**Temperature Effects on Early Life Stages of the North American Burbot:**

**Implications for Restoring the Lower Kootenai River Population**

A Dissertation

Presented in Partial Fulfillment of the Requirements for the

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by

Neil K. Ashton

Major Professor: Kenneth Cain, Ph.D.

Committee Members: James Nagler, Ph.D.; Brian Small, Ph.D.; Shawn Young, Ph.D.

Department Administrator: Lisette Waits, Ph.D.

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# Authorization to Submit Dissertation

This dissertation of Neil K. Ashton, submitted for the degree of Doctor of Philosophy with a Major in Natural Resources and titled "Temperature Effects on Early Life Stages of the North American Burbot: Implications for Restoring the Lower Kootenai River Population" has been reviewed in final form. Permission, as indicated by the signatures and dates below, is now granted to submit final copies to the College of Graduate Studies for approval.

Major Professor: _____Kenneth Cain_____ Date: ___5-12-21___

                              Kenneth Cain, Ph.D.

Committee Members: ____James J. Nagler_____ Date: _5-24-21_

                              James Nagler, Ph.D.

                              Brian C. Small_____ Date: _5-24-21_

                              Brian Small, Ph.D.

                              ___Shawn P. Young_____ Date: _5/24/21_

                              Shawn Young, Ph.D.

Department
Administrator: _____ Date: _____

                              Lisette Waits, Ph.D.

# Abstract

An imperiled population of endemic North American Burbot (*Lota lota maculosa*) inhabiting the lower Kootenai River in a transboundary region of Idaho and British Columbia struggles with recruitment failures linked to incompatibility between early life history patterns and upstream hydropower operations at Libby Dam in Montana. The loss of both river ice in winter and a flood pulse in spring has led to an unnatural post-dam thermal regime in spawning and early rearing habitats of Burbot. A series of laboratory experiments and river temperature modeling efforts were carried out to determine if simulated changes to the river thermal regime impact spawning, embryo development, and larval rearing. Contemporary warming in the post-dam regime between December−January does not appear to preclude functional gametogenesis, as river-adapted hatchery fish spawned viable gametes in February. However, elevated water temperatures of 4°C and 6°C shorten the spawning period to potentially coincide with suboptimal conditions for normal embryo development. Cold incubation temperatures are crucial during early embryogenesis, but the post-dam regime is often >3°C—and can be as warm as 5°C—which causes severe deformity and mortality of embryos. Later stages of embryonic development show reasonable tolerance to gradual warming and moderate thermal flux common in late winter and early spring. Larvae were observed inflating their swim bladders, foraging on rotifers, and exhibiting high survival through a critical period in waters <6°C, which is the typical post-dam condition in putative nursery habitats in April. However, such cold regimes also prolong resting behavior of yolk-sac larvae, delay swim bladder inflation, reduce feeding, slow growth, and diminish larvae performance. These important aspects of larval ecophysiology during a critical period of Burbot ontogeny could increase the risk of cumulative mortality and negatively impact recruitment. It was difficult to resolve a modeling solution of post-dam operations that better mimics pre-dam river temperatures in winter because daily changes in regional climate now explain most of the variance in river temperature between December−April. The unpopular option of restricting discharge from Libby Dam in December and January might return ice cover to the downstream meander reach, but a more realistic mitigation tool for Burbot conservation is restoring tributary, side-channel, and off-channel habitats potentially supportive of early life stages. Conservation aquaculture is rebuilding the population and improving opportunities for discovering the recruitment bottleneck(s). The focus of restoration, monitoring, and evaluation work should now be on improving embryo survival in tributaries and larvae survival through the critical period in nursery habitats of the meander reach. An unwavering commitment to hatcheries will also be a key management tool for combating the deleterious impacts of global warming on altered and natural spawning habitats of Burbot.

# Acknowledgements

# Dedication

This dissertation is dedicated to Dr. Paul J. Anders, who was among the vanguard Burbot biologists in the Idaho Panhandle to devote his brilliance to restoring these extraordinary fish and the natural ecology of the lower Kootenai River. Paul's legacy reverberates throughout the culture and community of the Kootenai region, University of Idaho, and greater Moscow area. His supernal departure from us has left a void in the hearts of those graced by his magnanimity, witticism, genius, and musical virtuosity. It is unfathomable that I write this dissertation without Paul's mentorship, but his spirit continues to help me see this work through to the end. I cherish the time I had with Paul in this world, and his opus, zeal for life, and verve to protect our environment is rooted in the memories of his family, friends, and colleagues.

A foremost reason for me undertaking a Ph.D. has been my daughter, Evelyn, who lightens my perceptions, fills my life with joy through nourishing respites from work, and who I hope will know that I could not have persevered through graduate school without her love and magical childhood these last seven years. My wife has supported me through countless adventures and endured my fascination with fisheries and aquaculture since college. More than anything, I hope the fruits of labor committed to this dissertation benefit these two companions in my life. I am immeasurably thankful to my family and friends for the beautiful experiences of living in the Pacific Northwest. I greatly appreciate the opportunities given to me by all past, present, and future fisheries professionals to pursue work that fulfills my curiosity and inspires me to have a beneficial influence on our natural resources.

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# Statement of Contribution

The following people and institutions contributed to publications as part of this dissertation:

**Candidate:** Neil K. Ashton, University of Idaho
**Author 1:** Tyler J. Ross, Idaho Department of Fish and Game
**Author 2:** Ryan S. Hardy, Idaho Department of Fish and Game
**Author 3:** Nathan R. Jensen, Kootenai Tribe of Idaho
**Author 4:** Shawn P. Young, Kootenai Tribe of Idaho
**Author 5:** Sarah M. Stephenson, British Columbia Ministry
**Author 6:** Valerie Evans, British Columbia Ministry
**Author 7:** Kenneth D. Cain, University of Idaho

Contribution of work by co-authors for each paper:

**Paper 1:** Located in Chapter 2

Ashton, N. K., N. R. Jensen, T. J. Ross, S. P. Young, R. S. Hardy, and K. D. Cain. 2019. Temperature and maternal age effects on Burbot reproduction. North American Journal of Fisheries Management 39:1192–1206.

- Conceived and designed experiment: Candidate, Author 3, Author 4
- Performed the experiment: Candidate
- Analyzed the data: Candidate
- Wrote the manuscript: Candidate, Author 1, Author 2, Author 3, Author 4, Author 7

**Paper 2:** Located in Chapter 3

Ashton, N. K., T. J. Ross, R. S. Hardy, S. M. Stephenson, V. Evans, N. R. Jensen, S. P. Young, and K. D. Cain. In press. Effects of temperature fluctuation on Burbot embryos: implications of hydropower and climate change. Transactions of the American Fisheries Society.

- Conceived and designed experiment: Candidate, Author 1, Author 2
- Performed the experiment: Candidate
- Analyzed the data: Candidate
- Wrote the manuscript: Candidate, Author 1, Author 2, Author 3, Author 4, Author 5, Author 6, Author 7

**Paper 3:** Located in Chapter 4

Ashton, N. K., N. R. Jensen, T. J. Ross, S. P. Young, R. S. Hardy, and K. D. Cain. In review. Development of Burbot larvae in cold regimes linked to hydropower discharge.

- Conceived and designed experiment: Candidate, Author 3, Author 4

- Performed the experiment: Candidate

- Analyzed the data: Candidate

- Wrote the manuscript: Candidate, Author 1, Author 2, Author 3, Author 4, Author 7

We, the undersigned, endorse the above stated contribution of work undertaken for each of the published (or submitted) peer-reviewed manuscripts contributing to this dissertation:

Major Professor: **Kenneth Cain**     Digitally signed by Kenneth Cain DN: cn=Kenneth Cain, o=UI, ou=FWS, email=kcain@uidaho.edu, c=US Date: 2021.05.12 06:22:24 -07'00'     Date: **5-12-21**

Kenneth Cain, Ph.D.

Committee Members: *James J. Nagler*     Date: **5-24-21**

James Nagler, Ph.D.

**Brian C. Small** Digitally signed by Brian C. Small Date: 2021.05.24 10:27:58 -06'00'     Date: **5-24-21**

Brian Small, Ph.D.

*Shawn P. Young*     Date: 5/24/21

Shawn Young, Ph.D.

Department
Administrator: _____ Date: _____

Lisette Waits, Ph.D.

# General Introduction

Burbot (*Lota lota*, Gadidae) is a unique freshwater cod found in cold lakes and rivers of North America and Eurasia. In these lacustrine and lotic environments, Burbot may be important predators and indicators of ecosystem balance. Populations across major portions of their current range are experiencing declines, imperilment, and extirpation. Numerous anthropogenic factors have been ascribed to these population declines including habitat degradation, pollution, invasive species, climate change, and overexploitation. The impact of dam installations and operations has especially affected populations in rivers and streams worldwide (McPhail and Paragamian 2000; Stapanian et al. 2010).

The lower Kootenai River (Kootenay in Canada) once supported a thriving native population of Burbot with major ecological and cultural importance to northern Idaho (USA) and southeastern British Columbia (Canada). The stock collapsed because of recruitment failures following river alterations, habitat loss, overfishing, and construction of Libby Dam (MT, USA) in 1972 (Hardy and Paragamian 2013). In 2005, regional stakeholders formally proposed an international conservation strategy to mitigate dam operations and restore a naturally producing, harvestable population (KVRI 2005; Ireland and Perry 2008). An aquaculture program was established as one of several approaches to rebuild the functionally extirpated population. Program collaborators include the Kootenai Tribe of Idaho (KTOI), Idaho Department of Fish and Game (IDFG), B.C. Ministry of Forests, Lands and Natural Resource Operations (BCMFLNRO), U.S. Fish and Wildlife Service (USFWS), and the University of Idaho Aquaculture Research Institute (UI–ARI). The program has reached an evaluative phase to determine the self-sustaining potential of hatchery fish sourced from an isolated donor population of broodstock in Moyie Lake (BC, Canada). Thousands of embryos, larvae, and tagged hatchery fingerlings are being stocked throughout the lower Kootenai River watershed (Ross et al. 2020).

The primary cause(s) for natural recruitment failure currently focus on changes to the river ecosystem (habitat, flow, temperature, nutrients, food web regimes) following construction and operation of Libby Dam (Hardy and Paragamian 2013). Recent monitoring shows that stocked juveniles (>3 months old) can survive to maturity and migrate to historic spawning grounds. Resource managers believe that a recruitment bottleneck(s) exists for early life stages occurring sometime between adult spawning and larvae metamorphosis (Ross et al. 2018). Temperature is a primary abiotic factor known to influence fish development, physiology, behavior, and food web productivity. Burbot are obligate mid-winter spawners across most their range and reproductive success may be particularly sensitive to temperature (Cott et al. 2013). Concomitantly, ongoing research is focused on

implications of Libby Dam flow and thermal regime effects on Burbot early life history. Winter and early-spring flows are now generally higher and fluctuate more frequently; likewise, river temperatures in winter oscillate with greater amplitude and frequency and are generally warmer. The river historically froze over in February, but it is mostly ice-free since construction of the dam (Paragamian et al. 2000).

Hydropower drafting of the cold, deep Koocanusa Reservoir upstream of Libby Dam has caused the downstream thermal regime to be cooler in April and May compared to pre-dam levels. Dam discharge between May−July is much less than historic natural flows characterized by a dominant unimodal flood pulse derived from snowmelt (Burke et al. 2009). The U.S. Army Corps of Engineers (USACE) conducted a statistical analyses of air temperature, tributary temperature and flow, and Kootenai River flow as potential factors influencing main-stem temperatures (location: Bonners Ferry [ID, USA]; months: April−June; years: 2000−2006; Easthouse 2009). Those study results infer that discharge and temperature have an inverse relationship in spring, but analyses of factors influencing winter river temperatures are lacking. Modeling is needed to potentially identify dam discharge profiles from December−March that provide adequate temperatures for adult migration, spawning, and embryo development.

Telemetry studies of adults suggest that migration and spawning of Burbot in the river is inhibited by higher winter discharges and warmer water temperatures (Paragamian and Wakkinen 2008). The contemporary post-dam thermal regime may spike to 5°C in February (peak adult spawning period) and 7°C in March (embryo development period). Conversely, downstream temperatures may drop to 3−5°C in April and May (i.e., larval development period). Previous research suggests these temperature regimes may adversely affect normal ontogeny of those respective life stages (Żarski et al. 2010; Lahnsteiner et al. 2012; Donner and Eckman 2011). Additionally, hatchery fish sourced from the donor lake population may exhibit migratory and physiological adaptations that are incompatible with contemporary conditions in the lower Kootenai River.

The objectives of this investigation were to identify potential bottlenecks to Burbot recruitment in the lower Kootenai River linked to post-dam thermal regimes affecting adult spawning, embryo incubation, and larval development. It is critical to establish temperature thresholds for normal ontogeny of these life stages relative to contemporary river conditions. Results from early life history investigations should also inform conclusions from modeling different factors impacting the post-dam thermal regime during a relatively unstudied winter period. The following research topics are the focus of my dissertation chapters and address my primary objectives:

1. Review the population collapse of wild Burbot and identify critical needs for research.

2. Evaluate spawning time and reproduction of river-adapted hatchery fish.

3. Test the effects of different winter post-dam temperature profiles on embryo incubation.

4. Test the effects of different spring post-dam temperature profiles on larval development.

5. Model different factors that explain variance in the winter/spring post-dam thermal regime.

The overarching deliverables from this dissertation provides resource managers with scientific information that helps determine if post-dam thermal regimes can support early life stages of Burbot, thereby enabling natural recruitment from a newly formed population mostly derived from river-adapted hatchery fish.

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# Chapter 1: The Near-Extirpation of Wild Burbot in the Lower Kootenai River: A Review of River Regulation and Life History Patterns

## Abstract

Regulation of large rivers has profound societal benefits including community preservation, regional prosperity, and cultural modernity. Large-scale flood pulses may be costly to industrialized landscapes, but they are essential to flourishment of biota that have co-evolved under natural paradigms of basin connectivity. The Kootenai River watershed has been a popular case study of river regulation impacts on indigenous flora and fauna. A native freshwater cod (Burbot, *Lota lota maculosa*, Gadidae) once persisted in this subbasin until the late 20th century despite widespread anthropogenic impacts dating back to the mid-1800s. In the 1970s, hydropower operations dramatically altered the natural regimes of the river with respect to temperature, hydrology, sedimentation, nutrient levels, and trophic ecology. These changes pushed the wild Burbot population to the brink of extirpation. This review investigates the clash between river regulation and Burbot life history patterns that ultimately prevented a natural population recovery. Critical needs for research are identified to help guide mitigation efforts, with particular emphasis on evaluations of post-dam thermal regime effects on early life stages of Burbot.

## 1.1. Introduction

Rivers have immense value to society for irrigation, transportation, power generation, fisheries, and recreation. Human exploitation of these benefits has severely altered river systems worldwide, with nearly 60% of major river basins fragmented by dams and river regulation (Revenga et al. 2000; Naiman et al. 2005). Floodplain wetlands and rivers are among the most threatened ecosystems worldwide (Junk and Wantzen 2004; Su et al. 2021). Concomitantly, an estimated 49% of freshwater fishes are imperiled in the U.S. (Master et al. 1998), with a similar estimate reported across other nations (MEA 2005). Regulation not only alters the thermal, hydrologic and sediment regimes of a river, but it also affects flora and fauna via changes in landscapes and basin connectivity. The magnitude of these anthropogenic impacts is unique to each system (Ligon et al. 1995; Poff and Hart 2002; Grant et al. 2003).

The Kootenai River located in western North America is a popular case study of regulation impacts on a riverine ecosystem. Its watershed has been subjected to numerous anthropogenic alterations dating from the mid-1800s to present day. Widespread industrialization of the drainage via mining, logging, diking, tributary diversion, main-stem channelization, floodplain farming, and

impoundment has profoundly impacted ecosystem processes. Important fisheries have been decimated since the 1970s, and two indigenous species are currently listed as "endangered" and "threatened" under the Endangered Species Act (ESA): White Sturgeon (*Acipenser transmontanus*) and Bull Trout (*Salvelinus confluentus*), respectively. Additionally, there is native population of North American Burbot (*Lota lota maculosa*) with cultural and ecological significance to the region. This population is considered imperiled by U.S. agencies and is Red Listed as extirpated, endangered, or threatened in Canada (Kootenai Tribe of Idaho and Montana Fish, Wildlife & Parks 2004). The lower Kootenai River and Kootenay Lake once supported a thriving endemic population of Burbot with major ecological and cultural importance to northern Idaho and southeastern British Columbia. A decade after construction of Libby Dam (Montana, USA), there was evidence of declining abundance in both the lake and river (Partridge et al. 1983; Andrusak 1987), and by the mid-1990s the population was approaching functional extirpation (Paragamian et al. 2000; Paragamian et al. 2008a; Hardy et al. 2013).

The objectives of this review are to 1) provide historical perspectives on the interactions between river regulation and Burbot life history, 2) incorporate additional details that provide further insights about the population decline, and 3) recommend ideas for future research that could influence restoration efforts.

## 1.2. Study Area

The Kootenai River is the third largest tributary to the Columbia River and drains a 41,910 km$^2$ subbasin comprising parts of British Columbia, Montana, and Idaho (70%, 23%, and 7% of subbasin area, respectively; Burke et al. 2009). The focus of this dissertation is on the subbasin portion downstream of barriers at Kootenai Falls and Libby Dam located at 310 and 352 river kilometers, respectively, from the confluence with the Columbia River near Castlegar, British Columbia. This system is known as the lower Kootenai River (spelled Kootenay in Canada) which flows west and north across the northern Idaho Panhandle and southeastern British Columbia into Kootenay Lake (Figure 1.0.1).

Snyder and Minshall (1996) describe three distinct sub-reaches of the lower Kootenai River between Libby Dam and Kootenay Lake: a long canyon reach (100 km) upstream of the Idaho−Montana border, a short braided reach (11 km) just upstream of Bonners Ferry, Idaho, and a long meander reach (122 km) downstream of Bonners Ferry that crosses into Canada. Kootenay Lake is a natural water body that has controlled water levels in the lower Kootenai River since glaciation. The lake covers 390 km$^2$ and stretches 107 kilometers from the top of the North Arm to the bottom of the South Arm, and has a 45-km-long West Arm. On average, the lake is 3.7 km wide, 94 m deep,

and 56% of the inflow to the lake is regulated by dams (Kootenai Tribe of Idaho and Montana Fish, Wildlife & Parks 2004).
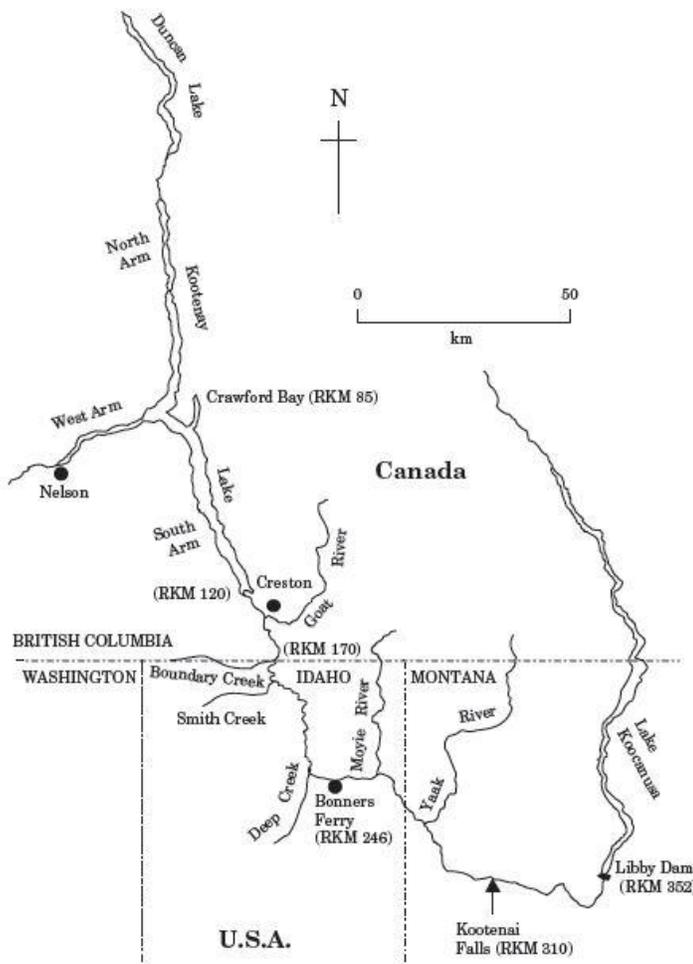


**Figure 1.0.1.** Map of the Kootenai River, Kootenay Lake, Koocanusa Reservoir, and major tributaries. The river distances from the northernmost reach of Kootenay Lake are in kilometers (RKM) and are indicated at important access points (illustration reprinted from Paragamian et al. 2005).

Channelization and enhancement of levees from the early 1900s to the 1970s dramatically reduced overbank flow and lateral connectivity in the lower Kootenai River watershed (Northcote 1973). These alterations enabled development of agricultural lands in the historic floodplain of the meander reach (Figure 1.0.2). Many tributaries along the meander were also highly impacted by mining, logging, and man-made diversions.

**Figure 1.0.2.** The meander reach of the lower Kootenai River with expansive agricultural development in the historic floodplain (image adapted from the Idaho Department of Transportation and Kootenai National Wildlife Refuge).

## 1.3. River Regulation

In the 1930s, Corra Linn Dam was constructed at the outlet of Kootenay Lake (near Nelson in the West Arm; Figure 1.0.1) for regional hydropower and flood control. Dam operations and enlargement of the lake outlet has allowed management of lake levels since 1939, with higher stages maintained during low-flow periods (September–February) to enhance energy production, and lower stages maintained during the high-flow spring and summer months to create flood storage. These regulation trends are opposite to natural conditions which follow a unimodal spring–summer flood pulse driven by snowmelt (Burke et al. 2009). Persistence of major floods (e.g., 1948, 1956, 1961) in the Columbia River Basin (CRB) led to commissioning of three dams in British Columbia (Duncan, Mica, Keenleyside) and one in Montana (Libby Dam) via the 1961 Columbia River Treaty between Canada and the USA. Two of these dams (Duncan and Libby) are in the lower Kootenai River subbasin. Duncan Dam is an earth-filled embankment without hydropower and constructed in 1967 for flood control. Completion of the dam severed upstream connectivity with Duncan Lake, Duncan River, and its tributaries. The U.S. Army Corps of Engineers (USACE) completed construction of Libby Dam in 1972 and created Koocanusa Reservoir (known as Lake Koocanusa) with a surface area of 188 km$^2$ at full pool. Its operations are mostly dictated by a combination of hydroelectric power generation, flood control, recreation, and special operations for the recovery of species listed under the ESA, including White Sturgeon, Bull Trout, and other salmonids in the CRB (Hoffman et al. 2002).

