration of degraded spawning habitat in other populations that have been affected by habitat degradation may be feasible. In future a more systematic and comprehensive spawning site study of the entire lake, including the south basin, would be useful to provide a more complete record of available habitat, habitat use and spawning sites.

This study has provided a total of 1001 marked individuals with Floy and PIT tags for future population estimates. Future sampling programs, following recommendations such as those in a recent review of this project (Schwarz, 2011), should provide reliable estimates of population size when sample size and time at large is sufficient to allow a strong mark recapture estimate.

Results from this study demonstrated that fertilizations from burbot collected at spawning locations is a viable option to replace or supplement captive adult broodstock for conservation aquaculture programs. Despite some small differences in methodologies in each year, eggs were successfully fertilized in the field and transported to the hatchery. We reliably captured both ripe males and females daily using angling as our primary capture technique. Further, our sampling corresponded with the period in which burbot were spawning naturally in the lake. Reports from anglers combined with captures of spent females indicated that spawning was already underway prior to the commencement of sampling and ripe females were captured the last several days of sampling each year, indicating that spawning continued beyond the period sampled. These results suggest the spawning period can be targeted effectively in future conservation efforts using this population. As well the success of holding tubes for maturation of green females proved to be a significant development in collection methods. Ripe males are easy to procure, but finding ripe females often proves to be challenging, and is the limiting factor to collection of gametes. With the ability to hold green females overnight, we were able to limit unnecessary handling

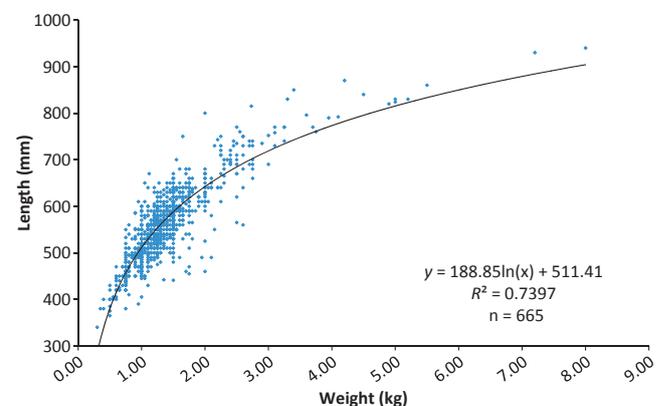


Fig. 4. Relationship between length and weight of burbot captured during angling efforts at spawning sites during conservation aquaculture gamete collection efforts (2009, 2010 and 2011 data)

of spawning individuals and reduce efforts needed to meet egg targets.

Survival of fertilized eggs was generally low upon arrival at the UI-ARI hatchery in the first 2 years of this project (mean of 30% for 2009 and 2010), and lower than the 56% from UI-ARI Moyie Lake captive broodstock hatchery operations during those years (Neufeld and Spence, 2009; Neufeld, 2010). Furthermore, the range in survival between batches of fertilized eggs was relatively large. Several practices likely contributed to these issues. Disinfection of eggs upon receipt at UI-ARI in 2009 (due to lake water use), egg transport stress (i.e. mechanical damage, temperature stress, etc.), time between fertilization arrival at UI-ARI (i.e. more than 36 h) the use of milt collected at varying times prior to being used in egg fertilizations and not evaluated for motility, as well as other transport related stressors. To avoid issues with disinfection caused mortality, eggs were treated following methods that are successfully used currently at UI-ARI including the use of pathogen free water to rinse and water-harden eggs and disinfection of eggs within an hour of fertilization (Polinski et al., 2010). In addition, an effort was made to deliver fertilized eggs to the U of I facility within 36 h of fertilization. Although earlier observation has shown that milt can remain viable for several days (J. Siple pers. comm., KTOI), milt contaminated with just a small volume of water when collected, will not be viable when latter. To increase fertilization success in 2010, milt was expressed directly from three or more ripe males as needed to fertilize each batch of eggs, or collected prior to fertilization and assessed using a microscope for viability near the time of fertilization. Use of a microscope to evaluate milt quality immediately prior to fertilization in 2011 likely eliminated milt quality as a factor in fertilization success. Also, to avoid other transport stressors such a mechanical damage, hard case drink coolers were used in 2011 to transport eggs as opposed to plastic bags used in the past. The coolers were filled close to the top to minimize excessive agitation, which may have also impacted egg survival. Improvements to fertilization and transport methods led to egg fertilization rates (live egg numbers) in 2011 that were on average 73% upon arrival at UI-ARI; a significant improvement from previous years (unpublished data on file). With this success it is believed that collection and fertilization of eggs in the field is feasible, and able to provide enough eggs to provide enough eggs for production level conservation aquaculture activities.