Libby Dam has undergone various phases of operation since its construction. Figure 1.0.3A shows the average daily storage of water in Koocanusa Reservoir for three distinct periods of river

regulation: 1) 1975−1994 includes the time from completion of reservoir filling until the USFWS Biological Opinion (BIOP) mandating increased springtime flows for ESA-listed White Sturgeon, 2) 1995−2002 represents the first phase of post-BIOP operations, and 3) 2003−2016 is the timeframe of the second phase of post-BIOP operations, otherwise known as Variable-Flow Flood Control (VARQ; USFWS 2006). The USACE began to increase water storage in autumn/winter of 1995 to provide greater flows in spring/summer, with the intention of improving sturgeon migrations to historical spawning grounds upstream of Bonners Ferry. Several years of these operations proved ineffective for stimulating migration, so in 2003 the USACE implemented VARQ to reduce the frequency of Koocanusa Reservoir refill failure and secure even more water for spring/summer flows (USFWS Biological Opinion 2006). As a result of VARQ, contemporary water storage in Koocanusa Reservoir has nearly doubled in the months of February and March when compared to historical dam operations.
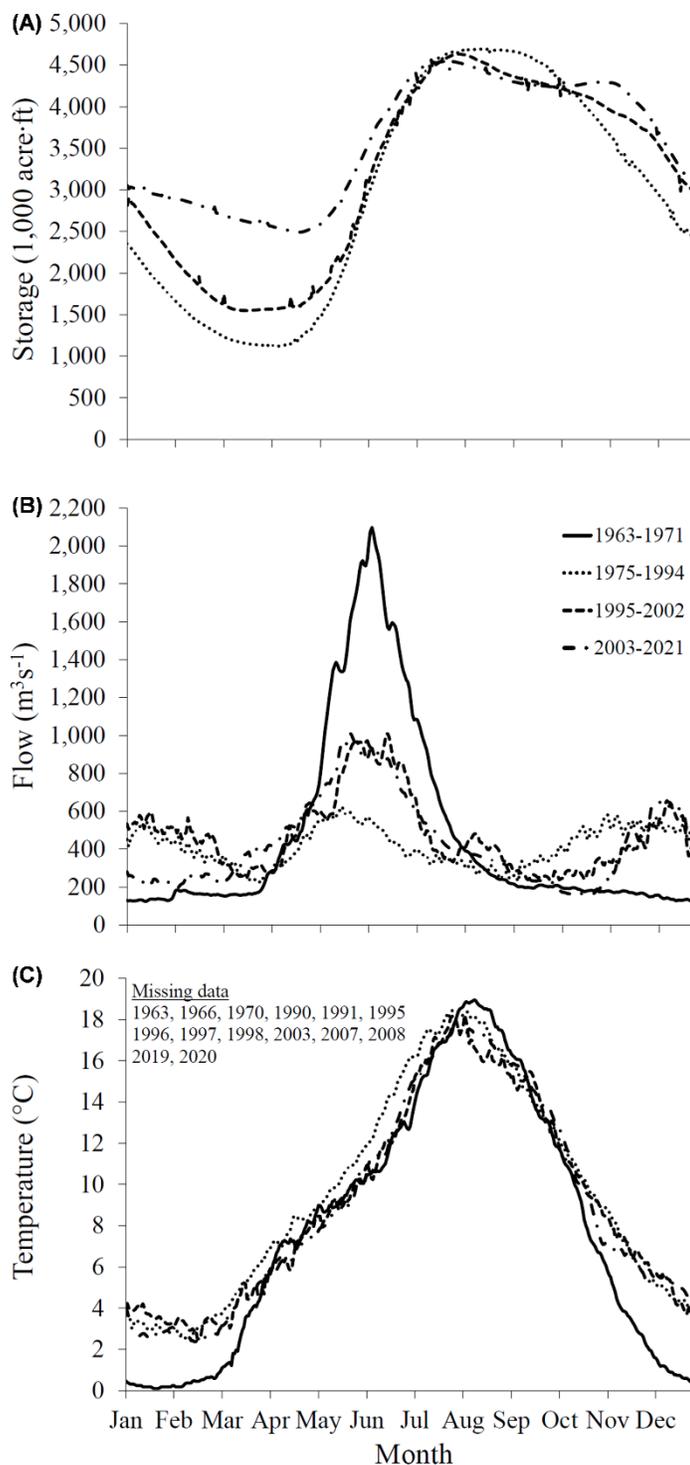
**Figure 1.0.3.** Panel A is mean daily water storage in Koocanusa Reservoir for three distinct operational phases from 1975−2021 (data source: U.S. Army Corp of Engineers). Panels B and C are mean daily flows and temperatures of the lower Kootenai River recorded at U.S. Geological Survey stations 12322001 and 12322000 (Porthill, Idaho). Pre- and post-Libby Dam operations coincide with 1963−1971 and 1975−2021. Data from 1972−1974 is excluded because reservoir filling was not completed until July 1974 (Hoffman et al. 2002). Missing data for some years is due to recording malfunctions at the station.

The post-BIOP and VARQ operations at Libby Dam have had a substantial impact on mean daily flows in the meander reach (Figure 1.0.3B). Spring/summer discharges nearly doubled since the sturgeon BIOP was implemented in 1995. Efforts to improve Koocanusa Reservoir refill via VARQ subsequently reduced February flows by almost half. Contemporary discharge in spring/summer still does not approach the scale of the pre-dam flood pulse (i.e., 1963−1971), but the post-dam regime in February is more like pre-dam levels that were characterized by low winter flows. Present-day VARQ discharges in early spring have increased relative to prior phases of dam operation and late-autumn flows continue to be three-fold or greater than pre-dam levels.

A full assessment of VARQ impacts on the main-stem thermal regime have been hampered by an incomplete record of river temperatures dating back to pre-dam levels; additionally, year-to-year changes in regional climate and spatiotemporal differences between recording stations have been confounding factors (Easthouse 2009). Figure 1.0.3C shows the mean daily temperature in the meander reach at difference phases of dam operations. Unlike the hydrograph, the river thermograph has been relatively consistent across different operational phases at Libby Dam. One definitive trend is that average autumn/winter river temperatures in the meander increased by 3°C after completion of Libby Dam.

Thermal capacitance of Koocanausa Reservoir stores heat over summer, and subsequent discharges for hydropower or flood control in the fall/winter produce artificially high flows and temperatures downstream between November−March (Figures 1.0.3B, 1.0.3C). Abrupt changes in hydropower discharge is commonly known as "hydropeaking," and can lead to rapid spikes in downstream temperatures or "thermopeaking" in winter (Zolezzi et al. 2011). The early years of Libby Dam operations were characterized by many instances of hydropeaking. However, past instances of oscillating discharge at the dam has not always produced thermopeaking events in the meander reach. For example, in 1980, no obvious relationship exists between the hydrograph and thermograph between January−March at the U.S. Geological Survey station 12322000 in Porthill, Idaho (Figure 1.0.4). Despite the fact that Libby Dam has mostly eliminated downstream ice cover, there are caveats to correlating dam discharge with fluctuations in river temperature in the meander reach, and more modeling research is needed to identify factors influencing the winter post-dam thermal regime that effects Burbot migration, spawning, and embryo incubation in the main channel.
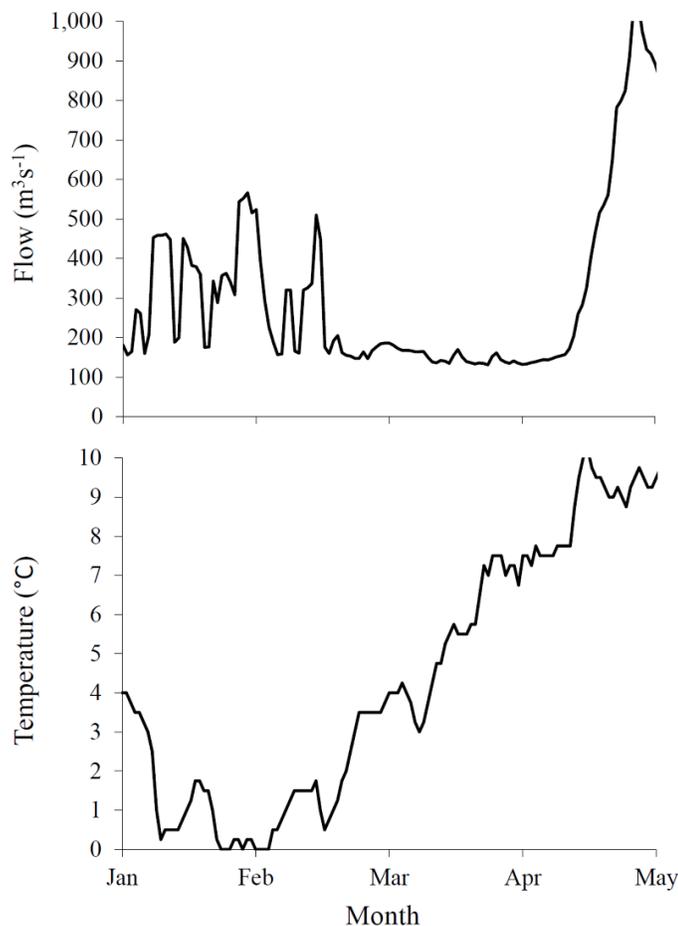
**Figure 1.0.4.** Mean flows and temperatures in the lower Kootenai River recorded at U.S. Geological Survey station 12322001 and 12322000 (Porthill, Idaho) during the winter/spring of 1980.

## 1.4. Burbot Fishery

Burbot once thrived in the lower Kootenai River, providing an important seasonal staple for Native Americans and early European settlers, as well as more recent residents of the Kootenai Valley in the 1920s–1960s (Kootenai Tribe of Idaho and Montana Fish, Wildlife & Parks 2004; KVRI 2005). In the early- to mid-1900s, tributaries along the meander reach were replete with spawning Burbot that were speared beneath the ice for winter subsistence and sale in markets at Bonners Ferry (Hardy and Paragamian 2013). Partridge et al. (1983) reported anecdotally that Burbot once entered shallow sloughs throughout the historic floodplain to feed on forage fish in the evenings; it was also presumed that significant winter spawning occurred in quiet backwaters and side channels of the river. After the 1960s, diking and channelization of tributaries—along with fishing regulations—eliminated the spear fishery in Idaho, but wintertime exploitation persisted in the lower Kootenai River via set lines and angling through the ice. Paragamian and Whitman (1997) reported that Burbot in the Idaho portion of the Kootenai River once supported annual harvests as high as

50,000 lbs. in the 1960s and 1970s. This traditional main-stem ice fishery was essentially eliminated in 1975 after Libby Dam operations altered the natural river regime. Historically, the river had extensive ice cover in the meander reach during most winters (Paragamian et al. 2000; Partridge et al. 1983); however, it has remained mostly ice-free up to present day, except for periodic extreme winters.

One of the largest Burbot harvests in North America also occurred in Kootenay Lake (mostly in the West Arm near Balfour, British Columbia) via a boat fishery between February−July. Martin (1976) found that Burbot first recruited to this fishery at age-4 and were fully recruited at age-7. Andrusak and Crowley (1978) suggested that the concentration of Burbot in the West Arm was either due to available spawning habitat and/or the presence of feeding grounds. The inlet to the West Arm near Balfour is characterized by a shallow shelf or sill which forms a food trap for introduced Mysid shrimp (*Mysis relicta*; a major prey item for adult Burbot; Martin and Northcote 1991). This sill entrains and mixes water from the epilimnion of Kootenay Lake, so the West Arm is often isothermal across all depths and generally warmer than the average lake temperature (Schindler et al. 2009). The fishery harvest appeared to be synched with high flows in May−June (Martin 1976), which may have entrained greater numbers of Mysids into the West Arm (Redfish Consulting 1998). Both the Idaho and British Columbia fisheries rapidly declined after construction of Libby Dam in 1972, with river and lake harvests being closed in 1992 and 1997, respectively (Paragamian et al. 2000; Hardy and Paragamian 2013). Figure 1.0.7 shows the annual number of Burbot harvested, catch rates, and timing of the fishery collapse from 1968–1983 in the West Arm.
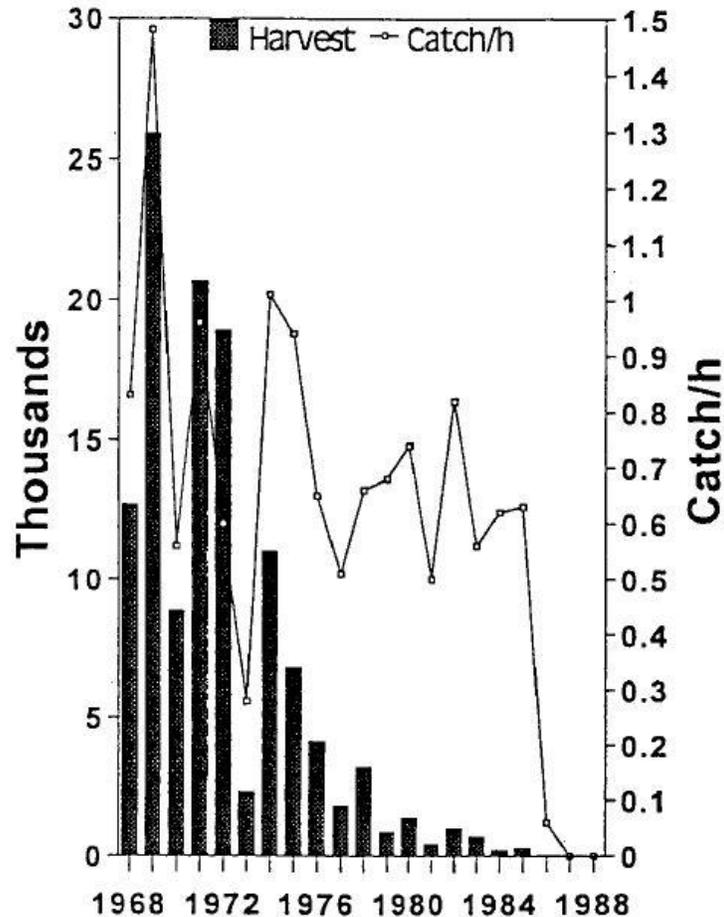
**Figure 1.0.5.** Harvests (thousands of fish) and catch rates (catch/hr) for Burbot from the West Arm of Kootenay Lake, British Columbia (graph reprinted from Paragamian et al. 2000).

Over the last two decades, a Burbot conservation aquaculture program has been developed by the Kootenai Tribe of Idaho (KTOI) to mitigate for population declines of wild fish caused by Libby Dam and other anthropogenic changes to the river system (KVRI 2005; Hardy et al. 2015). Stocking of hatchery fish has increased the abundance of Burbot in the meander reach of the lower Kootenai River (Figure 1.0.6) and expanded joint monitoring and evaluation goals involving KTOI, IDFG, and BCMFLNRORD. Additionally, the success of the hatchery program has recently enabled reopening the Burbot fishery to recreational angling in Idaho (Ross et al. 2020).
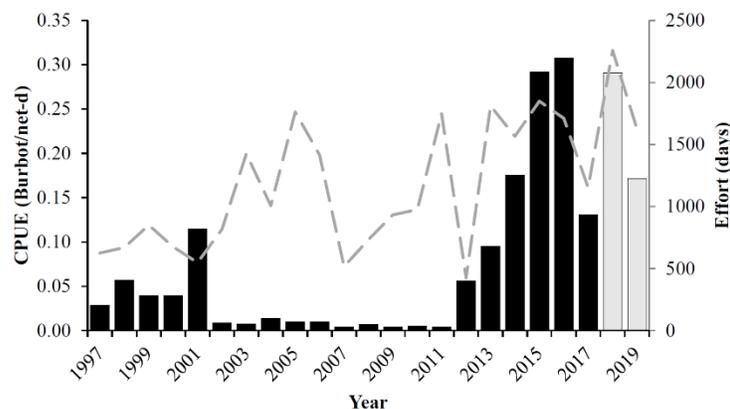
**Figure 1.0.6.** Catch-per-unit-of-effort (Burbot/net-day) of hoopnet sampling for index sites in the lower Kootenai River between 1992−2019. The gray dashed line shows hoopnetting effort (days). The gray bars represent catches affected by juvenile stocking from Twin Rivers Hatchery starting in 2015. Data from sites upstream from Bonners Ferry are not included. Annual sampling started December 1 and ended March 31 (graph reprinted from Ross et al. 2020).

## 1.5. Collapse of the Burbot Population in Kootenay Lake/Kootenai River

Resource managers were aware of the population decline in the West Arm and enacted fishing regulations that purportedly reduced harvests to sustainable levels (Martin 1976), however, these actions proved ineffective. Other investigators have implicated overfishing as a major factor causing Burbot population declines in different systems (Lafferty and Bernard 1993; Hubert et al. 2008). There are also Burbot stocks that have been resilient to fishing pressure (Klein et al. 2016a; Lewandoski et al. 2017), but these cases of exploitation are arguably not equivalent to the Kootenai/Kootenay fishery with respect to scale.

Interestingly, Paragamian et al. (2000) did not implicate overfishing as the primary cause for the Burbot fishery collapse. Sampling efforts throughout the 1990s found an increasing proportion of larger fish in the catch. The disappearance of smaller, younger fish suggested a recruitment bottleneck (Paragamian et al. 2000). Ahrens and Korman (2002) modeled Burbot population dynamics in relation to environmental indices within the West Arm. They reported that the fishery collapse was accelerated by unsustainable harvest, but ultimately caused by recruitment failure. Similarly, investigations of overfished Burbot populations in Alaska have concluded that high levels of exploitation are not sustainable when unfavorable environmental conditions are impacting survival or recruitment (Taube and Bernard 2001). Stapanian et al. (2010) concluded that Burbot populations are fully capable of recovering from overexploitation once fishing pressure is relaxed.

Any synopsis of exploitation must be weighed in the context of decimating large spawning aggregations—which was the case in the tributaries and mainstem of the lower Kootenai River (Redfish Consulting 1998; Kootenai Tribe of Idaho and Montana Fish, Wildlife & Parks 2004)—and

may have occurred in the West Arm of Kootenay Lake (Martin 1976). In general, adult Burbot exhibit life history traits that should be resilient to exploitation. For example, gonad maturation can first occur as early as age-2 (or as late as age-7, depending on the population and its environment) with males typically maturing earlier than females (Bonar et al. 2000). Adults can exhibit high survival rates (Klein et al. 2016) and may be long-lived iteroparous spawners with enormous fecundity and inordinate spermiation (Bjorn 1939; Bailey 1972; McPhail and Paragamian 2000). The case of the lower Kootenai River and Kootenay Lake Burbot population crash is perplexing because closure of both fisheries for two decades failed to result in any detectable recovery (Hardy et al. 2013).

Ahrens and Korman (2002) identified juvenile recruitment anomalies occurring in the late-1960s, and linked recruitment failure to a crash in cladoceran densities after 1964: presumably due to increases in densities of nonnative Mysid shrimp. However, more recent studies suggest that cladocerans may not be the most prevalent or important prey item for larvae and juvenile Burbot (Hardy et al. 2008; Klein 2016). Reductions in river nutrient loadings that started in the mid-1970s were linked to effluent remediation at a fertilizer plant in the upper Kootenai River, and nutrient trapping in reservoirs impounded by Libby Dam and Duncan Dam (Daley et al. 1981; Woods 1982; Snyder and Minshall 1996). Paragamian et al. (2000) found a significant positive correlation between ortho-phosphate levels in the lake and Burbot harvests occurring four years later (i.e., Burbot recruit to the fishery at age-4 and older). Diminished nutrient loadings and river productivity were implicated as potential factors limiting recruitment of juvenile Burbot to adulthood (Hardy et al. 2013). Ultra-oligotrophication of the lower Kootenai River watershed has also been identified as a key factor causing the decline of other fisheries including Kokanee (*Oncorhynchus nerka*), Rainbow Trout (*Oncorhynchus mykiss*), White Sturgeon, and Bull Trout (Ashley et al. 1997; Paragamian et al. 2002). Consequently, a nutrient addition program has been in effect for more than two decades (Kruse et al. 2019; Schindler et al. 2020).

Partridge (1983) and Paragamian (1995) initially believed that Burbot in the river and lake were from the same stock. Both investigators implicated winter discharges from Libby Dam as a major cause of the population crash. Increased winter flows downstream of the dam (see Figure 1.0.4) may have exceeded the sustainable swimming performance of Burbot (Jones et al. 1974) and potentially thwarted adult migrations to key spawning grounds in the mainstem and its tributaries. There is evidence from telemetry studies to support this conclusion (Paragamian et al. 2005), however interpretations of migratory behavior came from a small number of individuals that were a remnant of the historical population. Other investigators have suggested that spatiotemporal differences in reproductive patterns between the West Arm, North Arm, and South Arm potentially relate to three

distinct Burbot stocks (Martin 1976; Redfish Consulting 1998; Paragamian et al. 2000; Kootenai Tribe of Idaho and Montana Fish, Wildlife & Parks 2004).

It is plausible that different factors caused the collapse of three different stocks. For example, the extirpation of Burbot in the West Arm could be ascribed to a reduction in lake productivity following impoundment, pollution abatement, and degradation of the lower Kootenai River floodplain. The dramatic decline in Burbot abundance in the South Arm, lower Kootenai River, and its tributaries (presumed to be one stock; Paragamian et al. 1999) could be due to a multitude of factors including decades of fishing pressure on spawning aggregations, habitat degradation, and hydropower alterations to the natural regimes of the river (thermal, hydrologic, sediment and nutrient). Low population estimates in the North Arm could be a result of Duncan Dam construction in 1967, which severed upstream connectivity with Duncan Lake, Duncan River, and its tributaries. No historical data is available to confirm if this earth-filled dam isolated important adfluvial spawning grounds for Burbot inhabiting the North Arm (BC Hydro 2015).

The demise of Burbot in both the lake and river after the mid-1970s implicates a convenient assumption that a single stock was functionally extirpated by impoundment and hydropower operations. However, this may be an overly simplistic assessment given the spatiotemporal overlap of numerous causalities. A recent review by biologists in British Columbia surmised that collapse of the Kootenay Lake/Kootenai River Burbot population likely resulted from a combination of interlinked factors including unsustainable harvest rates, impoundment of the Kootenai and Duncan rivers, spawning stream habitat degradation, and reductions in lake and river productivity for early life stages (BC Hydro 2015). Changes in the thermal regime of the river are integral to the latter three factors and will be the focus of this investigation, particularly with respect to identifying thermal tolerances and preferences throughout the early life history of Burbot.

## 1.6. Life History Patterns of Burbot in Kootenay Lake/Kootenai River

### 1.6.1. Adult Spawning

Three life history patterns (lacustrine, adfluvial, riverine) are recognized for Burbot in the lower Kootenai River subbasin (Paragamian and Willis 2000; Kootenai Tribe of Idaho and Montana Fish, Wildlife & Parks 2004; Paragamian et al. 2008). There may also be fidelities to specific spawning strategies (e.g., deep vs. shallow lacustrine; tributary vs. main-stem adfluvial/riverine) that are not yet identified. Research has mostly been conducted after the mid-1970s and focused on a remnant population of adfluvial adults that presumably feed in Kootenay Lake, then spawn in the lower Kootenai River and its tributaries (Partridge 1983; Paragamian et al. 2000; Paragamian et al. 2005). The scarcity of lacustrine Burbot in Kootenay Lake is a paradox that continues to perplex

fisheries biologists. Sampling mortality due to barotrauma has hampered past evaluations of lacustrine Burbot in Kootenay Lake, particularly in deep waters (Baxter et al. 2002a; Neufeld et al. 2005). Current sampling methods with baited traps use protracted decompression stops to avoid barotrauma, but less cumbersome options like bottom camera bait stations are being discussed for future monitoring (S. M. Stephenson, British Columbia Ministry of Forests, Lands, Natural Resource Operations, and Rural Development [BCMFLNRORD], personal communication).

The vast shorelines of Kootenay Lake are characterized by substrates and winter temperatures that appear suitable for spawning and egg development. Additionally, drawdown of the lake by Corra Linn Dam/Kootenay Canal during winter is often less than three meters and should not impact many potential spawning sites (S. M. Stephenson, BCMFLNRORD, personal communication). The North and South Arms are typically isothermal from late fall to early spring, while the West Arm can be isothermal at epilimnion temperatures throughout the year due to its riverine morphology. Water temperatures and dissolved oxygen levels respectively range from 3−4°C and 15−20 mg/l between January−March at stations throughout the lake at depths ranging from 0−50 meters. The hypolimnion is generally >40 meters deep (depending on the season) and might also support Burbot spawning (hypolimnetic temperatures and dissolved oxygen levels have respective year-round ranges of 4−6 °C and 8−15 mg/l; Schindler et al. 2007; 2007a; 2009). Laboratory studies have shown that Burbot spawning and egg incubation can be successful between 0−4 °C (Jäger 1981; Taylor and McPhail 2000; Vught et al. 2008; Lahnsteiner et al. 2012). However, disparity exists between these studies with regards to optimal incubation temperature, so there may be adaptive thermal preferences across different populations. Little is known about groundwater exfiltration into Kootenay Lake at depths relevant for Burbot spawning, which is presumably deeper than Kokanee spawning sites in the West Arm (Andrusak and Andrusak 2011). It is possible that underflow does not benefit demersal Burbot eggs because it may lead to incubation temperatures >5°C and reduce oxygen levels, but more research is need on this topic to make any definitive assessment.

Martin (1976) evaluated gonads from a 1968 creel survey in the West Arm and recorded ripe, mature fish throughout March and early April; spent fish were not observed until the first week of April. Mature fish were largely absent from the catch during mid-May but were observed again from June−July. These data provided evidence of potential spawning in the West Arm and/or utilization of the Balfour site as a feeding ground before and after spawning (Redfish Consulting 1998). The possibility of historical spawning in the West Arm between March−April correlates with spawning time recently documented in nearby Arrow Lakes Reservoir (Robichaud and Glova 2014). However, it is somewhat delayed relative to peak spawn timing in nearby Moyie Lake, which typically occurs in February. Cott et al. (2013) concluded that February spawning was a conserved life history pattern in

various Burbot populations across Canada. In contrast, Martin (1976) suggested that spawning may have occurred as late as July in the West Arm. An evaluation of temperature data collected in 2006 (source: Schindler et al. 2009) suggests that the entire depth of the West Arm was >4°C by mid-April and would be lethal to Burbot embryos by June (i.e., >6°C). Unfortunately, it is not known if similar conditions existed in the West Arm during the pre-dam flood pulse.

Past observations of mature fish in July could support a theory that Burbot used the West Arm as a feeding ground (Redfish Consulting 1998), but then spawned elsewhere. Delayed spawning in spring/summer has been reported in other deep lakes (Fischer 1999; Bonar et al. 2000; Probst and Eckmann 2009; Donner and Eckmann 2011; Jude et al. 2013). This atypical spawning strategy may be an adaptation to improve recruitment during extreme environmental change or it could be a transition to the ancestral gadid life history mode (Jude et al. 2013). Burbot spawning was documented in early March along shorelines in the North Arm (Spence 1999), but these aggregations were small and were not observed again in follow-up studies (Baxter et al. 2002a; Neufeld 2005).

Burbot in the South Arm are presumed to be mostly adfluvial with a February spawning time in both the Goat River (near Creston, British Columbia) and the mainstem/tributaries of the lower Kootenai River (Partridge 1983; Paragamian et al. 2000). Little is known about the spatiotemporal patterns of wild Burbot spawning in the subbasin due to extremely low abundance and risks of sampling mortality (Paragamian 1994; Spence 1999; Paragamian et al. 2000; Paragamian and Whitman 2000; Baxter et al. 2002a; Neufeld 2005), however, recent monitoring and evaluation of mature, river-adapted hatchery Burbot in the subbasin is providing valuable information about upriver migration and confirms the importance of tributaries to the meander reach for spawning (Ross et al. 2020). This new data also infers a peak spawning time of mid-February in the Idaho portion of the lower Kootenai River.

*1.6.2. Anthropogenic Changes Affecting Early Life History*

There are at least five distinguishable pattern changes to the regime of the lower Kootenai River since construction of Libby Dam that potentially impact early life stages of Burbot: 1) higher and more variable fall/winter flows, 2) warmer and more variable fall/winter temperatures, 3) intermittently cooler and more variable early-spring flows, 4) lower spring/summer flows, and 5) reduced nutrient loading and zooplankton productivity. There is some evidence to support the theory that higher flows and warmer temperatures in fall/winter discourage upriver spawning migrations, but little is known about how these changes influence gonad maturation, gamete quality, and spawning success. There is some disagreement between studies regarding the thermal tolerances of embryos, and insufficient knowledge about incubation success under fluctuating temperatures. Even less is

known about larvae rearing success in mainstem and lake habitats that are potentially cooler, less productive, and no longer have substantial spring/summer connectivity with the historical floodplain.

It has been hypothesized that larval fish recruitment is conditioned by a spatiotemporal match with zooplankton abundance: referred to as the match–mismatch hypothesis (Cushing 1972). Larvae must locate swarms of prey during this critical period before a time of irreversible starvation or point-of-no-return (Miller et al. 1988). Once larvae successfully initiate feeding, starvation risks dramatically decrease (Hunter 1981). Another issue arising from productivity changes is a possible shift in fish assemblages and predator/prey dynamics. For example, there is uncertainty about contemporary embryo and/or juvenile survival given that a large biomass of Northern Pikeminnow (*Ptychocheilus oregonensis*) and Largescale Sucker (*Catostomus macrocheilus*) now occupy former habitats and ecological niches of the Burbot (Baxter et al. 2002; Figure 1.0.7).



**Figure 1.0.7.** Large aggregations of suckers and Northern Pikeminnows in former Burbot habitat. Observations from remotely operated vehicle surveys of the Balfour area of Kootenay Lake (image reprinted from Baxter et al. 2002).

The historic spring/summer flood-pulse was potentially an important cue for downstream migration to feeding grounds. Burbot are known to make downstream migrations post-spawning (Sorokin 1971; Müller and Berg 1982; Hedin 1983). During the fishery collapse, peak flows in spring/summer declined three-fold with Libby Dam operations; the natural unimodal flood pulse driven by snowmelt was transformed by river regulation into an unnatural multimodal flow regime (see Figure 1.0.3). Year-over-year declines in downstream migration to productive feeding grounds could impair energetics important to reproduction.

Little is known about early life stages of wild Burbot in the system: no eggs and only a handful of juveniles have been sampled from the subbasin (Martin 1976; Partridge 1983; Paragamian et al. 1995; Spence 1999; Paragamian and Whitman 2000; Ross et al. 2020). Mark−recapture efforts associated with stocking pre-feeding larvae in early spring has demonstrated survival and growth in off-channel floodplain habitats, although no evidence exists of similar success in the mainstem. Conversely, stocking planktivorous-feeding larvae into the mainstem during late spring has produced some success with an average survival rate of 0.3% to age-1; older and larger juveniles stocked in summer/fall show 10% survival to age-1, but slower growth rates that persist into later years. Information gathered from this monitoring and evaluation program now point toward a recruitment bottleneck occurring sometime between spawning and early feeding of larvae (Ross et al. 2020).

Both Partridge (1983) and Paragamian et al. (2008) inferred that post-dam increases in winter river temperatures could be problematic for early life stages (i.e., adult spawning, embryo incubation and larval development). Cott et al. (2013) reported that the austere under-ice environment is an important aspect of Burbot life history. For example, ice cover may play a role in the mating system via vocalizations involving the swim bladder drumming muscles (Cott et al. 2014). Spawning under the ice could be a strategy to minimize egg predation and to combat infection of eggs by pathogens that are less tolerant of icy temperatures below 3°C (e.g., *Saprolegnia* spp. or water mold; Van West 2006). Embryo incubation in cold temperatures under the ice can also delay hatch timing (Taylor and McPhail 2000) and may factor into match−mismatch dynamics for larval feeding in spring. Prior to construction of Libby Dam, ice cover and low flows provided a cold, thermally stable environment for spawning and embryo incubation in the mainstem. In contrast, the post-dam thermal regime is destabilized by discharge of warmer water from Koocanusa Reservoir. Overall, the loss of historic winter ice cover in the lower Kootenai River may be of great ecological consequence to early life history of Burbot.

## 1.7. Conclusions

The Kootenai River Subbasin has incurred over a century of anthropogenic alterations linked to regional development and larger contingencies associated with economy and ecology of the Columbia River. Since 1972, operation of Libby Dam has been integral to river system management, but it has also severely impacted downstream flora and fauna in the lower Kootenai River and Kootenay Lake. A wild Burbot population was nearly extirpated because of changes possibly related to overfishing, dams, habitat degradation, and system ultra-oligotrophication. Libby Dam now operates under guidelines of VARQ which include greater water storage in Koocanusa Reservoir, less power generation, more flood control, and fisheries mitigation. VARQ has generally resulted in lower

discharge in winter and higher discharge in spring/summer compared to previous phases of dam operation. The effect of VARQ on the average post-dam thermal regime has been subtle, with slightly cooler winter/spring temperatures in the meander reach over the last two decades.

Regulation of Kootenai River flows for hydropower, flood control, and mitigation for other endangered or threatened fish may be affecting Burbot life history in ways that suppress natural recruitment. Increased autumn/winter discharges and loss of the unimodal spring/summer flood pulse has potentially altered the natural migration behavior of Burbot between feeding grounds and spawning habitats. Warmer post-dam temperatures in the autumn/winter could also be affecting gametogenesis, Previous studies about post-dam regime effects on wild spawning migrations were limited by low abundance and protections for imperiled fish. However, recent development of a Burbot conservation aquaculture program has provided an opportunity to gain more information about post-dam effects on river-adapted hatchery fish. It is recommended that new studies utilize this burgeoning population of stocked fish to help determine if the post-dam regime suppresses gonad maturation and gamete quality or alters spawning time and reproductive success.

VARQ has generally produced winter flows that more closely resemble pre-dam levels between January–February, but there is still an unnatural absence of ice cover in the lower Kootenai River that may be hindering successful Burbot reproduction. Elevated and fluctuating river temperatures during winter are especially concerning because they contrast with natural pre-dam conditions. More research is needed to determine the effect of different river temperatures on spawning and embryo incubation. Conversely, discharge of reservoir water in early spring dampens the natural warming trend of the river that likely coincides with hatching of larvae, swim bladder inflation, and transitioning from yolk to zooplankton food source.

Nursery habitats for Burbot larvae are now mostly restricted to the main channel and Kootenay Lake because constructed levees along the mainstem and tributaries prevent overbank flow into the natural floodplain. Side-channel habitats, backwaters, and floodplain lakes potentially provide larvae with different forage ecology, thermal gradients, growth patterns, and predator exposure. It is still unknown if newly hatched Burbot larvae can inflate their swim bladders, initiate feeding on zooplankton, grow, and recruit to the fishery in a post-dam thermal regime. Given the knowledge gap about a possible recruitment bottleneck(s) occurring between spawning and larvae metamorphosis, there is a need for research that demonstrates larvae can thrive in a thermal regime like post-dam conditions in the lower Kootenai River.

In summary, the present review emphasizes the importance of understanding how anthropogenic alterations in the Kootenai River Subbasin potentially impact early life stages of Burbot to affect recruitment. Implementation of field and laboratory research that compares pre-dam

and post-dam thermal profiles for critical stages of adult spawning, embryo incubation, and larvae development should be the initial focus of investigations seeking to explain recruitment failures possibly linked to Libby Dam operations. The contemporary goal of mitigating the wild Burbot population decline in lower Kooteniai River/Kootenay Lake will be to leverage knowledge gained from stocking hatchery fish to reveal any potential post-dam regimes that support natural recruitment.

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# Chapter 2: Temperature and Maternal Age Effects on Burbot Reproduction

## Abstract

River regulation alters the thermal regime of many freshwater ecosystems, and impoundments are known to negatively impact recruitment of fluvial Burbot *Lota lota* populations. Some conservation programs in North America and Eurasia have begun to incorporate stocking of hatchery Burbot to mitigate extirpation risks. Anthropogenic modification of river temperatures and population structure has unknown implications for Burbot reproduction. This study evaluated the effects of elevated water temperatures and different maternal ages on spawning and embryo development of hatchery Burbot. Juveniles stocked downstream of a hydroelectric dam were found to naturally mature in a warmer post-dam regime. The river-adapted hatchery adults volitionally spawned in a broodstock facility at 2, 4 and 6°C. The spawning period spanned 18 days at 2°C but was shortened to 6−7 days at 4 and 6°C. Survival of embryos to the eyed-stage was 86.7, 47.9 and 0.1% at incubation temperatures of 2, 4 and 6°C, respectively. Embryo deformity increased dramatically between 4−6°C. Young (age-3) and old (age-7+) females spawned 4−12 days later than 5- and 6-year-old females. Age-3 females spawned smaller eggs, but no relationship was found between maternal age and embryo vital rates. It was concluded that juvenile stocking supplemented the abundance of reproductive adults in the highly altered ecosystem of the lower Kootenai River. Spawning temperatures above 4°C potentially underlie Burbot recruitment bottlenecks in systems affected by impoundments, climate warming, or other barriers to cold-water spawning habitat. Contraction of the spawning period by warmer temperatures and/or truncated population age structure could synchronize spawning to coincide with suboptimal conditions for normal embryo development.

## 2.1. Introduction

Burbot (*Lota lota*; Linnaeus 1758; Gadidae) are the only freshwater gadid widely distributed across the Holarctic ecozone at latitudes greater than 40°N (Van Houdt et al. 2003). Many southern populations are imperiled as a result of man-made changes to freshwater ecosystems (Stapanian et al. 2010). In particular, elevated winter water temperatures downstream of reservoirs have been implicated as a major threat to riverine populations (Paragamian and Wakkinen 2008; Harrison et al. 2016a; Blabolil et al. 2018). Burbot are characterized as an extreme winter specialist (Shuter et al.

2012), and reproduction may be largely incompatible with spawning habitats that deviate from an isothermal, ice-covered environment (Cott et al. 2013a).

Temperature is oftentimes a controlling factor in fish gametogenesis (Wieland et al. 2000; Wang et al. 2010; Abdulfatah et al. 2013). For teleosts inhabiting temperate zones, temperature can have a primary role in final gonad maturation and gamete release (Anguis and Cañavate 2005; Wang et al. 2010). Abnormal or fluctuating temperatures shortly before, during, and after spawning may disrupt reproduction (Van Der Kraak and Pankhurst 1997; Tveiten et al. 2001; Targońska et al. 2014). Temperatures that outlie a species preferendum can either delay or accelerate final maturation by days or even weeks (Atse et al. 2002). Warm winter temperatures can induce early gonad maturation in species that typically spawn later in spring (Geraudie et al. 2010; Dorts et al. 2012), and cold temperatures often cause delays in peak spawning time (Wright and Trippel 2009). Conversely, ovulation may also be delayed or inhibited when final maturation occurs at high temperatures (Taranger and Hansen 1993; Brown et al. 1995; Pankhurst et al. 1996; Tveiten et al. 2001).

Burbot generally exhibit a fixity toward mid-winter spawning at very cold temperatures (McPhail and Paragamian 2000; Evenson 2000; Cott et al. 2013a). However, there is some evidence of plastic life histories with spawning times that extend between autumn through summer depending on the population and ecosystem, with most instances of non-winter spawning linked with very deep lakes and reservoirs (Clemens 1951; Fischer 1999; Bonar et al. 2000; Probst and Eckmann 2009; Donner and Eckmann 2011; Jude et al. 2013). Some disparity exists between various reports of temperature effects on Burbot spawning. Cott et al. (2013a) suggested that an endogenous reproductive clock drives reproduction, thereby making Burbot less dependent on environmental cues. However, Żarski et al. (2014) demonstrated the importance of temperature as a controlling factor for final maturation by spawning Burbot in captivity without year-round changes in photoperiod (i.e., constant 24-hour daylight). The timing and location of Burbot spawning in streams has been linked to accessibility of cold water (Farkas et al. 1993). Harrison et al. (2016b) found that Burbot actively select for 4−6°C habitat during the pre-spawning period but avoided > 2°C between spawning and post-spawning. In contrast, Vught et al. (2008) reported spawning at water temperatures between 4−6°C. Burbot have also been observed spawning in spring waters of 5−6°C (even though cooler habitats were accessible), and Taylor and McPhail (2000) suggested that factors extrinsic to water temperature could be important to spawning.

A more informative temperature threshold for Burbot reproduction might be extrapolated from embryo thermal tolerances. Fish oftentimes spawn at temperatures that align with the thermal preferendum of embryos (Rombough 1997; Pörtner and Peck 2010; Hu et al. 2017). However, there is some disagreement regarding optimal temperatures for Burbot embryo development. For example,

Jäger (1981) found that Burbot embryos survive at 1−7°C, but mortality increased sharply above or below 4°C. A similar trend was reported by Vught et al. (2008), except 8°C was the upper lethal limit. Contrastingly, Żarski et al. (2010) and Lahnsteiner et al. (2012) both concluded that cold, stable temperatures (i.e., ≤ 2°C) supported the highest embryo survival. Żarski et al. (2010) and Kujawa et al. (1999) reported 100% embryo mortality following incubation above 5.0 and 6.5°C, respectively. Overall, these reports provide some evidence that Burbot maturation, spawning and embryogenesis could be vulnerable to winter water temperature increases to 6°C.

Gametogenesis in gadids is also influenced by age (Hutchings and Meyers 1993), size (Ouellet et al. 2011), and condition factor (Hardardottir et al. 2001; Kurita et al. 2003; Scott et al. 2006). Exploitation typically reduces the number of large individuals, which results in a higher proportion of smaller, younger adults (Scott et al. 2006); concomitantly, a preponderance of first-time spawners can delay peak spawning time (Wieland et al. 2000; Wright and Trippel 2009). The spawning period of a population has important ramifications affecting fisheries recruitment through a variety of demographic, physiological and ecological mechanisms (Wright and Trippel 2009). A relatively fixed spawning period in ecosystems with variable productivity can lead to recruitment trends that follow a match−mismatch model (Cushing 1990; Cushing and Horwood 1994; Fortier et al. 1995; Gotceitas et al. 1996). Reductions in the spawning period can increase the likelihood of mismatch, especially if selective pressures exist for early and late birth dates (Scott et al. 2006).

Few studies have reported on the reproduction of stocked Burbot. Blabolil et al. (2018) detailed the difficulties of sampling *Lota lota lota* for evaluations of stocking efforts in the Czech Republic. Bosveld et al. (2015) briefly mentioned the possibility of reproducing hatchery Burbot in the Netherlands based on increased juvenile abundance after stocking adults. In Germany, there is evidence of population recovery following substantial stocking of juveniles; natural reproduction of hatchery fish is suspected, but still needs to be confirmed (Brackwehr et al. 2016). There have been reports of hatchery Burbot migrating in the lower Kootenai River (ID, USA) during winter, showing substantial changes in weight and condition between January−March, which indirectly suggests that spawning had occurred in either the main channel or its tributaries (Hardy et al. 2015; Ross et al. 2018).

The Burbot population in the lower Kootenai River provides an excellent case study of elevated temperature effects on reproduction. Operations at Libby Dam have warmed the downstream system between October−March, and the main channel no longer has extensive ice cover (Paragamian and Wakkinen 2008). Burbot demographics have also changed since the wild population was deemed functionally extirpated over a decade ago (Paragamian et al. 2008); contemporary abundance consists primarily of river-adapted hatchery fish (Hardy et al. 2015; Ross et al. 2018) after juvenile stocking

began in 2009 (Paragamian and Hansen 2011). The overarching goal of this study was to adduce the impacts of post-dam temperatures and post-hatchery demographics on Burbot reproduction. To accomplish this aim, two experiments were conducted to evaluate the effects of different spawning temperatures and maternal ages on fish morphometrics, proportion of spent fish, spawning time, relative fecundity, egg size, embryo deformity and survival. Findings from this research have important implications for Burbot population recovery efforts worldwide and could be particularly informative to conservation efforts that include juvenile stocking programs.

## 2.2. Methods

### 2.2.1. Study Area

The Kootenai River (spelled Kootenay in Canada) is a tributary to the Columbia River and drains a 41,910 km$^2$ basin comprising parts of British Columbia, Montana, and Idaho (Burke et al. 2009). The present study focuses on the river system downstream of Libby Dam located 352 river kilometers (rkm) from the confluence with the Columbia River. This system—known as the lower Kootenai River—flows west and north across northern Idaho and southeastern British Columbia into Kootenay Lake (Figure 2.0.1).

### 2.2.2. Broodstock Collection and Husbandry

The Idaho Department of Fish and Game (IDFG) conducts annual hoop net sampling of Burbot in the lower Kootenai River between December−March at 38 different sites extending from Creston, BC, Canada (rkm 144.5) to the Idaho−Montana border (rkm 276) (Figure 2.0.1). Details of the hoop netting methods are described by Ross et al. (2018). One of the sampling sites (i.e., Ambush Rock) is near a putative mainstem spawning location (Hardy et al. 2015), and several other sites (i.e., Deep Creek, Parker Creek and Goat River) are located near potential spawning tributaries (Partridge 1983; Paragamian et al. 2000). During the last three weeks of January in 2017 and 2018, the Kootenai Tribe of Idaho (KTOI) followed IDFG hoop netting efforts and relocated river-adapted hatchery Burbot to the Twin Rivers Hatchery (Moyie Springs, ID, USA) for use as broodstock. During transport adults were exposed to 25 mg/L $H_2O_2$ and 5 g/L NaCl for ≤ 60 minutes as a prophylactic treatment for external parasites.
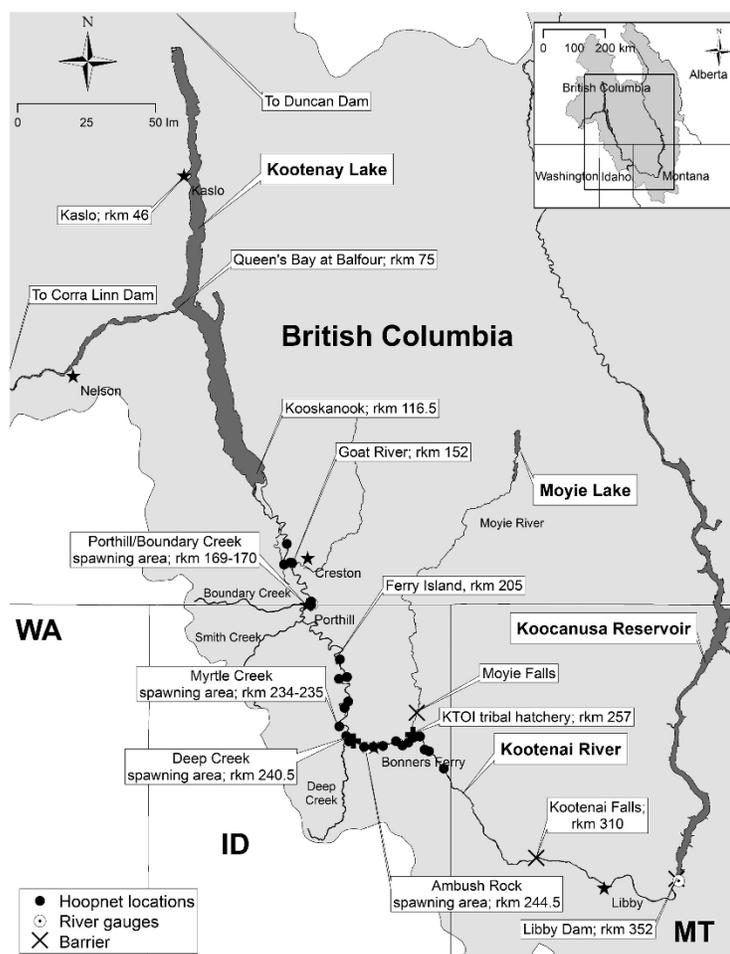
**Figure 2.0.1.** Map of the lower Kootenai River study area with labels identifying donor population source (Moyie Lake), hatchery location, hoop net sampling sites, major tributaries, key river kilometers (rkm), temperature gauging stations and significant barriers to migration.