We found that burbot gametes can be collected from adults during spawning and eggs successfully fertilized for a conservation aquaculture programs on an annual basis for hatchery production. To increase the success of these programs, work needs to focus on refining techniques and protocols to augment egg fertilization rates and subsequent survival during transport to the hatchery. Importantly, design of a long term breeding plan that can be implemented at Moyie Lake will be important to representing genetic diversity present in the wild population. This includes accurate records of breeding crosses and standardizing methods used with success elsewhere.

Spawning site surveys have demonstrated that the use of underwater cameras is a suitable method to locate spawning concentrations in lake populations of shoal spawning burbot, and qualitatively evaluate habitat conditions at these sites. The most heavily used spawning locations were observed on the east shore of Moyie Lake and spawning substrate was often comprised of rail deposited rock material. Use of this rail

grade material highlights the potential for spawning site rehabilitation in other burbot populations where suitable habitat conditions are lacking. Further investigations into the reproductive success of burbot spawning over this deposited material will be important.

Acknowledgements

This project was truly a collaborative effort between the many partners involved in burbot recovery, and could not would not have been a success without the field and planning assistance of many individuals including: Don Miller of Kootenay Wildlife Services Ltd., Jack Siple, Guadalupe Ponce, Josh Dunnington, and Justice Cree of the Kootenai Tribe of Idaho (KTOI), Jeff Burrows, John Bell, Kevin Heidt and Herb Tepper (MNRO), Vaughn L. Paragamian, Corie Laude (IDFG) and Greg Hoffman (USACE). Vaughn Paragamian (IDFG) and Sue Ireland (KTOI) provided administrative support, technical direction and provided staff for field sampling. Westslope Fisheries Ltd and BC Hydro provided administrative support and funding for analysis of age data, while Microtech (Juneau AK) completed otolith aging. Kootenay Trout Hatchery (KTH) staff provided well water for egg transport and occasional field assistance. The Bonneville Power Administration (Northwest Power and Conservation Council Fish and Wildlife Program through the Idaho Department of Fish and Game and the Kootenai Tribe of Idaho) provided funding for much of this project.

Conflict of interest

None of the authors have any conflicts of interest to declare.

References

- Ahrens, R.; Korman, J., 2002: What happened to the West Arm burbot stock in Kootenay Lake? Use of an age-structured population model to determine the possible causes for recruitment failure. Report prepared for the British Columbia Ministry of Environment, Nelson, BC, 30 p.
- Arndt, S. K. A.; Hutchinson, J., 2000: Characteristics of a tributary-spawning population of burbot from Columbia Lake. In: *Burbot: biology, management, and culture*. V. L. Paragamian, D. W. Willis (Eds). Am. Fish. Soc. Fish. Manage. Sec. 1, 48–60.
- Arndt, S.; Baxter, J., 2006: Status of burbot (*Lota lota*) in Arrow Lakes Reservoir. Report prepared for Columbia Basin Fish & Wildlife Compensation Program, Nelson, and British Columbia, 35 p.
- Baxter, J.; Spence, C.; Neufeld, M., 2002a: Kootenay Lake burbot studies progress 2000–2001. Report prepared for the Ministry of Water Land and Air Protection, Nelson, BC, 38 p.
- Baxter, J.; Spence, C.; Neufeld, M., 2002b: Kootenay Lake burbot studies progress 2001–2002. Report prepared for the Ministry of Water Land and Air Protection, Nelson.
- Boag, T. D., 1989: Growth and fecundity of burbot, *Lota lota* L., in two Alberta lakes. Master's Thesis, University of Alberta, Edmonton.
- Christensen, J. M., 1964: Burning of otoliths, a technique for age determination of soles and other fish. *J. Conserv.* **29**, 73–81.
- Clemens, H. P., 1951: The growth of the burbot, *Lota lota maculosa*, (LeSueur) in Lake Erie. *Trans. Am. Fish. Soc.* **80**, 163–173.
- Cope, S., 2010: Duncan Reservoir burbot monitoring annual report: 2009–10 (Interim Report 2). Duncan Dam Water Use Plan monitoring report prepared for BC Hydro, Burnaby, BC, Canada.
- Dillen, A.; Vught, I.; De Charleroy, D.; Monnier, D.; Coeck, J., 2008: A preliminary evaluation of reintroductions of burbot in Flanders, Belgium. In: *Burbot: biology, management, and culture*. V. L. Paragamian and D. H. Bennett (Eds). Am. Fish. Soc. Symp. **59**, 179–183.
- Harzevili, A. S.; De Charleroy, D.; Auwerx, J.; Van Slycken, J.; Dhert, P.; Sorgeloos, P., 2003: Larvae rearing of burbot (*Lota lota*) using