Within 24 hours of arriving at the hatchery, fish were anesthetized in a buffered (equal parts NaHCO₃) solution of 250 mg/L tricaine methanesulfonate (Tricaine-S®, Western Chemical Incorporated, Ferndale, WA, USA) for determining age (from PIT tag ID), total length (*TL*; mm), wet weight (*W*; g), fish health, spawning condition (i.e., expression of milt or ova) and sex (via ultrasound; Chison, Model ECO 3, with a Model L7M-A 7.5 MHz linear array probe, Wuxi, Jiangsu, China). Genetic sampling via fin clips and genotyping of single nucleotide polymorphic markers verified sex and age of fish prior to starting experiments (M. R. Campbell, IDFG, personal communication). In both study years, males and females were held separately in 5,000 L flat-bottom, circular tanks prior to starting the experiments. Pre-experiment holding temperatures were similar to the river thermal regime until the last day of broodstock collection but were then maintained between 2−5°C independent of river temperature until the start of experiments (Figure 2.0.2).
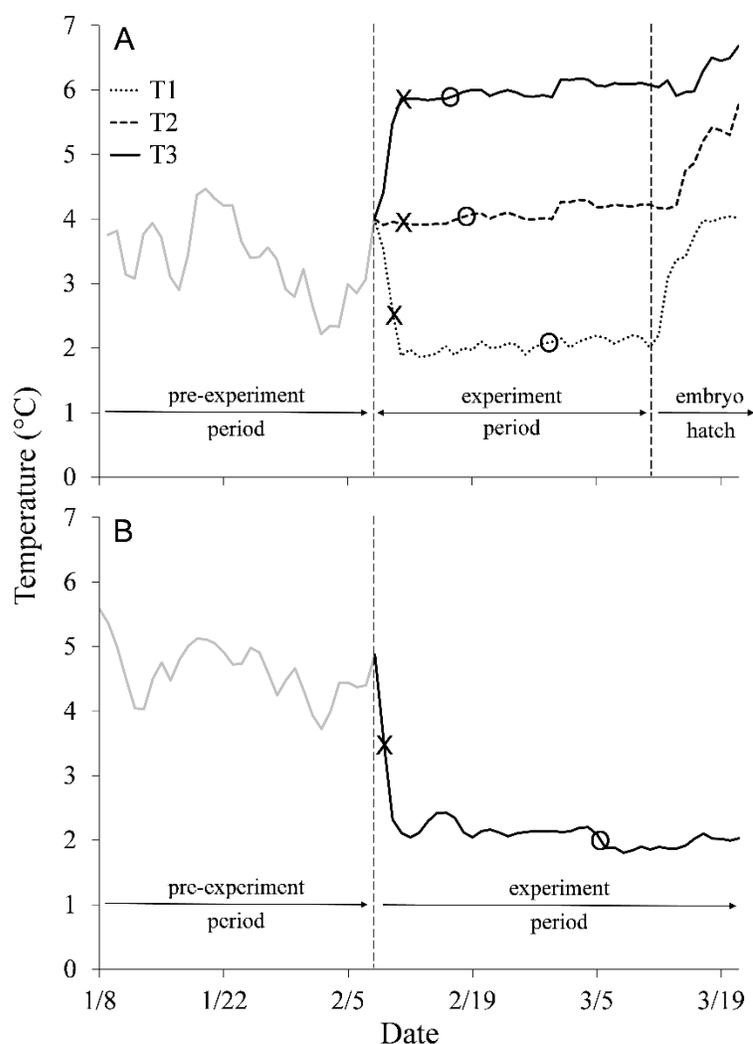
**Figure 2.0.2.** Thermographs for Burbot broodstock and egg incubation experiments at Twin Rivers Hatchery in 2017 and 2018. The pre-experiment period spanned the time from broodstock capture in the lower Kootenai River to the start of experiments at the hatchery. In 2017 (panel A), three temperature treatments (T1, T2 and T3) targeted 2, 4 and 6°C, respectively. A profile of ~2°C was used in 2018 (panel B) during an evaluation of maternal age-groups. Xs and circles mark the start and end of observed spawning, respectively.

Broodstock were not fed in the hatchery and experiments were limited to 3−4 weeks to avoid potential fish health complications. Minimal handling stress to broodstock was an overarching objective at the hatchery to better replicate natural spawning behavior and reproductive physiology. Adults spawned volitionally in partially sheltered tanks at low stocking densities and slow current velocities. Temperature control to within ± 0.5°C of target levels was achieved with portable refrigerant-based chillers (Frigid Units, Model BHC 1183-1, Toledo, OH, USA). Water was supplied in a flow-thru arrangement with inflows of 7.5 L per minute for each replicate broodstock tank. Dissolved oxygen levels were ≥ 9 mg/L according to a handheld meter (Oxyguard Handy Polaris 2, Oxyguard International, Farum, Denmark) and ammonia concentrations were below detectable levels

of a test kit (API®, Mars Incorporated, McLean, VA, USA). Skylighting provided a natural photoperiod with a peak intensity ≤ 1,000 lux at the water surface based on handheld meter readings (MW700 Portable Lux Meter, Milwaukee Instruments, Rocky Mount, NC, USA). Supplemental daylight-spectrum fluorescent lighting was used periodically for egg collection efforts.

### 2.2.3. Egg Collection and Incubation

Eggs were passively collected from the effluent of broodstock tanks via submerged nylon screen containers with 400 μm mesh. Spawns that began in the morning were collected and incubated in the afternoon; evening spawns were collected and incubated the next morning. Eggs collected from each replicate broodstock tank were incubated separately in 1 L Imhoff cone incubators (previously described by Jensen et al. 2008) that shared the same water source as the broodstock tanks within each treatment. Hydrogen peroxide was dosed via peristaltic pump into the incubation system at 500 mg/L for 15 minutes each day as a prophylactic treatment against fungal infection (Polinski et al. 2010).

### 2.2.4. Temperature Effects on Broodstock Spawning and Embryo Development

A 21-d experiment was initiated on February 8, 2017 to test the effects of three different temperature treatments (2, 4, and 6°C; Figure 2.0.2) on broodstock- and embryo-related response variables (Table 2.0.1). Fish were not randomly selected for the experiment because research efforts were linked to hatchery production goals; therefore, only individuals with satisfactory health, condition factor (≥ 0.50; $K$; Ricker 1975) and size ($TL$ ≥ 397 mm) were chosen as broodstock. A total of 45 males and 36 females were selected for the experiment (Table 2.0.2). With the exception of four fish (two males and two females), all ages were known from hatchery-origin PIT tags or parentage-based genetic markers; the four fish of unknown age were within the size range of other broodstock between 3−6 years old. Each treatment included three replicate 1,700 L flat-bottom, circular tanks with 4 females and 5 males per tank. Fish were stocked into the experiment at 4°C and gradually acclimated to the different temperature treatments over a two-day period (Figure 2.0.2). Observations of volitional spawning and egg collections ended on February 28, 2017. Each adult was anesthetized and measured for post-experiment morphometrics and spawning condition. Males were checked for presence of milt and females were categorized as unripe, ripe, or spent. All broodstock were returned to the lower Kootenai River by March 2, 2017.

Egg incubators shared the same temperature treatments as broodstock until the embryos reached the eyed stage. Incubation temperatures were increased on March 12, 2017 for hatchery operational requirements to synchronize embryo hatching (Figure 2.0.2). Observations of egg

incubation ended on March 21, 2017. Cumulative survival of embryos was estimated by differences in egg volumes measured at 0, 1, 4, 9, and 15 days post-spawning (dps). Final survival estimates were made at the eyed stage. Subsamples of eggs from the top, middle and bottom fractions of incubators ($n$ = 10 per fraction) were digitally imaged for analyses of egg diameters, embryo deformity and survival (AmScope™ SM-1TZ-RL-10MA trinocular microscope with MT1000-CK 10.7 megapixel digital camera, Irvine, CA, USA). The percentage of living embryos in the subsamples were used to improve the accuracy of survival estimates. Dead eggs were distinguished by large, dark inclusions and occasional gross distension in egg morphology. Embryo deformity was identified by malformation of the eyes, head, or vertebrae. A non-deformed or normal embryo was defined by the absence of any visible deformity identified via digital image analysis at the eyed stage (ImageJ, version 1.50b, National Institutes of Health, Bethesda, MD, USA).

*2.2.5. Maternal Age Effects on Adult Spawning and Embryo Development*

A 26-d experiment was initiated on February 8, 2018 to test the effects of four different maternal age-groups (age-3, 5, 6 and 7+) on broodstock- and embryo-related response variables (Table 2.0.1). A total of 64 males and 32 females were selected for the experiment (Table 2.0.2). Each treatment included two replicate 1,700 L flat-bottom, circular broodstock tanks with 4 females and 8 males per tank. Male broodstock ranging in age from 3−7 years were evenly distributed into the tanks. Female broodstock were segregated into tanks according to the four different maternal age-groups. The 7+ year-old females consisted of 2009 and 2011 year classes; two females were of unknown age, but their large size and weight (i.e., *TL*: 796 and 840 mm; *W*: 5.1 and 4.7 kg) clearly inferred an age of at least 7 years (Ross et al. 2018). Fish were stocked into the experiment at 4.9°C and gradually acclimated to 2.0−2.4°C (Figure 2.0.2). Spawning observations and egg collections were terminated on March 5, 2018. Each adult was anesthetized, measured for post-experiment morphometrics and evaluated for spawning condition. Broodstock were returned to the lower Kootenai River on March 6, 2018. Egg incubators were supplied with 1.7−2.2°C water from spawning until the eyed-staged (Figure 2.0.2). The previously described methods of sampling and imaging were used for estimating egg diameters, embryo deformity and survival. Observations of egg incubation ended on March 12, 2018.

*2.2.6. Data Analyses*

The experimental unit was the subpopulation of fish in each tank. Observational units included individual fish, eggs, and spawning events. Table 2.0.1 lists the various covariate, response and treatment variables included in statistical analyses.

**Table 2.0.1.** A list of covariate, response and treatment variables used in statistical analyses of results from study years 2017 and 2018.

| Variable | Description | Abbreviation | Definition or formula | Units |
|---|---|---|---|---|
| Covariate | Pre-experiment period | $t_{pre}$ | Experiment start date – fish capture date | d |
| | River kilometers | $RKM$ | Upriver distance of fish capture | km |
| | Fish age | $A$ | Age by PIT tag and genetic marker | years |
| | Fish sex | $m$ or $f$ | Sex by genetic marker | male or female |
| | Total length | $TL$ | Distance from tip of snout to tip of tail | Mm |
| | Wet weight | $W$ | Mass of fish in the atmosphere | G |
| | Condition factor | $K$ | $10^5 \cdot W/TL^3$ | g/mm$^3$ |
| | Relative weight[a] | $W_r$ | $W/W_s \cdot 100$ | % |
| | Biomass | $B$ | Total weight of fish per tank | G |
| | | | | |
| Response | Spawning time | $t_s$ | # of days into February | day |
| | Spawning period | $t_p$ | # of days between the first and last spawn | day |
| | Collected egg volume | $E_v$ | Total volume of collected eggs per tank | mL |
| | Relative fecundity | $F_r$ | $E_v/B_f$ | mL/kg |
| | Spent females | $SP_f$ | (# of spent females)/(# of females)·100 | % |
| | Percent weight loss | $WL$ | $\lvert (W_{post} - W_{pre})/W_{pre} \cdot 100 \rvert$ | % |
| | Specific weight loss | $SWL$ | $\lvert \log_e(W_{post} \div W_{pre})/days \cdot 100 \rvert$ | %/day |
| | Egg diameter | $E_d$ | cross-sectional distance of a live egg | mm |
| | Change in condition | $\Delta K$ | $\lvert K_{pre} - K_{post} \rvert$ | g/mm$^3$ |
| | Embryo deformity | $D$ | (# of deformed embryos)/(# of live embryos)·100 | % |
| | Embryo survival | $S$ | (mL of live embryos)/(mL of spawned eggs)·100 | % |
| | | | | |
| Treatment | Temperature | $T$ | Constant 2, 4 and 6°C | °C |
| | Maternal age | $A_m$ | 3, 5, 6 and 7+ years old | years |

[a]See Methods section for a definition of standard weight ($W_s$) used to calculate relative weight ($W_r$).

Fish robustness was defined by Fulton's condition factor ($K$; Ricker 1975) and relative weight ($W_r$; Wege and Anderson 1978) calculated from the standard weight ($W_s$) equation for *Lota lota maculosa* ($\log_{10}W_s = 2.898\log_{10}TL - 4.868$; Fisher et al. 1996). A Spearman's rank correlation coefficient ($\rho$) test examined relationships between covariate or response pairs. A Bonferroni correction was used to control type I error in multiple correlation tests involving the same variable. Log-linear models (negative binomial probability distribution and log link) were used to analyze data with offsets. Logistic models (binomial distribution and logit link) were performed on percentile or proportional data. Linear regression or analysis of deviance models (Gaussian distribution and identity link) were used in tests with continuous data. All generalized linear model analyses were validated with homoscedastic scatterplots of residuals versus fitted estimates. Assumptions of normal error distribution for simple linear models were verified with quantile–quantile plots. Power transformations determined by Box−Cox method normalized any non-normal data. Instances of overdispersion were adjusted with a Pearson scale parameter. Type I error ($\alpha$) was defined at 0.05 for all statistical tests. Tukey's honestly significant difference (HSD) post-hoc tests were performed to control error rate in multiple, pairwise comparisons between categorical treatments. Wald chi-square test statistics ($X^2$) were presented with degrees of freedom in subscripts. All statistical analyses were performed in SAS (Statistical Analysis Software program, PROC GENMOD, version 9.4; SAS Institute, Cary, NC, USA).

## 2.3. Results

### 2.2.6. Temperature Effects on Broodstock Spawning and Embryo Development

Temperatures in the lower Kootenai River between December 2016−January 2017 were 3−6 ºC warmer than pre-dam levels (Figure 2.0.3). The majority of broodstock were captured during late-January 2017 in the meander reach downstream of Bonners Ferry (i.e., < 250 rkm). No females spawned eggs during the pre-experiment period and 11% of males failed to express milt upon capture; however, only one of these individuals was not spermiating in post-experiment examinations. Broodstock survival was 100% for the duration of the 21-d experiment.

The spawning period occurred between February 10−28 at 2°C, but was between February 11−18 at 4 and 6°C; thus, the total range of spawning events was extended by 11 days at 2°C. A regression analysis estimated that mean ± SE spawning time ($t_s$) was delayed by 0.7 ± 0.2 days per °C decrease in temperature ($X^2_{1, 16} = 13.49$, $P < 0.01$). A similar result was found for the mean ± SE spawning period ($t_p$), which increased by 1.4 ± 0.1 days per °C decrease in temperature ($X^2_{1, 16} = 18.51$, $P < 0.01$). Temperature did not have a significant effect on collected egg volumes ($E_v$), relative

fecundity ($F_r$), or egg size ($E_d$) (Table 2.0.2). Both $E_v$ and $F_r$ failed to correlate with other response variables (i.e., $t_s$, $WL$, $SWL$, $\Delta K$ and $\Delta W_r$).
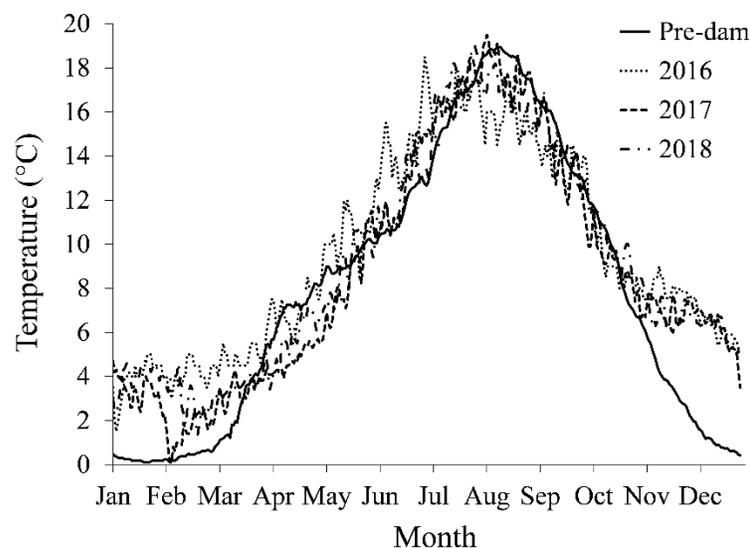


**Figure 2.0.3.** A comparison of average daily temperatures in the main channel of the lower Kootenai River for compiled pre-dam years (1963−1971) and three recent post-dam years (2016, 2017 and 2018). Temperature data for pre-dam years were sourced from the U.S. Geological Survey (stream site station 12322000, Porthill, ID), whereas thermographs for 2016, 2017 and 2018 were sourced from a nearby station (U.S. Geological Survey, stream site station 12309500, Bonners Ferry, ID).

Covariates among male and female subpopulations (i.e., $RKM$, $t_{pre}$, $A$, $TL$, $W$, $K$ and $W_r$) did not differ between treatments at the start of the experiment (Table 2.0.2). Female relative weights were close to average for *Lota lota maculosa*, while male $W_r$ was slightly lower than average (Table 2.0.2). All broodstock lost weight during the 21-d experiment as a result of spawning activity and lack of supplemental food. Females showed greater weight loss ($WL$ and $SWL$) and diminishing condition ($\Delta K$) than males ($X^2_{1, 16} = 31.05$, $P < 0.01$), but opposing trends in $\Delta K$ between sexes were observed at 4°C (Table 2.0.3).

Post-experiment examinations determined that one age-4 female at 4°C potentially skipped spawning; it showed no urogenital inflammation and had a $WL$ of 8% , which was much lower than average for the subpopulation (Table 2.0.3). Males at 2°C readily expressed viscous milt at the end of the experiment; however, at 4°C milt was less copious, and at 6°C only small droplets of milt were observed in clear fluid. Despite post-experiment qualitative differences in hand-stripped milt between treatments, analysis based on presence or absence failed to detect any temperature effect on spermiation. Changes in broodstock morphometrics and spawning condition did not significantly correlate with metrics of embryo development (i.e., $E_d$, $D$ and $S$).

**Table 2.0.2.** Pre- and post-experiment morphometrics[b] of Burbot broodstock in experiments with temperature and maternal age as treatments. Means and standard errors (in parentheses) are shown for male and female individuals (*N*) within a treatment subpopulation.

| Study year | Sex | Treatment | *N* | *A* (years) | *TL* (mm) | *W* (g) | *K* (g/mm$^3$) | $W_r$ (%) |
|---|---|---|---|---|---|---|---|---|
| Pre-experiment 2017 | Female | 2°C | 12 | 5.2 (0.3) | 562 (24) | 1,431 (241) | 0.73 (0.03) | 103 (5) |
| | | 4°C | 12 | 5.4 (0.3) | 550 (25) | 1,301 (208) | 0.72 (0.03) | 100 (4) |
| | | 6°C | 12 | 5.3 (0.3) | 558 (20) | 1,321 (172) | 0.71 (0.03) | 100 (4) |
| | Male | 2°C | 15 | 5.1 (0.2) | 512 (9) | 947 (64) | 0.69 (0.02) | 97 (3) |
| | | 4°C | 15 | 5.1 (0.2) | 494 (14) | 866 (77) | 0.69 (0.02) | 96 (3) |
| | | 6°C | 15 | 4.9 (0.3) | 512 (16) | 931 (84) | 0.67 (0.02) | 93 (2) |
| Post-experiment 2017 | Female | 2°C | 12 | - | 561 (25) | 1,084 (186) | 0.55 (0.02) | 78 (3) |
| | | 4°C | 12 | - | 551 (25) | 1,006 (154) | 0.55 (0.02) | 78 (2) |
| | | 6°C | 12 | - | 560 (20) | 997 (126) | 0.53 (0.01) | 75 (2) |
| | Male | 2°C | 15 | - | 512 (10) | 816 (55) | 0.59 (0.02) | 83 (3) |
| | | 4°C | 15 | - | 495 (14) | 721 (65) | 0.57 (0.02) | 79 (2) |
| | | 6°C | 15 | - | 511 (16) | 793 (71) | 0.57 (0.01) | 80 (1) |
| Pre-experiment 2018 | Female | Age-3 | 8 | 3.0 (0.0) | 471 (14) | 834 (78) | 0.79 (0.03) | 109 (4) |
| | | Age-5 | 8 | 5.0 (0.0) | 555 (14) | 1,216 (107) | 0.70 (0.02) | 99 (3) |
| | | Age-6 | 8 | 6.0 (0.0) | 676 (30) | 2,411 (308) | 0.75 (0.02) | 108 (3) |
| | | Age-7+ | 9 | 7.6 (0.4) | 722 (39) | 3,159 (578) | 0.76 (0.04) | 109 (7) |
| | Male | Age-3 | 16 | 4.5 (0.4) | 482 (25) | 894 (153) | 0.71 (0.03) | 98 (4) |
| | | Age-5 | 16 | 4.6 (0.4) | 501 (24) | 873 (107) | 0.65 (0.02) | 90 (2) |
| | | Age-6 | 16 | 4.6 (0.4) | 482 (22) | 837 (119) | 0.67 (0.02) | 93 (3) |
| | | Age-7+ | 16 | 4.5 (0.4) | 483 (25) | 849 (121) | 0.69 (0.02) | 95 (2) |
| Post-experiment 2018 | Female | Age-3 | 8 | - | 472 (13) | 667 (63) | 0.63 (0.03) | 87 (5) |
| | | Age-5 | 8 | - | 552 (15) | 939 (87) | 0.55 (0.02) | 77 (3) |
| | | Age-6 | 7 | - | 670 (32) | 1,816 (267) | 0.58 (0.02) | 83 (3) |
| | | Age-7+ | 9 | - | 711 (39) | 2,620 (548) | 0.64 (0.04) | 92 (7) |
| | Male | Age-3 | 16 | - | 481 (25) | 742 (123) | 0.59 (0.02) | 82 (2) |
| | | Age-5 | 16 | - | 495 (23) | 745 (95) | 0.57 (0.01) | 79 (2) |
| | | Age-6 | 15 | - | 488 (23) | 756 (104) | 0.60 (0.02) | 82 (3) |
| | | Age-7+ | 14 | - | 500 (24) | 780 (105) | 0.58 (0.01) | 81 (2) |

[b]See Table 2.0.1 for descriptions of morphometric variable abbreviations.

Embryos incubating at 2°C showed the lowest deformity (*D*) and highest survival (*S*) up to the eyed-stage (Table 2.0.3; *D*: $X^2_{2,6} = 42.56$, $P < 0.01$; *S*: $X^2_{2,6} = 202.08$, $P < 0.01$). Embryo deformity at the eyed stage was extremely high at 6°C, less severe at 4°C, and rarely observed at 2°C (Figure 2.0.4). The odds of deformity increased by a factor of $3.78 \pm 0.86$ per °C increase between the range of 2−6°C (log_temperature = $1.33 \pm 0.23$; $X^2 = 34.46$, $P < 0.01$); concomitantly, odds of normal embryo survival decreased by a factor of $0.27 \pm 0.05$ (log_temperature = $-1.31 \pm 0.19$; $X^2 = 47.49$, $P < 0.01$; Figure 2.0.5). A repeated measures analysis detected differences in embryo survival between treatments by 4 dps ($X^2_{2,6} = 265.32$, $P < 0.01$), and mortality primarily occurred between 0−9 dps (Figure 2.0.6). Generally, there was more variability in survival estimates at 4°C compared to 2 or 6°C. Some fertilization occurred at 6°C, as live zygotes were observed at 9 and 15 dps. Only a few hundred embryos survived to hatch at 6°C, whereas approximately 5.9 and 1.4 million embryos

hatched from 2 and 4°C treatments, respectively. Spawning temperature had no detectable effect on egg size, and diameters did not change significantly between the 1-cell and eyed-stage (Table 2.0.3).
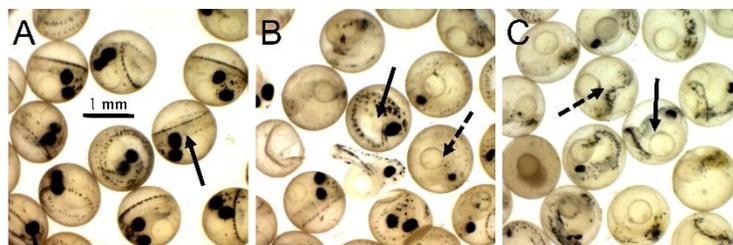


**Figure 2.0.4.** Images of Burbot (*Lota lota maculosa*) embryos from the lower Kootenai River incubating at 2, 4, and 6°C (panels A, B, and C, respectively). The scale bar for all images is shown in panel A. Solid arrows indicate embryos categorized as normal and without overt signs of deformity. Dashed arrows point to examples of deformed embryos with patent eye malformation (panel B) or arrested development (panel C).
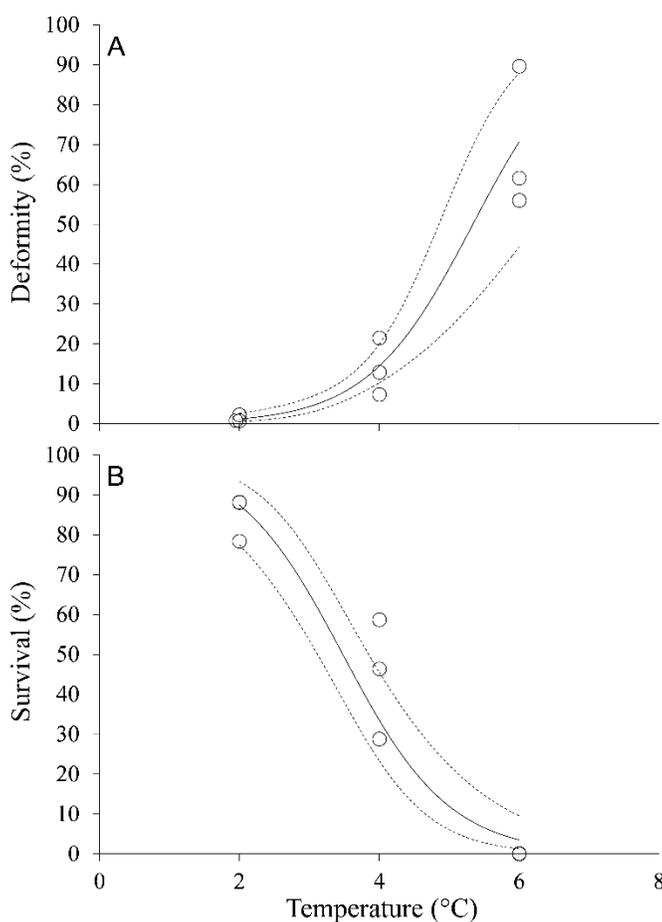


**Figure 2.0.5.** Logistic regression models of Burbot embryo deformity (panel A) and normal embryo survival (panel B) against incubation temperatures of 2, 4, and 6°C. Circles represent observed data from replicate (*n* = 3) broodstock subpopulations. Solid lines are the expected mean predicted by modeling. Dotted lines represent 95% confidence intervals for the predicted mean.
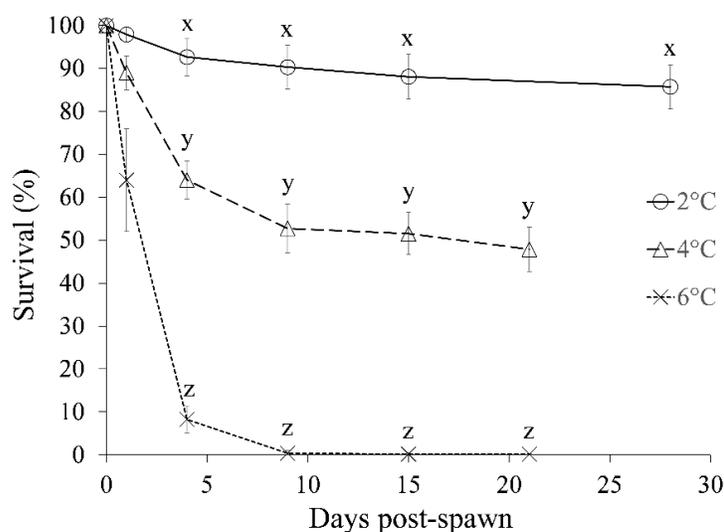
**Figure 2.0.6.** Cumulative survival of Burbot embryos from spawning to the eyed stage at incubation temperatures of 2, 4, and 6°C. Circles, triangles and Xs represent the estimated mean embryo survival from replicate ($n = 3$) broodstock subpopulations at the respective temperatures. Error bars show the standard error of the mean. Lowercase letters indicate significant difference in embryo survival between the temperature treatments ($P < 0.05$).

### 2.2.6. Maternal Age Effects on Broodstock Spawning and Embryo Development

Temperatures were again elevated in the lower Kootenai River between December 2017−January 2018 relative to average pre-dam levels (Figure 2.0.3). Broodstock were primarily captured during mid-January 2018 in the meander reach downstream of Bonners Ferry (<250 rkm). Most males expressed milt during the pre-experiment period and only two individuals were not spermiating in post-experiment examinations. There were three cannibalism events over the experiment duration; each case involved an age-3 male being eaten by an older, larger female (ages 6 and 7+). Additionally, one female (age 6) died of unknown causes, but exophthalmia was noted.

Maternal age significantly affected spawning time ($X^2_{3, 4} = 26.93$, $P < 0.01$). Young (age-3) and old (age-7+) females had a $t_s$ that was 4−12 days later than 5- and 6-year-olds (Table 2.0.3). Generally, spawning events in 2018 were more dispersed and occurred later in February compared to 2017. The mean ± SE percentage of spent females ($SP_f$) for age-3, -5, -6 and -7+ treatments was 75 ± 25, 100 ± 0, 75 ± 25 and 53 ± 28%, respectively; maternal age did not significantly affect $SP_f$. A positive correlation ($\rho > 0.89$, $P < 0.01$) existed between $SP_f$ and weight loss metrics ($WL$ and $SWL$), as well as relative fecundity ($F_r$). Maternal age did not significantly affect the total volume of collected eggs ($E_v$) or $F_r$ (Table 2.0.3). Variability in $E_v$ was high (except for age-5 females) and collected egg volumes (mean ± SE; mL) across treatments were 1,357 ± 550 (age-3), 2,179 ± 214 (age-5), 3,050 ± 1,010 (age-6) and 2,908 ± 2043 (age-7+). Interestingly, subpopulations with larger, older

**Table 2.0.3.** A summary of measured responses[c] for Burbot broodstock and embryos in experiments with temperature and maternal age as treatments. Means and standard errors (in parentheses) are shown for replicate broodstock subpopulations ($n$) within each treatment. Lowercase letters following column values indicate significance difference between treatments within a study year ($P \leq 0.05$).

| Study year | Treatment | $n$ | Sex | $WL$ (%) | $t_s$ (days) | $F_r$ (mL/kg) | Embryo stage | $E_d$ (mm) | $D$ (%) | $S$ (%) |
|---|---|---|---|---|---|---|---|---|---|---|
| 2017 | 2°C | 3 | Female | 24.4 (1.0) w | 15.2 (0.8) x | 0.55 (0.05) | 1-cell | 1.21 (0.02) | - | - |
| | 4°C | 3 | | 21.6 (1.0) x | 13.1 (0.8) xy | 0.41 (0.10) | | 1.20 (0.01) | - | - |
| | 6°C | 3 | | 24.0 (0.7) w | 12.5 (0.5) y | 0.44 (0.09) | | 1.22 (0.01) | - | - |
| | 2°C | 3 | Male | 13.6 (1.9) z | - | - | Eyed | 1.21 (0.01) | 1.1 (0.5) x | 85.7 ( 3.5) x |
| | 4°C | 3 | | 17.0 (0.6) y | - | - | | 1.21 (0.01) | 15.7 (3.9) y | 47.9 (7.3) y |
| | 6°C | 3 | | 14.5 (0.4) yz | - | - | | 1.22 (0.01) | 66.3 (8.8) z | 0.1 (0.0) z |
| 2018 | Age-3 | 2 | Female | 20.0 (3.5) | 25.4 (2.4) x | 0.40 (0.16) | 1-cell | - | - | - |
| | Age-5 | 2 | | 22.9 (0.6) | 13.3 (0.2) y | 0.45 (0.05) | | - | - | - |
| | Age-6 | 2 | | 22.2 (0.9) | 17.2 (1.2) z | 0.40 (0.18) | | - | - | - |
| | Age-7+ | 2 | | 15.3 (4.2) | 21.3 (0.2) x | 0.21 (0.13) | | - | - | - |
| | Age-3 | 2 | Male | 15.6 (2.0) | - | - | Eyed | 1.15 (0.01) y | 1.9 (0.6) | 86.7 (4.5) |
| | Age-5 | 2 | | 15.4 (0.2) | - | - | | 1.25 (0.01) z | 1.7 (0.2) | 71.1 (5.2) |
| | Age-6 | 2 | | 12.9 (0.2) | - | - | | 1.24 (0.01) z | 3.1 (2.3) | 69.5 (23.6) |
| | Age-7+ | 2 | | 15.2 (0.9) | - | - | | 1.24 (0.02) z | 3.4 (0.5) | 71.1 (6.2) |

[c]See Table 2.0.1 for descriptions of response variable abbreviations.

females and more broodstock biomass did not spawn significantly more eggs. Correlations between $F_r$ and other response metrics followed the same trends as those previously described for $SP_f$.

Covariates that were uncorrelated with maternal age (i.e., $RKM$, $t_{pre}$, $K$ and $W_r$) did not differ between treatments at the start of the experiment (Table 2.0.2). Like the previous year, a higher pre-experiment $K$ was observed in females as compared to males ($X^2_{1, 14} = 12.25$, $P < 0.01$). Females were older ($X^2_{7, 8} = 60.01$, $P < 0.01$) and weighed more than males ($X^2_{7, 8} = 62.40$, $P < 0.01$) in the age-6 and 7+ treatments (Table 2.0.2). The mean ± SE female biomass (kg) across treatments were 3,338 ± 78 (age-3), 4,862 ± 115 (age-5), 9,645 ± 335 (age-6) and 12,635 ± 1,938 (age-7+); male biomass and age did not differ between treatments and averaged 6,905 ± 154 kg and 4.53 ± 0.04 years, respectively. Female broodstock showed greater changes in morphometrics (i.e., $WL$, $SWL$ and $\Delta K$) over the experiment period relative to males (Table 2.0.3). Grouping by maternal age had no detectable effect on body metric changes in either sex.

Age-3 females spawned eggs with smaller diameters (Table 2.0.3; $X^2_{3, 4} = 16.04$, $P < 0.01$). No correlation was found between egg diameter and deformity ($D$) or survival ($S$). Generally, the youngest and oldest females spawned eggs with the highest and lowest percentage of normal embryos, respectively (Table 2.0.3).

## 2.4. Discussion

This study provides the first empirical account of reproduction from hatchery Burbot released into an ecosystem altered by hydropower operations. Stocked juveniles matured in a warmer post-dam regime, and these river-adapted hatchery fish also volitionally spawned at different temperatures (2, 4 and 6°C) and maternal ages (3, 5, 6 and 7+ year-olds) following relocation to a broodstock facility. Adults exhibited rate functions (i.e., size- and age-at-maturity) and relative weights similar to other wild populations (Clemens 1951; Chen 1969; Bailey 1972; Muth and Smith 1974; Evenson 1990; Fisher et al. 1996; Arndt and Hutchinson 2000; Bonar et al. 2000; Katzman and Zale 2000; McPhail and Paragamian 2000; Cott et al. 2013a). Percent weight loss of both sexes during the spawning period was comparable to reports from wild fish (Kainz and Gollmann 1996; Arndt and Hutchinson 2000). Live eggs were homogeneous with respect to yolk transparency and oil globule polarization—two critical ova maturation criteria for Burbot (Foltz et al. 2012). Egg size and embryo survival to the eyed stage was also within reported ranges from other investigations (Jäger 1981; Kainz and Gollmann 1996; Kujawa et al. 1999; Vught et al. 2008; Taylor and McPhail 2000; Żarski et al. 2010; Lahnsteiner et al. 2012). Overall, there was strong evidence of reproductive potential in a hatchery population of Burbot inhabiting the highly altered ecosystem of the lower Kootenai River.

A spawning temperature of 2°C delayed spawning time and extended the spawning period for adult Burbot; additionally, 2°C supported the lowest deformity and highest survival of embryos to the eyed stage. Broodstock held at 4 and 6°C also spawned, but the average spawning time and overall spawning period were shortened compared to adults held at 2°C. Egg incubation at 4 and 6°C caused moderate and high embryo mortality, respectively. Survival of normal embryos incubating at a constant 5°C was estimated to be very poor based on regression modeling. This finding is particularly important because temperate spring waters, deep reservoir discharges and profundal zones of deep lakes can be a near-constant 5°C in winter (Taylor and McPhail 2000; Paragamian and Wakkinen 2008; Donner and Eckmann 2011; Harrison et al. 2016a; Blabolil et al. 2018). Similar observations of temperature-dependent spawning time, embryo deformity and survival have been observed in other wild Burbot populations. Żarski et al. (2010) observed a 17-d spawning period at < 2°C, but only 10 days of spawning occurred in a warming episode from 2→7°C; the quality of eggs released at higher temperatures appeared normal, but embryo mortality was 100%. Taylor and McPhail (2000) tested three egg incubation profiles (3, 4 and 5°C), and observed hatch survivals of 44−71, 28−35 and 5−14%, respectively. These survival ranges are within the regression model estimates from the present study. Other researchers observed higher embryo survival at 6°C (26−45%); however, eggs were fertilized at lower temperatures (1.2−3.8°C) and their experiments did not account for thermal tolerances of zygotes (Lahnsteiner et al. 2012; Vught et al. 2008). In the present study, 4 and 6°C did not inhibit spawning, but further synchronized gamete release to coincide with suboptimal thermal conditions for normal embryo development. Recruitment failures among Burbot populations affected by impoundments and/or climate change could be tied to limited access to spawning habitats that do not have ice-cover in mid-winter and are consistently warmer than 4°C.

The approach of winter stimulates reproduction in many cold-water fish species (Shuter et al. 2012), and the autumn−winter period is critical for adult Burbot growth and maturation (Chen 1969; Pulliainen and Korhonen 1990; Mustonen et al. 2002; Cott et al. 2013b). Concerns exist that elevated river temperatures from October−March due to impoundment, hydropower, flood management and climate change act as bottlenecks to Burbot reproduction (Paragamian and Wakkinen 2008; Harrison et al. 2016a). Prior to our sampling of broodstock in 2017 and 2018, the lower Kootenai River averaged 3−6 ºC higher than pre-dam levels between November−December. Temperatures also fluctuated between 2−5°C during the pre-experiment period; in contrast, the river was often ice-covered and below 1°C during the monitored pre-dam era (Paragamian and Wakkinen 2008). Despite major autumn−winter thermal deviations from natural conditions, the river-adapted hatchery broodstock released viable gametes from early-February to early-March, which overlaps with the anecdotal spawning period of wild fish prior to installation of Libby Dam (Partridge 1983;

Paragamian et al. 2000). Although the extent of broodstock exposure to mainstem river temperatures prior to capture was unknown, recent telemetry work suggests that most adults reside in the river year-round (S. M. Stephenson, British Columbia Ministry of Forests, Lands, Natural Resource Operations and Rural Development [BCMFLNRORD], personal communication). Żarski et al. (2010) observed successful reproduction of Burbot immediately after abrupt cooling from month-long temperatures of 6−7°C. Their findings support the results herein, which show that a warmer post-dam regime between autumn−winter is unlikely to prevent functional gametogenesis in the lower Kootenai River hatchery Burbot population.

A positive correlation exists between temperature and gametogenesis in several gadids (Kjesbu 1994; Wright and Trippel 2009; Kjesbu 2010); therefore, a warmer autumn−winter regime could lead to earlier spawning of Burbot. However, data from the present study does not support this inference. Hatchery adults recaptured from the warmer post-dam regime spawned in mid-February, coinciding with the spawning time of the donor population inhabiting Moyie Lake, which is often ice-covered and colder in mid-winter (S. M. Stephenson, BCMFLNRORD, personal communication). Further research is needed to 1) determine if elevated pre-spawning temperature alters Burbot spawning time, and 2) establish a thermal threshold(s) for inhibition of gametogenesis, final maturation, and spawning. Testing the effects of constant 2, 4 and 6°C from December−March on adult gonad development could elucidate broader implications for maturation and gamete quality in the context of climate warming and altered ecosystems.

The recapture of river-adapted hatchery fish of known year-class enabled tests of maternal age effects, which were found to significantly impact spawning time, but not embryo deformity or survival up to the eyed-stage. On average, young (age-3) and old (age-7+) females spawned later than 5- and 6-year-olds. Unlike the other tested age groups, 100% of the age-5 females spawned in this study. Individuals entering their second or third year of maturity may be the most consist spawners in this population. Arndt and Hutchinson (2000) observed a one-week delay in a wild Burbot spawning run within a spring-fed tributary, and this coincided with an increased proportion of younger, smaller individuals. It is also common for a minor percentage of adults to skip spawning, or to show low body condition throughout the spawning season (Evenson et al. 1990; Pulliainen and Korhonen 1993; Cott et al. 2013a). Similar patterns of delayed or skipped spawning among youngest and oldest adults, and more consistent reproduction from middle-aged individuals has been reported in other marine gadids (Hutchings and Meyers 1993; Trippel 1998; Wieland et al. 2000; Scott et al. 2006; Wright and Gibb 2005; Wright and Trippel 2009; Skjæraasen et al. 2012).

For many species of fish, different age cohorts spawn at different times and locations within a system (Berkeley et al. 2004; Hixon et al. 2013). Expansion of the spawning period can reduce the

possibility for mismatch between ecosystem productivity and juvenile fish recruitment (Scott et al. 2006). In Atlantic cod, truncation of year class diversity shortens the spawning period and reduces the likelihood of spatiotemporal matching between birth date, embryo hatch, larval first-feeding, and peak zooplankton abundance (Hutchings and Meyers 1993). The early life history characteristics of Burbot are rooted in marine gadoid ancestry (Teletchea et al. 2009), and future conservation efforts should consider the implications of preserving or restoring population age structure in the context of match−mismatch recruitment modeling. Year class strength of more heavily studied temperate freshwater fishes (e.g., Yellow perch *Perca flavescence*; Northern pike *Esox lucius*; Walleye *Sander vitreus*) is oftentimes driven by match−mismatch dynamics impacting early life stages (Jolley et al. 2010; Hühn et al. 2014; Boehm et al. 2016; Dembkowski et al. 2017).

It was determined that age-3 females produced smaller eggs than older conspecifics, but this had no detectable effect on embryo deformity or survival to the eyed-stage. Kainz and Gollmann (1996) also observed a reduction in egg size among smaller female Burbot. In other marine gadids, a positive correlation exists between egg diameter and maternal age or size; additionally, these broodstock factors can affect egg quality, hatching success and larvae size (Chambers and Waiwood 1996; Trippel 1998; Wright and Gibb 2005). Similar relationships may exist between broodstock and early life stages of Burbot but were not detected by the present experimental design because it excluded the smallest, least robust adults, and did not include evaluations of larvae. Further research on the topic of maternal effects on offspring fitness is needed for Burbot.

## 2.5. Conclusions

This study found that stocked juvenile Burbot matured in the highly altered ecosystem of the lower Kootenai River, and became reproductive broodstock for the Twin Rivers Hatchery. There were no indications that the warmer post-dam regime impaired functional gametogenesis in the sampled population. Adults volitionally released gametes at 2, 4 and 6°C, but elevated water temperatures did contract the spawning period and reduced survival of normal embryos. Predictions of severe embryo deformity and mortality at temperatures constantly above 4°C underscore a possible scenario for recruitment failure. A diversity of maternal age-groups ranging from 3 to 7+ year-olds was important to expanding the spawning period. Many factors influence recruitment in any given system but defining the abiotic and biotic factors affecting reproduction is a crucial step toward managing a fish population. Burbot conservation aquaculture programs are not yet widespread but results from this study demonstrate the efficacy of supplementation as a management tool for gaining insights about ecophysiology while rebuilding an imperiled or extirpated stock.