- Brachionus calyciflorus* rotifer as starter food. *J. Appl. Ichthyol.* **19**, 84–87.
- Ireland, S. C.; Perry, P. N., 2008: Burbot restoration in the Kootenai River basin: using agency, tribal, and community collaboration to develop and implement a conservation strategy. In: *Burbot: biology, management, and culture*. V. L. Paragamian and D. H. Bennett (Eds). *Am. Fish. Soc. Symp.* **59**, 251–256.
- Jensen, N. R.; Williams, S. R.; Ireland, S. C.; Siple, J. T.; Neufeld, M. D.; Cain, K. D., 2008a: Preliminary captive burbot spawning observations. In: *Burbot: ecology, management, and culture*. V. L. Paragamian and D. H. Bennett (Eds). *Am. Fish. Soc. Symp.* **59**, 155–165.
- Jensen, N. R.; Ireland, S. C.; Siple, J. T.; Williams, S. R.; Cain, K. D., 2008b: Evaluation of egg incubation methods and larval feeding regimes for North American burbot. *N. Am. J. Aquacult.* **70**, 162–170.
- Jensen, N. R.; Zuccarelli, M. D.; Patton, S. J.; Williams, S. R.; Ireland, S. C.; Cain, K. D., 2008c: Cryopreservation and methanol effects on burbot sperm motility and egg fertilization. *N. Am. J. Aquacult.* **70**, 38–42.
- Johnson, T., 1981: Biotope changes and life cycle of *Lota lota* in the Bothnian Sea and a coastal river. *Oesterriechische Fischereiverband* **34**, 6–9.
- Kootenai Valley Resource Initiative (KVRI) Burbot Committee, 2005: Kootenai River/Kootenay Lake burbot conservation strategy. Prepared by Kootenai Tribe of Idaho and S. P. Cramer and Associates, Bonners Ferry Idaho, 77 p.
- McCrimmon, H. R., 1959: Observations on spawning of burbot in Lake Simcoe, Ontario. *J. Wildl. Manage.* **23**, 447–449.
- McPhail, J. D.; Paragamian, V. L., 2000: Burbot biology and life history. In: *Burbot: biology, management, and culture*. V. L. Paragamian and D. W. Willis (Eds). *Am. Fish. Soc. Fish. Manage. Sec.* **1**, 11–23.
- Neufeld, M., 2005: Kootenay sturgeon and burbot recovery progress in British Columbia 2004–2005. Report prepared for the British Columbia Ministry of Environment, Lands and Parks, Nelson, BC, 49 p.
- Neufeld, M. D., 2008: Moyie Lake burbot: population assessment 2007. British Columbia Ministry of Environment, Nelson, BC, 24 p.
- Neufeld, M., 2010: Moyie Lake burbot program 2009–10: conservation aquaculture gamete collection and spawning surveys. British Columbia Ministry of Environment, Nelson, BC, 38 p.
- Neufeld, M.; Spence, C., 2004: Kootenay sturgeon and burbot recovery progress 2003–04. Report prepared for the British Columbia Ministry of Environment, Lands and Parks, Nelson, BC, 99 p.
- Neufeld, M.; Spence, C., 2009: Moyie Lake burbot program: sport fishery, juvenile sampling and conservation aquaculture/spawning surveys. Report prepared for the British Columbia Ministry of Environment, Nelson, BC, 49 p.
- Neufeld, M. D.; Cain, K. D.; Jensen, N. R.; Ireland, S. C.; Paragamian, V. L., 2011a: Movement of Lake Origin Burbot Reared in a Hatchery Environment and Released into a Large River. *N. Am. J. Fish. Manage.* **31**, 56–62.
- Neufeld, M. D.; Davis, C. A.; Paragamian, V. L.; Ireland, S.; Anders, P.; Jensen, N.; Cain, K., 2011b: Kootenai River burbot – 5 year operational research plan. Report completed for the KVRI Burbot Sub-Committee, 32 p.
- Paragamian, V. L.; Wakkinen, V. D., 2008: Seasonal movement and the interaction of temperature and discharge on burbot in the Kootenai River, Idaho, USA and British Columbia, Canada. In: *Burbot: biology, management, and culture*. V. L. Paragamian, D. H. Bennett (Eds). *Am. Fish. Soc. Symp.* **59**, 55–77.
- Paragamian, V. L.; Whitman, V.; Hammond, J.; Andrusak, H., 2000: Collapse of the burbot fisheries in Kootenay Lake, British Columbia, Canada, and the Kootenai River, Idaho, USA, post-Libby dam. In: *Burbot: biology, management, and culture*. V. L. Paragamian and D. W. Willis (Eds). *Am. Fish. Soc. Fish. Manage. Sec.* **1**, 155–164.
- Paragamian, V. L.; Pyper, B. J.; Daigneault, M. J.; Beamesderfer, R. P.; Ireland, S. C., 2008: Population dynamics and extinction risk of burbot in the Kootenai River, Idaho, USA and British Columbia, Canada. In: *Burbot: biology, management, and culture*. V. L. Paragamian, D. H. Bennett (Eds). *Am. Fish. Soc. Symp.* **59**, 213–234.
- Polinski, M. P.; Jensen, N. R.; Cain, K. D.; Johnson, K. A.; Ireland, S. C., 2010: Assessment of formalin and hydrogen peroxide use during egg incubation of North American burbot. *N. Am. J. Aquacult.* **72**, 111–117.
- Powell, M.; Paragamian, V. L.; Dunnigan, J., 2008: Mitochondrial variation in western North American burbot with special reference to the Kootenai River in Idaho and Montana. In: *Burbot: biology, management, and culture*. V. L. Paragamian and D. H. Bennett (Eds). *Am. Fish. Soc. Symp.* **59**, 3–28.
- Prince, A., 2007: East Kootenay burbot population assessment. Report prepared for the British Columbia Ministry of Environment, Nelson, BC, 25 p.
- Schwarz, C. J., 2011: Review of mark-recapture sampling program to estimate abundance of Burbot in Moyie Lake, British Columbia. Report prepared for the British Columbia Ministry of Forests, Lands and Natural Resource Operations, Nelson, BC, 14 p.
- Stapanian, M. A.; Paragamian, V. L.; Madenjian, C. P.; Jackson, J. R.; Lappalainen, J.; Evenson, M. J.; Neufeld, M. D., 2010: World-wide status of burbot and conservation measures. *Fish Fish.* **11**, 34–56.
- Vught, I.; Harzevili, A. S.; Auwerx, J.; De Charleroy, D., 2008: Aspects of reproduction and larviculture of burbot under hatchery conditions. In: *Burbot: biology, management, and culture*. V. L. Paragamian and D. H. Bennett (Eds). *Am. Fish. Soc. Symp.* **59**, 167–178.
- Worthington, T.; Kemp, P.; Osborne, P. E.; Howes, C.; Easton, K., 2010: Former distribution and decline of the burbot (*Lota lota*) in the UK. *Aquat. Conserv.* **20**, 371–377.

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