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# Chapter 3: Effects of Temperature Fluctuation on Burbot Embryos: Implications of Hydropower and Climate Change

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## Abstract

Burbot *Lota lota* populations across the Holarctic ecozone are facing imperilment from environmental warming, particularly in ecosystems impacted by hydropower and climate change. We conducted a series of laboratory experiments to evaluate tolerance of Burbot embryos to various thermal regimes. Both cyclical and constant warming between 4.0−5.5°C during the initial 25 °C·d of embryo development led to significant deformity and mortality of protolarvae, with faster rates of temperature increase (i.e., 0.4°C per day) causing greater harm than gradual rates of warming. Random family effects interacted with incubation temperature of embryos to account for significant variability in deformity and survival of protolarvae. These findings have management implications for identifying and/or augmenting the thermal fitness of populations in modified ecosystems, particularly in Burbot populations where aquaculture production supports abundance. A model of hatching time based on degree-days of embryo development was also developed to improve predictions of natural emergence, peak larvae abundance, and optimal timing for larvae stocking. It is recommended that hydropower operations avoid thermopeaking during winter spawning of Burbot, especially if reservoir discharges exceed 4°C. Maintaining water temperatures below 3°C for at least the first two weeks of embryogenesis is critical for Burbot reproduction and underscores the importance of ice-covered spawning habitats. Global climate trends are expected to exacerbate warming in rivers, and many challenges lie ahead for conservation efforts seeking to mitigate the risks of Burbot extirpation.

## 3.1. Introduction

Water temperature is a critical factor in fish ecophysiology (Fry 1971; Brett 1979), and effective fisheries management requires understanding how thermal alterations impact fish and their ecosystems (Caissie 2006; Olden and Naiman 2010). Discharge of water from deep, large reservoirs often causes abnormal warming of downstream flows in winter, which can result in the loss of natural ice cover in southern boreal rivers impounded by hydroelectric dams (Caissie 2006; Paragamian and Wakkinen 2008). Abrupt changes in hydropower discharge is commonly known as "hydropeaking," and can lead to rapid spikes in downstream temperatures or "thermopeaking" in winter (Zolezzi et al. 2011). As river impoundment and climate warming exacerbate the problem of displaced ice

phenology in modified ecosystems, the risks of local extinction among winter-specialist species in Holarctic regions is predicted to increase along southern zoographic boundaries (Shuter et al. 2012; Coulter et al. 2014).

One of the most extreme examples of a winter specialist among freshwater fish is the Burbot *Lota lota* (Gadidae), which typically spawns December−March in spawning aggregations under the ice (McPhail and Paragamian 2000; Shuter et al. 2012). The occurrence of Burbot in lowland waters increases its vulnerability to anthropogenic alterations (Walters et al. 2018)—particularly changes in water temperature (Pandakov et al. 2020). Warming of some freshwater ecosystems has been linked to population declines along the southern extent of the species' distribution (Stapanian et al. 2010). Harrison et al. (2016) reviewed the topic of hydropower impacts on Burbot and surmised that post-dam thermal regimes were a paramount threat to riverine populations, and that more research is needed to better define thermal requirements for Burbot reproduction in environments altered by dams and climate change.

The earliest embryonic stages of fish often exhibit greater sensitivity to thermal stress than later life stages (Brooks et al. 1997; Puvanendran et al. 2015). Underlying physiology has been linked to temperature-sensitive molecular processes involving cellular migration, differentiation, and proliferation which occur simultaneously, but at different rates during the blastula and gastrula stages (Skjærven et al. 2011; Bobe 2015). Consequently, adult fish often spawn at temperatures that align with optimal thermal conditions for early embryogenesis (Pörtner and Peck 2010; Hu et al. 2017). Żarski et al. (2010) reported that Burbot can spawn at water temperatures ranging from 0.0−7.0°C; however, 6.0°C represents the upper lethal limit for early-stage embryos of some North American (Taylor and McPhail 2000; Ashton et al. 2019) and Eurasian populations (Kujawa et al. 1999; Vught et al. 2008). It has been theorized that variable and elevated incubation temperatures diminish Burbot recruitment via higher mortality in embryos (Taylor and McPhail 2000; Żarski et al. 2010).

Limited information exists regarding the effects of temperature variability on small fish embryos in cold water, as most research has focused on discrete incubation temperatures (Puvanendran et al. 2015; Lim et al. 2017). Embryos from other coolwater or warmwater species have demonstrated resilience to temperature changes due to seasonal weather or human disturbances (Schneider et al. 2002; Landsman et al. 2011); however, the narrow thermal tolerance of embryos from coldwater species likely makes them more vulnerable to temperature fluctuations (Thome et al. 2016; Lim et al. 2017). Prior research on thermal tolerances of Burbot embryos has focused on hatchery incubation regimes with constant temperatures (Bjorn et al. 1940; Jäger et al. 1981; Taylor and McPhail 2000; Ashton et al. 2019), or in some cases abrupt temperature changes (i.e., no acclimation) (Kujawa et al. 1999; Vught et al. 2008; Lahnsteiner et al. 2012). There have been

general observations of Burbot embryo survival under fluctuating thermal conditions (Kainz and Gollmann 1996; Żarski et al. 2010; Polinski et al. 2010), but these studies did not include empirical tests of incubation temperature.

The lower Kootenai River (spelled Kootenay in Canada) located in the transboundary region of northern Idaho and southeastern British Columbia provides a case study for the effects of post-dam thermal regimes on Burbot reproduction (Ashton et al. 2019). This river once supported a large endemic population and fishery that eventually collapsed after numerous anthropogenic ecosystem alterations; natural recruitment has been low since completion of Libby Dam in 1972 (Hardy et al. 2015), and contemporary abundance mostly consists of river-adapted hatchery fish (Ross et al. 2020). The river has mostly lost its natural winter ice cover, resulting in elevated and variable water temperatures in main-stem spawning habitats of Burbot (Paragamian and Wakkinen 2008; Ashton et al. 2019). The post-dam thermal regime between mid- to late-winter typically fluctuates around an average of 3.5°C with a range of 0.0−5.0°C. Evidence of survival to adulthood among hatchery Burbot released into the river as 3-month-old larvae implies that a recruitment bottleneck(s) is occurring sometime between spawning and larvae metamorphosis. An empirical determination of Burbot embryo tolerance to temperature fluctuation could assist investigations of hydropower and climate change impacts on reproduction and recruitment in the lower Kootenai River and other Holarctic ecosystems.

Our experimental objectives were to test the effects of elevated and/or fluctuating incubation temperatures on 1) timing and percentage of hatched Burbot embryos, 2) early-protolarvae deformity and survival, and 3) late-protolarvae swim bladder inflation and survival. The overarching goal of this study was to further elucidate causative factors that potentially underlie recruitment failure of Burbot in the Kootenai River and other systems experiencing similar extirpation risks.

## 3.2. Methods

### 3.2.1. Broodstock and Egg Collection

Burbot eggs were obtained from a donor population of wild adults ($F_0$ generation) inhabiting Moyie Lake (British Columbia) during the third week of February in 2016, 2017, and 2018. Procedures for broodstock capture, egg fertilization, water hardening, and disinfection have been described by Neufeld et al. (2011), with the exception that eggs were not disinfected with iodophor in 2018 due to concerns that it increases egg mortality. Five different families (one female and male parent per family) were spawned each study year. Approximately 20 mL of eggs were randomly subsampled from each family for incubation experiments. Eggs were fertilized at ~1.5°C in 2016 and 2017, and at 2.0 and 4.0°C in 2018. Additional Burbot eggs were obtained on March 5, 2018, from

hatchery adults ($F_1$ generation) caught in the lower Kootenai River and held at the Kootenai Tribe of Idaho's Twin Rivers Hatchery (Moyie Springs, Idaho). Details of river-adapted broodstock capture and communal holding have been described by Ashton et al. (2019). Adult Burbot were observed volitionally spawning in the holding tank at 4.0°C and eggs were collected from the tank bottom using a siphon.

*3.2.2. Egg Transport and Incubation*

Water hardened eggs from Moyie Lake and Twin Rivers Hatchery were transported in oxygenated 2-L thermoses to the University of Idaho Aquaculture Research Institute (UI−ARI; Moscow, Idaho). Each family of eggs from Moyie Lake had a separate thermos, whereas eggs from Twin Rivers Hatchery were pooled as mixed families. Eggs were kept at ± 0.5°C of fertilization temperature during 6−12 hours of transit. In 2016 and 2017, each thermos was gently poured into separate egg incubators consisting of a fluidized 1-L Imhoff cone (see Jensen et al. 2008). The eggs were incubated overnight at 2.0−3.0°C with 0.3 L/min water flow per incubator using a municipal water source (pretreated with activated carbon filtration, sodium thiosulfate dechlorination, and aeration). In 2018, eggs in each thermos were allowed 60 minutes for acclimation to 4.0°C upon arriving at UI−ARI, then gently poured into separate incubators, each consisting of a polyvinyl chloride tube (18 mm inside diameter × 76 mm long) with 400-µm nylon mesh sealed to the bottom. The incubator tubes were attached to a purpose-built apparatus with a timer-controlled, pneumatic-driven actuator that slowly raised and lowered the submerged eggs by 40 mm every 3 minutes in a 4-L water bath supplied with 0.3 L/min pretreated municipal water. A 200-Watt submersible heater wired to a temperature controller (Willhi® WH1436, Shenzhen Willhi Electronics Company, Shenzhen, Guangdong, China) maintained the water bath overnight at 4.0°C. Temperature data loggers (Elitech® RC-5, Elitech Technology Incorporated, Milpitas, California) waterproofed in sealed polyethylene bags provided a thermographic record during incubation (see Figure 3.0.1A for an illustration of the egg incubation apparatus). All controllers and data loggers were calibrated to 0°C using an ice-water slurry with readings confirmed by a mercury thermometer and two digital meters (YSI® 30 and 55 meters with probes, YSI Incorporated, Yellow Springs, Ohio).

Following overnight acclimation in all study years, a 3-mL polyethylene transfer pipet (narrow tip removed) was used to randomly subsample and stock eggs into incubator tubes within replicate blocks of the incubation array. Each subsample consisted of ~0.4 mL of eggs, which was transferred to a labeled well within a polystyrene 12-well cell culture plate (Corning®, Corning Incorporated, Corning, New York). The flat-bottom well plates were digitally scanned for egg enumeration (HP® Scanjet 8200, Hewlett-Packard Incorporated, Palo Alto, California); afterwards,

eggs from each well were pipetted into a labeled incubator tube in the array. Throughout embryo incubation, hydrogen peroxide was dosed into the pretreated municipal water supply via peristaltic pump at 300−400 mg/L for 15 minutes as a daily prophylactic treatment against fungal infection (Polinski et al. 2010). Overhead fluorescent lighting (daylight spectrum) with a timer-controlled photoperiod set for Moscow (Idaho) provided a light intensity of 800−1,200 lx at the top of the incubator tubes (MW700 portable lux meter, Milwaukee Instruments, Rocky Mount, North Carolina).
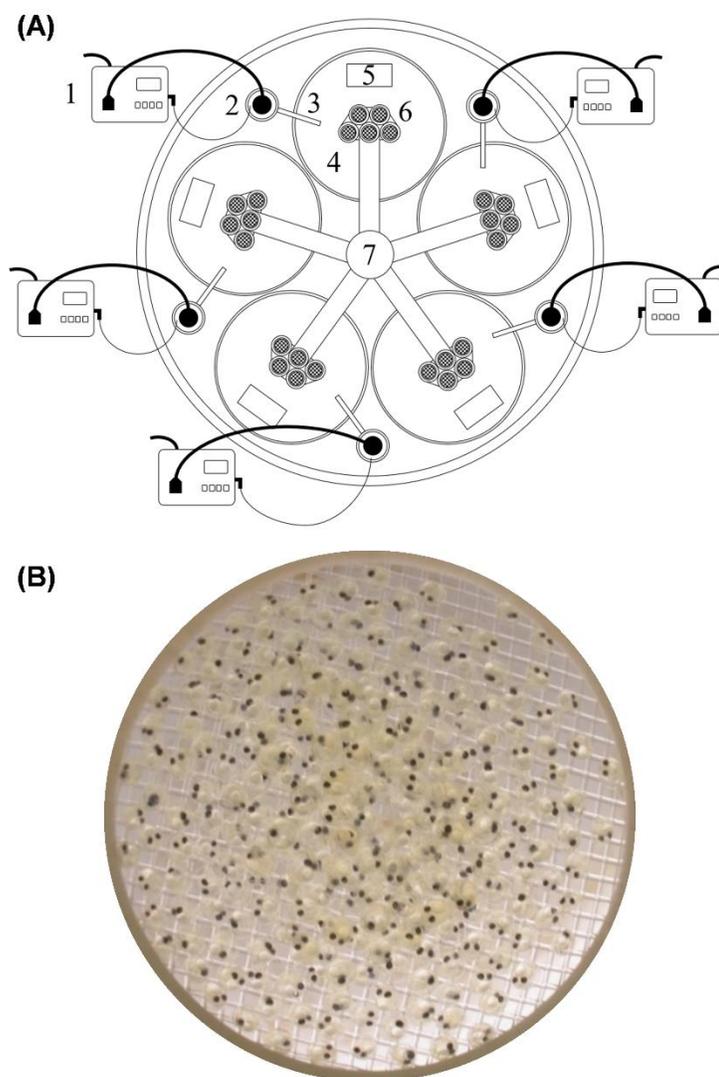


**Figure 3.0.1.** A top-view illustration of a single replicate block from the incubation array used to rear Burbot embryos (panel A). Number labels coincide with the following equipment descriptions: temperature controller (1), heat exchanger (2), water inlet (3), water bath (4), temperature data logger (5), incubator tubes (6), and pneumatic-driven actuator (7). Panel B shows an enlarged image of embryos rearing in an incuabtor tube.

*3.2.3. Experimental Design and Temperature Treatments*

Studies with Moyie Lake eggs included five temperature profiles and five families replicated across four blocks. Experiments were conducted with a split-plot design with randomized whole-plot and sub-plot units; the water baths were temperature treatment whole-plots and the incubator tubes were family sub-plots. A more simplified experimental design of 4−5 replicate incubator tubes per water bath was used for Kootenai River eggs sourced from a mixture of $F_1$ parents. Thermal treatments in the UI−ARI incubation array were selected based on pre-dam and post-dam thermographs recorded from the main channel of the lower Kootenai River (Figure 3.0.2). Temperature set points were manually inputted into the controllers and adjusted by 0.2−0.5°C increments. The first experiment in 2016 evaluated profiles simulating pre-dam conditions (T1), average trends for cold (T2) and warm (T4) post-dam regimes, and thermal fluxes around those trends (T3 and T5, respectively; Table 3.0.1). The coldest treatment (T1) exposed embryos to water temperatures <3°C until 35 days post-fertilization (dpf); other treatments (T2−T5) began to warm at 5 dpf (Figure 3.0.3A). Thermal amplitudes, cyclical frequencies, and rates of warming/cooling for T3 and T5 were extrapolated from river thermographs with moderate to high thermal flux in March of 2004 and 2013, respectively (U.S. Army Corps of Engineers, BFEI stream site station, Bonners Ferry, Idaho). At the time of the experiment, the best estimate of peak spawning time of river-adapted hatchery Burbot in the lower Kootenai River was early-March (Hardy et al. 2015).

A follow-up experiment in 2017 specifically tested the effects of warming on early embryogenesis (Table 3.0.1.). For the experiment, all fertilization occurred at 1.5°C. A simulated pre-dam profile maintaining incubation temperatures below 3°C until 21 dpf (T6) was compared against other profiles (T7−T10) with different rates of warming (i.e., 1.5→5.5°C) over a period ranging from 1−10 dpf (Figure 3.0.3B). The slowest rate of warming (T7) simulated a 0.5°C per day change often observed in the main channel of the Kootenai River; the fastest rate of warming (T10) simulated a thermal scenario where eggs were spawned in a tributary under the ice but drifted downstream into a main-stem temperature of 5.5°C.

The final experiment in 2018 tested the combined effects of elevated fertilization temperature (4°C) and thermal fluctuations on embryo development (Table 3.0.1). Embryos experienced profiles with rapid cooling (T11), thermal cycles in antiphase (T12 and T13), gradual warming (T14), and rapid warming (T15) (Figure 3.0.3C). Eggs were also fertilized at 2°C to provide control treatments for the T11 and T14 profiles. Embryos sourced from $F_1$ broodstock from the lower Kootenai River experienced the same temperature treatments, except there were no comparisons with 2°C fertilization because volitional spawning at the hatchery occurred at 4°C.

*3.2.4. Larval Rearing and Data Collection*

Sampling at hatch involved gently rinsing embryos and early-protolarvae from each incubator tube into a 200-mL polystyrene weigh boat, which was then placed on an illuminated base for overhead imaging (Canon® PowerShot SX620 HS 20.2 megapixel digital camera, Canon Incorporated, Melville, New York). The contents of the weigh boat were then carefully poured into a 3-L nursery tank (Aquatic Habitats®, MBK Installations Limited, Calverton, United Kingdom) partially filled with 1-L of pretreated municipal water with light aeration for further observations of larvae development. Incubator tubes from each temperature-controlled bath were pooled after hatch. Sampling of early- and late- protolarvae for all study years occurred between 186−215 and 244−304 °C·d, respectively. Nursery tank temperatures were increased to 8°C for more comparable observations of swim bladder inflation in 2017 and 2018. Dead eggs and larvae in the tanks were removed every other day with a transfer pipette. All experiments were terminated after sampling of late-protolarvae and involved similar sampling methods used at hatch. High resolution images from sampling were then analyzed for counts of alive, hatched, and deformed individuals using image analysis software (ImageJ, version 1.50b; National Institutes of Health, Bethesda, Maryland). Deformity was defined by visible malformation of the eyes, head, or vertebrae. Swim bladder inflation was determined by presence of a gas bubble in the bladder.

*3.2.5. Data Analyses*

Pre-dam and post-dam temperatures were compared over a time series using a log-linear repeated measures regression model (gaussian distribution and log link) with autoregressive covariance structure. Tested response variables in 2016, 2017, and 2018 experiments included percent hatch, deformity, survival, and swim bladder inflation. Logistic regression model analyses (binomial distribution and logit link) were performed on all percentile data with temperature as a fixed effect. Analyses included a blocking factor when it improved model fit. The number of embryos stocked per incubator was not a significant covariate in any model building efforts and was excluded from all analyses. Incubator, family, and the interaction of temperature with family were considered random effects in analyses involving eggs from Moyie Lake. An a priori assumption was that broodstock from Moyie Lake were random individuals from the donor population; additionally, each clutch of eggs within an incubator tube was a random subsample of the fertilized egg mass, and each subsample was randomly assigned to the incubation array within each temperature treatment. No random effects were included in analyses of eggs sourced from Kootenai River broodstock because the simplified experimental design did not segregate families or replicate water baths. The relationship between average incubation temperature ($T_i$) and time to first hatch (expressed as days

postfertilization [$Y_{dpf}$] and degree-days of development [$Y_{dd}$]) for pooled study observations were best fit with logarithmic regression equations:

$$Y_{dpf} = -24.90 \cdot \log_e(T_i) + 71.34$$

$$Y_{dd} = 49.56 \cdot \log_e(T_i) + 81.47$$

All models in this study were validated with likelihood-ratio tests and homoscedastic scatterplots of residuals versus fitted estimates. Instances of overdispersion were adjusted with a Pearson scale parameter. Type I error ($\alpha$) was defined at 0.05 for all statistical tests. Tukey's honestly significant difference post hoc tests were performed to control the error rate in multiple pairwise comparisons between categorical treatments. Wald chi-square test statistics ($X^2$) are presented with degrees of freedom in subscripts. All statistical analyses were performed in SAS version 9.4 (PROC GLIMMIX; SAS Institute, Cary, North Carolina).

## 3.3. Results

### 3.3.1. Pre-dam versus Post-dam Regimes

A comparison of thermographs from pre-dam and post-dam regimes of the lower Kootenai River illustrates the impacts of hydropower on downstream temperatures during winter. February temperatures in the main channel were consistently <2.5°C during seven pre-dam years (1964−1972; Figure 3.0.2A), whereas February temperatures during seven recent post-dam years (2010−2016; Figure 3.0.2B) fluctuated more and were mostly >2.5°C (except for 2014). In some post-dam years, river temperatures exceeded 4.5°C for several days in February with fluctuations of ± 1.0°C or more at rates of approximately 0.5°C per day. A regression analysis comparing daily average main-stem temperatures revealed differences between pre-dam and post-dam regimes from February to mid-March ($X^2_{1,\,911} = 606.33$, $P < 0.01$; Figure 3.0.2C). The illustrated thermographs are more similar between March−April, except for greater fluctuation or cycles during post-dam years (Figures 3.0.2A and 2B). The expected mean temperature of the pre-dam regime showed a faster rate of warming by March 1st, and by early-April warming occurred at a rate four times greater than that of the post-dam regime (Figure 3.0.2C).

### 3.3.2. Study year 2016: Simulated Pre-dam and Post-dam Incubation Temperatures

The quality (e.g., survival up to hatch) of eggs sourced from Moyie Lake broodstock in 2016 was extremely variable between the five sampled families. For example, one group had 69% survival up to hatch, while another group in the same treatment only had 3% survival. Additionally, differences in deformity of larvae (2 to 53%) occurred among families. Four of the five families had poor embryo survival in all temperature treatments. The random effect of families predominated,
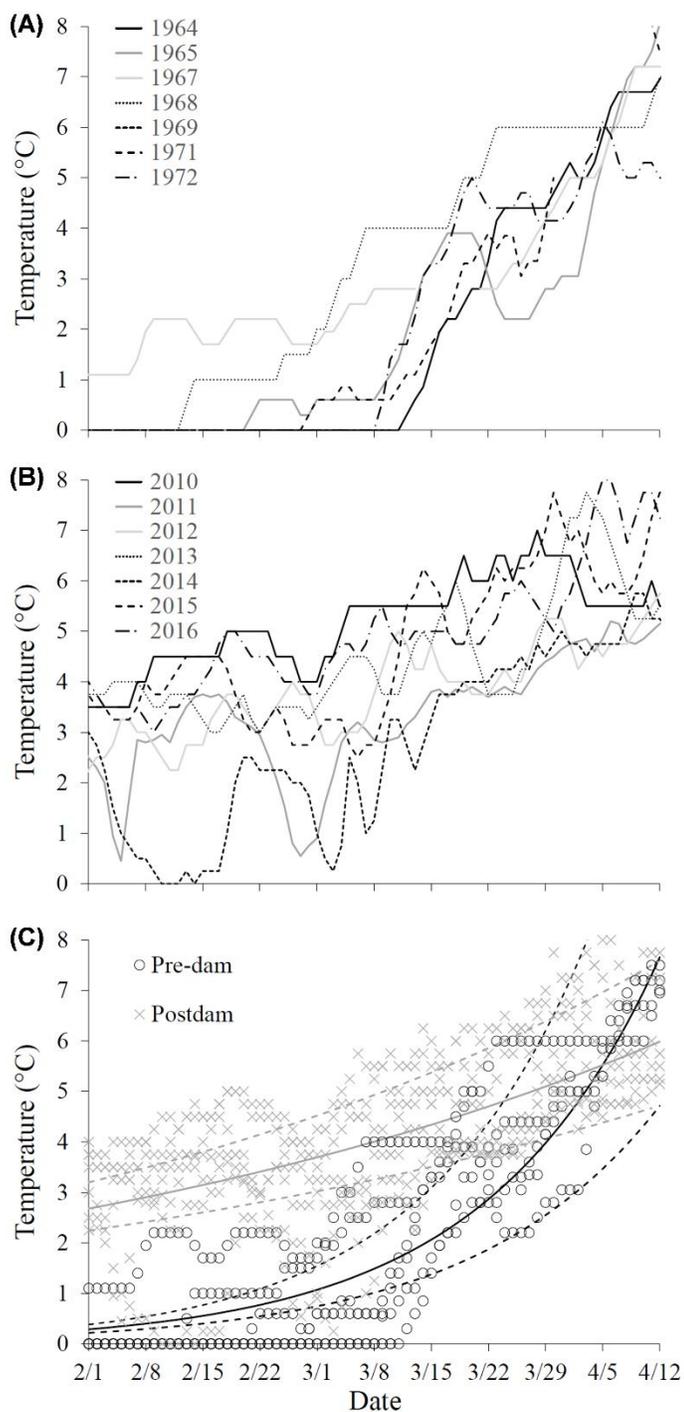
**Figure 3.0.2.** A comparison of average daily temperatures during winter−spring in the main channel of the lower Kootenai River for seven pre-dam years (1964−1972; panel A) and post-dam years (2010−2016; panel B). Panel C shows expected mean temperatures and 95% confidence intervals (solid and dashed lines, respectively) for pre-dam and post-dam regimes (black and grey lines, respectively). Temperature data were sourced from the U.S. Geological Survey (USGS station 12322000 at Porthill, Idaho). Line gaps in panel A indicate missing data. Temperatures recorded in 1969 were a constant 0°C between February 1−March 10 (later data missing). Temperatures recorded after March 20, 1972, were influenced by operations at Libby Dam (Libby, Montana).

accounting for 67−97% of the variance in analyses of hatching percentage ($H$), early-protolarvae deformity ($D$) and survival ($S$). Interactions between families and temperatures accounted for a small proportion of the observed variance (≤8%). The effect of random incubators or egg clutch samples within treatments was substantial in analyses of $H$, but not for $D$ or $S$ (26, 0, and 3% of variance, respectively).

Hatching percentage was highest in the cold incubation profile (T1) ($X^2_{4, 12} = 47.16$, $P < 0.01$; Table 3.0.1) despite similar degree-days of embryo development across treatments ($197 \pm 1$ °C·d; mean $\pm$ SE); however, embryos in T1 also experienced the longest incubation period (62 d) and highest hatching temperature (6.7°C) (Figure 3.0.3A). Embryo deformity was lower in the colder treatments (T1, T2, T3) ($X^2_{4, 12} = 79.56$, $P < 0.01$). Survival to the early-protolarvae stage was low (22 $\pm$ 1%) across treatments (Table 3.0.1). The highest percentage of larvae with inflated swim bladders ($SBI$) coincided with embryo incubation under gradual warming (T2) compared to moderate thermal flux (T3) or elevated temperatures (T4), and high thermal flux or spikes (T5) resulted in the lowest $SBI$ ($X^2_{4, 15} = 45.23$, $P < 0.01$). Survival of non-deformed larvae ($S_n$) at the late-protolarvae stage was higher when embryos incubated without thermal flux (T2, T4) as opposed to high flux (T5) ($X^2_{4, 12} = 16.71$, $P < 0.01$). Periodic temperature spikes to 4.7, 5.9 and 7.0°C occurring in T5 at respective embryo development rates of 10, 20 and 37 dpf (31, 78 and 163 °C·d; Figure 3.0.3A) resulted in a 31% decrease in $S_n$. More frequent but moderate thermal flux (T3) during embryo incubation had no detectable effect on $S_n$, but slightly diminished or delayed swim bladder inflation.

### 3.3.3. Study year 2017: Different Rates of Warming During Early Incubation

Random family effects accounted 54−91% of the variance in analysis of $H$, $D$, and $S$. A substantial proportion of $H$ and $S$ variance (40 and 34%, respectively) also came from interactions between families and temperatures. The effect of incubators was ≤12% of variance.

Faster rates of warming from 1.5→5.5°C during the first week of embryogenesis reduced $H$, $S$, $SBI$, and $S_n$, whereas the opposite trend was recorded for deformity (Table 3.0.1). Hatching percentage was highest in a cold incubation profile (T6) and lowest when embryos experienced moderate to abrupt warming early in development (T8, T9, T10) ($X^2_{4, 11} = 107.25$, $P < 0.01$). Early protolarvae deformity was lowest in T6 and highest in T9 and T10 ($X^2_{4, 11} = 323.16$, $P < 0.01$). Survival at hatch was higher when embryos were not exposed to rapid or abrupt warming in the first week of embryogenesis ($X^2_{4, 14} = 80.03$, $P < 0.01$). Late-stage protolarvae from T6 had the highest $SBI$; the lowest $SBI$ coincided with T9 and T10 ($X^2_{4, 14} = 502.70$, $P < 0.01$). Survival of non-deformed larvae was highest in T6 ($68 \pm 2$%) and lowest in T10 ($2 \pm 1$%). Overall, embryo exposure to

warming from 1.5→5.5°C between 1−5 dpf (3−20 °C·d) resulted in a 47−97% decrease in $S_n$ compared to incubating below 3°C up to 22 dpf (59 °C·d). Gradual warming (T7) of 0.4°C per day from 1.5→5.5°C by 9 dpf (39 °C·d) had no significant effect on $S_n$ but did reduce or delay swim bladder inflation.
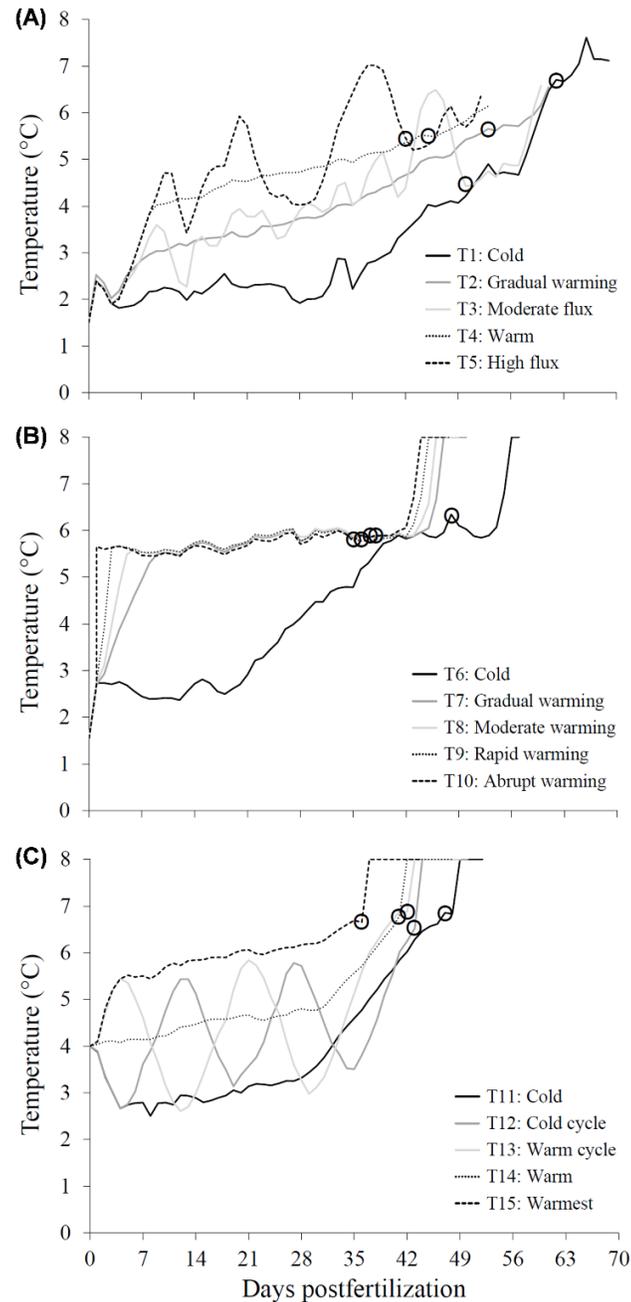


**Figure 3.0.3.** Daily average temperatures between egg fertilization and early-protolarvae sampling of Burbot for different incubation treatments (T1−T15) tested in 2016, 2017, and 2018 experiments (panels A, B, and C, respectively). Circles indicate sampling at hatch when early-protolarvae were enumerated and relocated to nursery tanks. Experiments were terminated after sampling late-protolarvae between 44−61 days postfertilization.

**Table 3.0.1.** A summary of measured responses for Burbot protolarvae after embryo rearing in replicate incubators (*n*) under different temperature treatments[a]. Means (SE in parentheses) of percent hatch (*H*), deformity (*D*), and survival (*S*) are shown for early-protolarvae; percent swim bladder inflation (*SBI*) and non-deformed survival ($S_n$) are shown for late-protolarvae. Lowercase letters next to column values indicate significant differences between treatments within a broodstock category and study year ($P \leq 0.05$).

| Year | Broodstock | Treatment | Incubator regime | *n* | *H* (%) | *D* (%) | *S* (%) | *SBI* (%) | $S_n$ (%) |
|------|-----------|-----------|------------------|-----|---------|---------|---------|-----------|-----------|
| 2016 | Moyie L. | $T1_{1.5}$ | Cold | 4 | 98 (1) y | 16 (5) y | 23 (5) | 79 (4) xy | 13 (3) yz |
| | | $T2_{1.5}$ | Gradual warming | 4 | 74 (5) z | 18 (5) y | 26 (5) | 86 (2) x | 14 (1) y |
| | | $T3_{1.5}$ | Moderate flux | 4 | 71 (6) z | 17 (4) y | 21 (5) | 75 (3) y | 11 (1) yz |
| | | $T4_{1.5}$ | Warm | 4 | 77 (4) z | 27 (6) z | 23 (5) | 74 (3) y | 14 (2) y |
| | | $T5_{1.5}$ | High flux | 4 | 74 (4) z | 32 (7) z | 19 (5) | 60 (2) z | 9 (1) z |
| 2017 | Moyie L. | $T6_{1.5}$ | Cold | 4 | 96 (1) w | 13 (4) w | 74 (3) y | 74 (1) w | 68 (2) w |
| | | $T7_{1.5}$ | Gradual warming | 4 | 95 (1) wx | 22 (6) x | 69 (3) y | 55 (3) x | 56 (4) w |
| | | $T8_{1.5}$ | Moderate warming | 4 | 86 (3) x | 33 (5) y | 54 (3) y | 31 (3) y | 37 (2) x |
| | | $T9_{1.5}$ | Rapid warming | 4 | 77 (4) y | 72 (5) z | 33 (4) z | 14 (3) z | 9 (1) y |
| | | $T10_{1.5}$ | Abrupt warming | 3 | 66 (4) z | 82 (6) z | 24 (3) z | 8 (1) z | 2 (1) z |
| 2018 | Moyie L. | $T11_{2.0}$ | Cold | 4 | 91 (3) w | 2 (0) y | 89 (2) x | 96 (1) v | 80 (2) v |
| | | $T11_{4.0}$ | Cold | 4 | 77 (3) yz | 3 (0) y | 82 (5) xy | 93 (2) vw | 72 (1) w |
| | | $T12_{4.0}$ | Cold cycle | 4 | 72 (4) z | 3 (0) y | 80 (5) xyz | 91 (1) wx | 69 (2) w |
| | | $T13_{4.0}$ | Warm cycle | 4 | 70 (5) z | 12 (5) z | 63 (8) yz | 54 (4) y | 47 (2) y |
| | | $T14_{2.0}$ | Warm | 4 | 89 (2) wx | 2 (0) y | 80 (4) xyz | 90 (1) wx | 63 (2) wx |
| | | $T14_{4.0}$ | Warm | 4 | 82 (4) xyz | 4 (0) y | 75 (7) xyz | 85 (2) x | 61 (3) x |
| | | $T15_{4.0}$ | Warmest | 4 | 85 (2) wxy | 13 (0) z | 60 (7) z | 24 (1) z | 34 (1) z |
| 2018 | Kootenai R. | $T11_{4.0}$ | Cold | 5 | 80 (1) x | 5 (1) w | 59 (3) y | 89 | 47 |
| | | $T12_{4.0}$ | Cold cycle | 5 | 70 (1) y | 7 (1) wx | 65 (2) x | 85 | 48 |
| | | $T13_{4.0}$ | Warm cycle | 5 | 58 (2) z | 21 (1) y | 56 (1) y | 61 | 37 |
| | | $T14_{4.0}$ | Warm | 5 | 66 (3) y | 12 (1) x | 64 (0) x | 61 | 42 |
| | | $T15_{4.0}$ | Warmest | 5 | 79 (2) x | 51 (4) z | 50 (1) z | 38 | 24 |

[a]Subscripts indicate egg fertilization temperature in °C

*3.3.4. Study year 2018: Different Phases of Temperature Change During Incubation*

Random family effects were again the major variance component (64−86%) in analyses of $H$, $D$, and $S$. Interactions between families and temperatures were a small proportion of the variance (11 and 14%, respectively) in models of $H$ and $D$, but were substantial (35%) in analyses of $S$. Random incubator effects were insignificant in analysis of $D$, and otherwise small (0 and 9% of the variance, respectively) in best fit models of $H$ and $S$.

Embryos exposed to thermal cycling (T12, T13) had slightly lower hatching percentages ($X^2_{6, 18}$ = 73.86, $P < 0.01$; Table 3.0.1). T13 and T15 shared a 0.4°C per day rate of warming from 4.0→5.4°C by 4 dpf (Figure 3.0.3C), which caused higher deformity in early protolarvae ($X^2_{6, 23}$ = 332.71, $P < 0.01$). Survival at hatch was highest when incubation temperatures did not warm above 4.1°C during the first week of embryogenesis (T11, T12, T14) ($X^2_{6, 21}$ = 26.30, $P < 0.01$). The coldest (T11) and warmest (T15) treatments had the highest and lowest incidence of *SBI*, respectively ($X^2_{6, 21}$ = 786.34, $P < 0.01$). T11 and T12 shared a 0.4°C per day rate of cooling from 4.0→2.7°C by 4 dpf (Figure 3.0.3C). T12 cycled through two thermopeaks to 5.4−5.8°C between 12−28 dpf while T11 remained <3.5°C, yet no difference was detected in $H$, $D$, $S$, *SBI* and $S_n$ between T11 and T12 (Table 3.0.1). Survival of non-deformed larvae was highest in T11 and T12, and lowest in T15 ($X^2_{6, 18}$ = 867.08, $P < 0.01$). Overall, moderate warming of 0.4°C per day from 4.0→5.4°C by 4 dpf (24 °C·d) resulted in a low $S_n$ (34−47%) in T13 and T15, whereas moderate cooling of 0.4°C per day from 4.0→2.7°C by 4 dpf (17 °C·d) led to a relatively high $S_n$ (69−72%) in T11 and T12.

Egg fertilization at 2°C (as opposed to 4°C) increased $H$ and $S_n$ in T11 but had no detectable effect in T14 (Table 3.0.1). Further analyses revealed that one family showed much higher survival in T11 and T14 when fertilization occurred at 2°C; this same family had 0% survival in T13 and T15, whereas the other four families had ≥60% survival (Figure 3.0.4).

The effects of T11−T15 treatments on embryos from river-adapted hatchery adults ($F_1$ broodstock from the Kootenai River) paralleled results for embryos from wild adults ($F_0$ broodstock from Moyie Lake) (Table 3.0.1). Hatching percentage was lowest in T12 (cold cycle) and T13 (warm cycle) ($X^2_{4, 20}$ = 147.95, $P < 0.01$), which reaffirmed that cyclical incubation temperatures can extend degree-days to hatching. Embryos exposed to early warming (T13, T15) had more deformity ($X^2_{4, 20}$ = 461.64, $P < 0.01$). Prolonged exposure of embryos to temperatures >5.5°C (T15) resulted in the lowest survival at hatch ($X^2_{4, 20}$ = 95.70, $P < 0.01$) and diminished *SBI* and $S_n$. Nursery tanks were not replicated for late stage protolarvae from Kootenai River broodstock, thus standard errors were not produced for *SBI* and $S_n$ in Table 3.0.1.
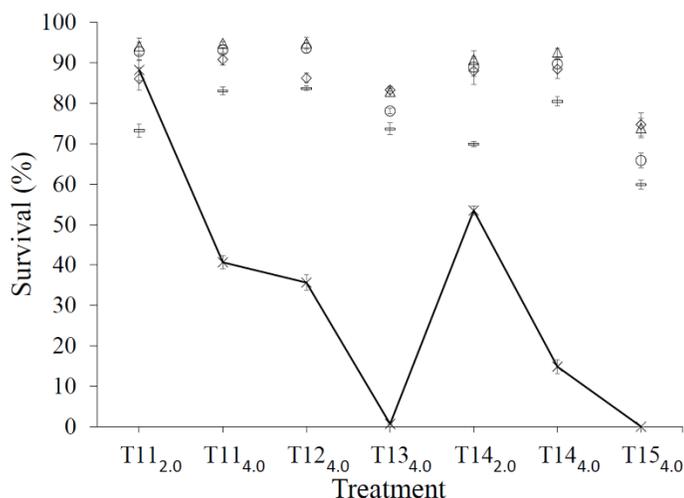
**Figure 3.0.4.** Survival of Burbot embryos from five unique families up to the early-protolarvae stage for different incubation temperature treatments in the 2018 experiment (T11−T15 defined in Figure 3C). Discrete symbols represent mean (±SE) embryo survival from replicate ($n = 4$) incubators for each family. The solid line emphasizes a single family of embryos with poor tolerance to elevated fertilization temperature (4°C; T11 and T14) and further warming by 4 days postfertilization (4.0→5.4°C; T13 and T15). Treatment subscripts on the abscissa indicate egg fertilization temperature in °C.

### 3.3.5. Timing of Embryo Hatch

Regressions analyses predict that warmer thermal regimes shorten the incubation period of Burbot embryos (Figure 3.0.5A; $X^2_{1, 58} = 464.84$, $P < 0.01$); however, incubation at higher temperatures requires more degree-days to first hatch (Figure 3.0.5B; $X^2_{1, 58} = 87.40$, $P < 0.01$). Extrapolating the $Y_{dpf}$ function to daily pre-dam temperature data recorded from the main stem of lower Kootenai River (Figure 3.0.2A) predicts that first hatch ranged from 41−53 dpf (Table 3.0.2). Records of spawning dates do not exist for pre-dam years, and it was assumed that peak spawning in the main channel occurred on February 15th of each year. A similar estimation for post-dam years predicts that first hatch ranged from 31−46 dpf. Main channel river temperatures associated with these predicted first hatch dates were 5.0−7.4°C and 3.8−6.3°C for pre-dam and post-dam regimes, respectively. Generally, Table 3.0.2 emphasizes the possibility that eggs spawned in mid-February during pre-dam years hatched on average later in the spring and at warmer temperatures; however, it also suggests that hatch time and main-stem temperatures could be similar for certain pre-dam and post-dam years (e.g., 1967 vs. 2014; 1968 vs. 2015).
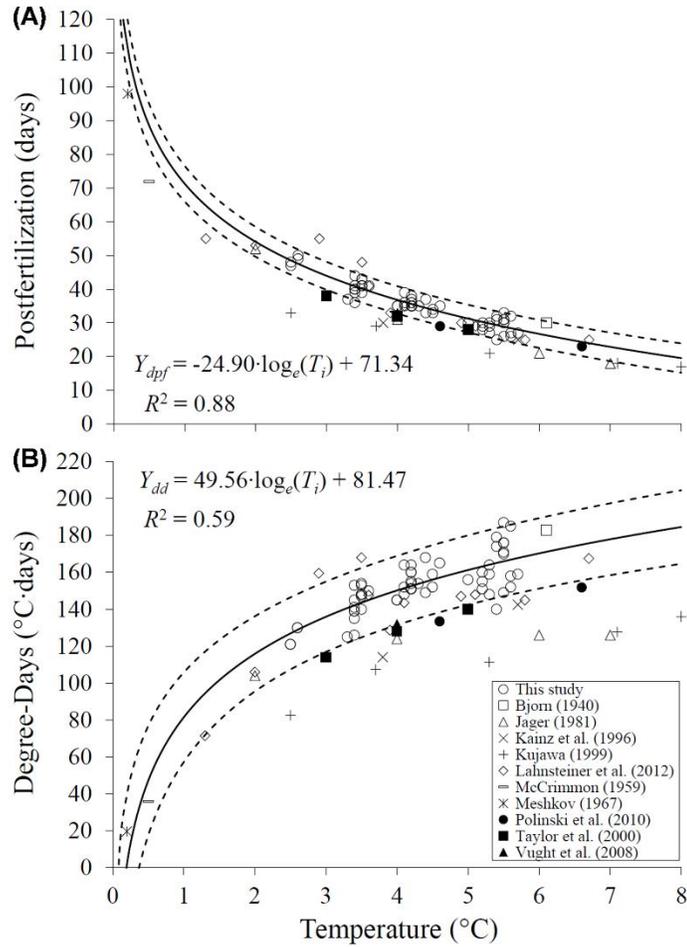
**Figure 3.0.5.** Burbot embryo development to first hatch expressed as days postfertilization (panel A) and degree-days (panel B) at different incubation temperatures. Expected means (solid lines) and 95% confidence intervals (dashed lines) are estimated from pooled observations of embryo hatching in 2016, 2017, and 2018 experiments (regression equations and coefficient of determination [$R^2$] are shown). $T_i$ represents average incubation temperature on the abscissa; $Y_{dpf}$ and $Y_{dd}$ are the expected means of days postfertilization and degree-days on the ordinate, respectively. Burbot embryo development to first hatch in other studies are shown for comparison.

**Table 3.0.2.** Estimates of Burbot embryo development rate to first hatch (expressed as days postfertilization [dpf], °C·d, and date [month-day]) in the main channel of the lower Kootenai River for pre-dam and post-dam years. $T_i$ is the cumulative average temperature of the river between the presumed spawning date (February 15th) until first hatch (predicted from logarithmic regression; see Figure 5). $T_h$ is the average daily temperature of the river at the time of predicted first hatch. Temperature data were sourced from the U.S. Geological Survey (USGS station 12322000 at Porthill, Idaho).

| River regime | Year | $T_i$ (°C) | $T_h$ (°C) | dpf | °C·d | Hatch date |
|---|---|---|---|---|---|---|
| Pre-dam | 1964 | 2.1 | 6.7 | 53 | 119 | 4-09 |
| | 1965 | 2.2 | 7.2 | 53 | 120 | 4-09 |
| | 1967 | 2.7 | 5.0 | 46 | 131 | 4-02 |
| | 1968 | 3.3 | 6.0 | 41 | 140 | 3-28 |
| | 1971 | 2.2 | 7.4 | 51 | 121 | 4-07 |
| | 1972 | 2.3 | 5.3 | 51 | 123 | 4-07 |
| | | | | | | |
| Post-dam | 2010 | 5.0 | 6.0 | 31 | 161 | 3-18 |
| | 2011 | 3.1 | 4.5 | 43 | 138 | 3-30 |
| | 2012 | 3.7 | 4.0 | 38 | 147 | 3-25 |
| | 2013 | 4.0 | 3.8 | 37 | 150 | 3-24 |
| | 2014 | 2.8 | 4.8 | 46 | 132 | 4-02 |
| | 2015 | 4.1 | 6.3 | 36 | 151 | 3-23 |
| | 2016 | 4.6 | 4.8 | 33 | 157 | 3-20 |
| | | | | | | |
| Pre-dam average | | 2.5 | 6.3 | 49 | 127 | 4-05 |
| Post-dam average | | 3.9 | 4.9 | 38 | 148 | 3-24 |

## 3.4. Discussion

### 3.4.1. Pre-dam Versus Post-dam Regimes and Embryo Thermal Tolerance

Thermographs from the main channel of the lower Kootenai River between 1964−2016 show that water temperatures downstream of Libby Dam in February are warmer and more variable than pre-dam conditions. Reservoir discharges between mid-March and mid-April also produce a river regime that typically warms more slowly in the spring than natural ice-off conditions. Similar trends have been reported in other northern-temperate rivers below large reservoirs impounded by hydroelectric dams (Caissie 2006; Olden and Naiman 2010). Regulation of rivers in winter is believed to have profound impacts on Burbot reproduction (Stapanian et al. 2010; Harrison et al. 2016; Ashton et al. 2019); concomitantly, our findings suggest that reservoir discharges responsible for lost ice cover and thermopeaking above 4°C potentially threaten normal development of early-stage embryos of Burbot.

Our evaluation of embryo tolerance to thermal fluctuations determined that water temperatures below 3.0°C between egg fertilization to 50 °C·d of development were preferable for incubation. Rapid warming from 4.0→5.5°C during the first week of embryogenesis led to significant deformity and mortality at hatch, whereas cooling from 4.0→2.7°C benefited survival of non-deformed protolarvae. At later stages of embryogenesis, gradual warming above 4.0°C and moderate

fluctuations between 4.0−7.0°C were tolerated without noticeable harm. These findings agree with other studies of Burbot embryo incubation that similarly observed poor tolerance to thermal stress during the earliest stages of development. Lahnsteiner et al. (2012) reported high survival of non-deformed embryos when water temperatures were ≤2.5°C until 25 °C·d, but then gradually peaked to 6.5°C between 35−56 °C·d. Conversely, warming before 35 °C·d of development has led to poor embryo viability and survival at hatch (Kujawa et al. 1999; Vught et al. 2008; Lahnsteiner et al. 2012). Żarski et al. (2010) recorded 100% embryo mortality when incubation temperatures increased from 2.0→5.5°C at 13 °C·d and fluctuated between 5.0−7.8°C two weeks. However, Polinski et al. (2010) documented protolarvae survival of 59−72% after thermal fluctuations between 3.5−4.5°C during the first week of development, followed by moderate warming to 5.0−5.5°C by 42−48 °C·d.

*3.4.2. Family Effects and Embryo Viability*

Random family effects were found to interact with incubation temperature of embryos to account for significant variability in deformity and survival of protolarvae. Family traits strongly influence egg quality in marine gadids (Dahlke et al. 2016), and it follows that similar trends might apply to Burbot. Dahlke et al. (2016) also determined that genetics underlie thermal reaction norms for embryo viability of Atlantic Cod *Gadus morhua*, which is important for adaptive responses to climate-driven warming. Tolerance of Burbot embryos to different temperatures could be an important indicator of a population's thermal fitness to modified ecosystems. Conservation aquaculture programs may need to manage the genetic diversity of broodstock in ways that account for changes in embryo thermal tolerance across different families. In nature, preservation of ice-covered spawning habitats could be critical for certain families—or possibly even populations—intolerant of elevated temperatures during spawning and embryo rearing.

Variability in embryo quality should be expected during hatchery production of Burbot, even with use of standard aquaculture techniques that include manual egg stripping, sperm motility checks prior to fertilization, and embryo incubation in clean, cold water. We observed substantial differences in embryo viability at cold temperatures across three consecutive study years with 15 different families. Similarly, this pattern has been routinely observed during hatchery operations in Idaho over the past two decades. Embryo viability could also be linked to non-genetic issues such as phenotypic traits (e.g. maternal body composition, ovary ripeness, egg quality, sperm quality, etc.) and physical factors (e.g. spawning procedures, transport conditions, water chemistry, etc.) that confound with random family effects.

*3.4.3. Temperature Effects on Embryo Development Rate*

Interestingly, Burbot embryos incubating at cold temperatures require fewer °C·d to hatch than embryos incubating at warm temperatures. For various fish species a decrease in embryo °C·d until emergence can occur at extremely low and high incubation temperatures (Hamel et al. 1997; Lim et al. 2017). The phenomenon has been attributed to thermal regimes that outlie the normal physiological range for a species (Neuheimer and Taggart 2007). However, results from our research and other studies show that °C·d to hatch decrease as incubation temperatures become more optimal for Burbot embryos (Taylor and McPhail 2000; Lahnsteiner et al. 2012). This pattern of embryonic development has also been observed in the winter-spawning Arctic Cod *Boreogadus saida* (Laurel et al. 2018). We also confirmed that more °C·d are required to hatch Burbot embryos incubating in fluctuating or elevated thermal regimes. This agrees with observations of embryo development in Walleye *Sander vitreus* (Allbaugh and Manz 1964; Piper et al. 1982), but opposite trends were reported with Northern Pike (Kuznetsov and Lukiyanov 2014), or no effect of temperature fluctuations on °C·d to emergence in Lake Whitefish (Lim et al. 2017).

Understanding the interaction between embryo development rate (°C·d) and water temperature is critical for predicting the hatch of larvae from an estimated peak spawning time. The timing of Burbot larvae hatch in relation to spatiotemporal patterns of winter adult spawning and spring zooplankton abundance is believed to be critically important for juvenile recruitment (Koporikov and Bogdanov 2019). Our model extrapolations of temperature-dependent embryo development to the main channel of the lower Kootenai River infer that an average post-dam thermal regime potentially advances embryo hatching time by 1−2 weeks to coincide with early-spring river temperatures that average 1−2°C cooler than pre-dam conditions. The impacts of spring discharge cooling on Burbot larvae are poorly understood. Prior research on temperature effects on feeding, growth, and survival of Burbot larvae has been limited to warmer conditions in hatcheries (i.e., ≥10°C; Barron et al. 2012). We recommend that future research of Burbot recruitment bottlenecks in impounded drainages focus on investigating the effects of cold, nutrient-poor waters on larval ecophysiology, particularly during the protolarvae transition to exogenous feed.

Use of our embryo development model in concert with spatiotemporal knowledge of adult spawning patterns could be an effective approach for predicting peak abundance of larvae and focusing efforts to monitor young-of-year production. Our predictions of pre-dam and post-dam hatch timing in the lower Kootenai River depend on an assumption that peak spawn timing (i.e., February) has remained consistent between the 1960s and 2000s. This assumption is supported by anecdotal observations (Kootenai Tribe of Idaho and Montana Fish, Wildlife and Parks 2004) and a preponderance of February spawning among various Burbot populations in North America and

Eurasia (Ashton et al. 2019). Brackwehr et al. (2016) theorized that timing of hatch should coincide with ice melt and higher water levels in lowland rivers to provide Burbot larvae access to nursery grounds in floodplain lakes and backwaters. Monitoring of pelagic larvae after ice-off in spring likely has major advantages over egg sampling in substrates during winter; concomitantly, there are more publications about field techniques for Burbot larvae sampling in lakes and rivers (Saunders et al. 2014; McCullough et al. 2015; Koporikov and Bogdanov 2019; Toivonen et al. 2020).

## 3.5. Conclusions

Our research suggests that a water temperature below 3°C is optimal for Burbot embryos until 50 °C·d of development, resulting in fewer deformities and higher survival of protolarvae. This finding emphasizes the importance of implementing management strategies that conserve cold spawning habitats for Burbot reproduction in altered, impounded, and natural ecosystems. During later stages of embryo development, we observed that embryos from $F_0$ (wild) and $F_1$ (hatchery) broodstock showed reasonable tolerance to gradual warming and moderate temperature fluctuations representative of a typical post-dam winter regime in the main channel of the lower Kootenai River. Consequently, biologists working in the Kootenai Basin now have greater confidence that contemporary hydropower operations at Libby Dam can—in some years—produce downstream temperatures supportive of Burbot embryo survival until emergence. However, thermal stress on embryos is likely a major bottleneck to survival during years when dam discharges routinely peak or stay above 4°C in February and early-March. Evidence of strong, family-dependent thermal tolerances among embryos underscores a further need to test broodstock traits (e.g., parental age) against different incubation temperatures to identify and/or augment the thermal fitness of the Burbot population in the Kootenai River. In a broader context, identifying any population-specific adaptations that either increase or decrease a stock's vulnerability to environmental warming is paramount to managing Burbot extirpation risks caused by river regulation and climate change. Global climate warming is predicted to cause more intense and frequent localized climactic shifts (IPCC 2018), and subsequent increases in the magnitude, frequency, and rate of warming between February−March could negatively impact Burbot survival in both regulated and unregulated rivers. Lastly, our model of temperature-dependent embryo development can be used to predict emergence and peak larvae abundance in natural systems, which should assist with juvenile recruitment monitoring. This model can also be used to estimate hatch timing under various incubation temperatures in a hatchery setting and improve predictions of optimal timing for larvae stocking. Ultimately, the efforts of resource managers to develop effective Burbot conservation strategies will greatly improve with increased knowledge of early-life-stage ecophysiology.

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# Chapter 4: Development of Burbot Larvae in Cold Regimes Linked to Hydropower Discharge

## Abstract

Hydroelectric dams operating in boreal rivers can cause downstream cooling in spring which potentially impacts nursery habitats of some imperiled Burbot *Lota lota* populations. The goal of this study was to elucidate recruitment implications from larval ecophysiology of Burbot in cold regimes associated with deep reservoir discharges and/or natural ice-off conditions. We found that waters <6.0°C present significant inefficiencies in larvae activity and growth during a critical period of development up to 28 days posthatch (dph). Most larvae showed signs of swim bladder inflation and horizontal swimming by 70 °C·dph (3.6−4.0 mm TL) when reared between 4.0−9.0°C. Larvae at 150 °C·dph (3.8 mm TL) foraged on rotifers between 3.9−9.3°C, but rearing at <6.0°C reduced feeding activity. At 250 °C·dph (4.3 mm TL), waters from 3.9−5.5°C caused a major reduction in feeding on larger, faster prey like *Artemia* nauplii compared to 9.3−11.0°C. Larvae reared at <7.5°C until 28 dph failed to gain weight, and those reared at <6.0°C showed weight loss despite high rotifer feeding incidence and survival. We concluded that cold regimes 1) prolong the resting behavior of protolarvae, 2) delay swim bladder inflation, 3) reduce feeding activity, 4) slow growth, and 5) diminish larvae performance. Altogether, these outcomes in nature could lead to cumulative mortality that jeopardizes recruitment. A preflexion larvae size of 4.6 mm TL was suggested as a developmental threshold coinciding with starvation risks linked to "point of no return" (PNR)—as opposed to temporal metrics like dph or °C·dph—due to possibilities of different incubation temperatures confounding development stage at hatch. Excellent survival observed in this study highlights the feasibility of improving conservation or commercial aquaculture techniques for Burbot, which has been hampered by mass mortality of larvae during the critical period. Overall, these findings emphasize the importance of researching Burbot larvae ecophysiology between hatching and first feeding to better understand how different abiotic and biotic factors interact with temperature-dependent ontogeny to influence recruitment in both altered and natural ecosystems.

## 4.1. Introduction

Growth of fish larvae strongly correlates with water temperature (Houde 1987; 1989), and when this dependent relationship interacts with spatiotemporal patterns of zooplankton productivity it can affect larvae survival and subsequent year class strength via match−mismatch dynamics (Ellertsen et al. 1989; Cushing 1990; Jude et al. 2013; Siddon et al. 2013; Boehm 2016). Unusually

cold waters have been shown to reduce recruitment of age-0 fish in different freshwater and marine populations (Hunter 1984; Rutherford and Houde 1995; Betsill and Van den Avyle 1997; Galloway et al. 1998; Kaemingk et al. 2014; Laurel et al. 2017; Dembkowski et al. 2017). In some northern-temperate rivers, habitats downstream of hydroelectric dams are artificially cooled in spring by discharge from deep reservoirs (Ward and Stanford 1979; Whitfield and Woods 1984; Webb and Walling 1993; Caissie 2006; Easthouse 2009; Olden and Naiman 2010). Additionally, impoundment and channelization of regulated rivers potentially reduces nutrient levels, floodplain connectivity, zooplankton abundance, fish larvae growth, and fisheries recruitment (Nunn et al. 2007; Hardy et al. 2008; Kemp 2016; Latli et al. 2019). Researchers now believe such conditions may be an underlying cause for successive recruitment failures of Burbot *Lota lota* (Gadidae) in some rivers of North America and Europe (Fisher 2000; Stapanian et al. 2010; Bosveld et al. 2015; Hardy et al. 2015; Brackwehr et al. 2016; Ross et al. 2020).

Burbot are extreme winter specialists that inhabit cold, freshwater ecosystems throughout the Holarctic ecozone (McPhail and Paragamian 2000; Shuter et al. 2012). The altered thermal regimes of impounded rivers threaten many populations of Burbot along its southern range (Paragamian and Wakkinen 2008; Stapanian et al. 2010; Hardy and Paragamian 2013; Hardy et al. 2015; Harrison et al. 2016; Blabolil et al. 2018; Pandakov et al. 2020). Temperature tolerance of Burbot is very narrow in the earliest life stages and widens with ontogeny (Shiri Harzevili et al. 2004). Embryos typically incubate at temperatures between 0−6°C (McPhail and Paragamian 2000; Cott et al. 2013; Ashton et al. 2021). Age-0 fish are often found in warmer environments that coincide with pelagic and littoral ecosystems in spring/summer/fall (Fischer 1999; Hofmann and Fischer 2002; Kjellman and Eloranta 2002; Probst and Eckmann 2009; Koporikov and Bogdanov 2011). Swimming larvae are known to withstand a temperature range of 0−24°C (Shodjai 1980; Mansfield et al. 1983; Wolnicki et al. 2002; Miler and Fischer 2004; Probst and Eckmann 2009; Donner and Eckmann 2011) and putatively show greater tolerance to thermal fluctuations than larvae of other fish species in similar habitats (Sorokin 1976). There is oftentimes a correlation between larvae size, growth, and mortality that influences recruitment (Miller et al. 1988; Houde 1997), and faster larvae growth has been shown to increase recruitment in some Atlantic Cod populations (Campana 1996; Meekan and Fortier 1996). Although freshwater fish generally produce larger larvae at hatch and exhibit higher survival compared to marine fish (Miller et al. 1988; Houde 1994), Burbot are a unique "freshwater cod" with early life history traits linked to ancestral marine cod inhabiting high-latitude waters (Jäger et al. 1981; Teletchea et al. 2009; Cott et al. 2013); hence, the potential for similarities in recruitment mechanisms.

Optimal bioenergetics for swimming, feeding and growth of larval fish may depend on successful onset of swim bladder inflation. A properly inflated swim bladder in larvae also reduces the risk of suffocation in fine substrates, increases dispersal, and improves predator avoidance (Taylor and McPhail 2000; Czesny et al. 2005). The effects of temperature on Burbot larvae swim bladder inflation has not been empirically tested in any known publication; however, a review of literature infers a pattern of reduced time to inflation with warming (Fischer 1999; Taylor and McPhail 2000; Vught et al. 2008; Palińska-Żarska et al. 2014). Even though problems with swim bladder inflation are widespread in hatchery production of physoclistous fish, the topic is relatively unstudied in the fields of fisheries ecology and recruitment (Czensy et al. 2005). Burbot larvae mortality in hatcheries can exceed 60% between emergence and first feeding and is often linked to improper inflation (Taylor and McPhail 2000; Kucharczyk et al. 2004; Żarski et al. 2009; Palińska-Żarska et al. 2014). Low survival during this "critical period"—as first reported by Hjort (1914)—has also been observed for fish larvae in natural settings, but the underlying cause(s) is often confounded by other factors such as poor zooplankton availability, predation, and suboptimal hydrodynamics (Hunter 1981; Ghan and Sprules 1991; Hardy et al. 2008; Stoll and Beeck 2012; China and Holzman 2014; Minto et al. 2014; Dahlke 2016). Developmental deformities caused by warm temperatures during embryo incubation and/or broodstock effects can prevent normal swimming and inflation, which ultimately lead to starvation and severe larvae mortality (Lahnsteiner et al. 2012; Palińska-Żarska et al. 2014; Ashton et al. 2021). Distinct swimming behaviors associate with major larval stages of Burbot including swim up or emergence, swim bladder inflation, first feeding, metamorphosis and settlement; concomitantly, these stages coincide with different depth strata and temperature exposures (Mansfield et al. 1983; Ghan and Sprules 1991; Fischer 1999; Taylor and McPhail 2000; Miler and Fischer 2004; Donner and Eckmann 2011; Palińska-Żarska et al. 2014).

The effects of cold water (i.e., <10°C) on Burbot larvae development, feeding, growth and survival are largely unknown. Most research has focused on warmer temperatures in hatchery settings (Wolnicki et al. 2001, 2002; Shiri Harzevili et al. 2003, 2004; Jensen et al. 2008; Wocher et al. 2011; Trabelsi et al. 2011; Barron et al. 2012; Furgala-Selezniow 2014; Palińska-Żarska et al. 2015). However, Burbot can be among the first species of fish larvae to appear in ichthyoplankton sampling efforts in early spring after ice-off when water temperatures are cold in both lotic and lentic environments (Mansfield et al. 1983; Ghan and Sprules 1991; Wang and Appenzeller 1998 ; Miler and Fischer 2004; Probst and Eckmann 2009; George et al. 2013; McCullough et al. 2015; Tucker et al. 2018; Korporikov et al. 2019; Toivonen et al. 2020). Embryo development time to hatching are inversely related to temperature; warmer water accelerates and synchronizes hatching, whereas colder water delays and protracts hatching (Żarski et al. 2009a; Lahnsteiner et al. 2012). Jäger et al. (1981)

reported that protolarvae would not initiate feeding on *Artemia* nauplii and died of starvation when temperatures were <8°C. Shodjai (1980) also found that larvae digestion and growth are strongly suppressed by low temperatures. These findings have led to postulations that young larvae must migrate or passively drift to warmer habitats to grow and survive (Kjellman 2003; Miler and Fischer 2004; Koporikov and Bogdanov 2011). Increased zooplankton abundance in warmer surface waters of lakes is important to larvae feeding, growth and survival (Ryder and Pesendorfer 1992; Miler and Fischer 2004); likewise, foraging on zooplankton in shallow, warm floodplains can produce faster growth in rivers (Koporikov and Bogdanov 2011). Overall, favorable abiotic and/or biotic conditions that support growth and survival of larvae are believed to be primary factors influencing Burbot recruitment (Kjellman 2003; Brackwehr et al. 2016; Koporikov and Bogdanov 2011; 2019). Ashton et al. (2021) cautioned that hydropower operations may cause unusual warming in winter during embryo incubation, followed by abnormal cooling in spring during emergence, which might present suboptimal conditions for early life history of Burbot.

Seminal research by Hunter (1981) identified three main mortality factors in fish larvae including (1) starvation due to mismatch between the gape size of larvae and the body size of planktonic prey, (2) predation/cannibalism, and (3) energetic inefficiencies due to extreme temperatures. Our study pertains to this third factor, with a primary objective to evaluate Burbot larvae development in cold water, and to investigate the existence of a putative thermal threshold (≥8°C) for larvae feeding. We examined larval swim bladder inflation, first feeding, prey ingestion, growth, and survival in a series of laboratory temperature trials. The overarching goal was to elucidate recruitment implications from larvae ecophysiology in cold regimes associated with deep reservoir discharges and/or natural ice-off conditions in spring.

## 4.2. Methods

### 4.2.1. Egg Collection and Incubation

During the third week of February in 2017 and 2018, eggs from 5−6 different families (one female and male parent per family) were obtained from wild adult Burbot inhabiting Moyie Lake (British Columbia). Procedures for broodstock capture, egg fertilization, water hardening, and disinfection have been described by Neufeld et al. (2011), with the exception that eggs were not disinfected with iodophore in 2018 due to concerns that it increases embryo mortality. Approximately 100 mL of eggs were transported to the University of Idaho Aquaculture Research Institute (UI−ARI; Moscow, Idaho) following methods described by Ashton et al. (2021), and then pooled into a 1-L Imhoff cone and incubated at 2.8−3.5°C until hatch according to procedures detailed by Jensen et al. (2008).

*4.2.2. Experimental Design*

A series of larval rearing trials were conducted at UI−ARI between April−May in 2017 and 2018. Larvae were randomly stocked into larviculture units (Figure 4.0.1) within an array consisting of 3−4 replicate blocks and 4−5 experimental units randomly assigned within each block. Stocking of larvae involved subsampling from the hatching tank with a 200-mL white polystyrene tray and enumerating larvae via overhead imaging (Canon® PowerShot SX620 HS 20.2 megapixel digital camera, Canon Incorporated, Melville, New York). Each larval rearing tank consisted of a 0.5-L polystyrene container (externally lined with opaque, black polyethylene sheeting). A 400-µm nylon mesh was sealed to the bottom of the container to retain larvae. Water and zooplankton recirculated at 0.10 L/min via an airlift pump constructed from a 1.6 mm diameter glass air-tube and 12.0 mm diameter chlorinated polyvinyl chloride (CPVC) pipe. Water recirculation was necessary to maintain zooplankton suspension in the larval rearing zone for extended periods at cold temperatures (see below for more details of live zooplankton feeding for each trial). A drip-flow of influent water (municipal source pretreated with activated carbon filtration, dechlorination, aeration, and chilling/heating) at 0.03 L/min flushed out wastes and prevented formation of oil films at the water surface. Zooplankton were fed to the larvae three times per day between 09:00−17:00 hours with a ration of ~5 prey/mL per feeding. Influent water was stopped for one hour at the start of each feeding to prevent immediate flushing of zooplankton. The rearing tank was partially submerged within inner and outer water baths made of high-density polyethylene (0.75 L and 3.5 L, respectively). Influent water flowed into the inner bath at 0.30 L/min via 4.8 mm diameter PVC flexible tubing.

A 200-Watt submersible heater wired to a temperature controller (Willhi® WH1436, Shenzhen Willhi Electronics Company, Shenzhen, Guangdong, China) maintained temperatures in each water bath. Temperature data loggers (Elitech® RC-5, Elitech Technology Incorporated, Milpitas, California) waterproofed in sealed polyethylene bags provided a thermographic record. All controllers and data loggers were calibrated to 0°C in an ice-water slurry with readings confirmed by a mercury thermometer and two digital meters (YSI® 30 and 55 meters with probes, YSI Incorporated, Yellow Springs, Ohio). Temperature set points were manually inputted into the controllers and adjusted by 0.2−0.5°C increments. Hydrogen peroxide was dosed at 100 mg/L for 15 minutes as a daily prophylactic treatment against fungal infection (Polinski et al. 2013). Overhead fluorescent lighting (daylight spectrum) with a timer-controlled photoperiod set for Moscow (Idaho) provided a light intensity of 800−1,200 lux at the top of rearing units (MW700 Portable Lux Meter, Milwaukee Instruments, Rocky Mount, North Carolina).
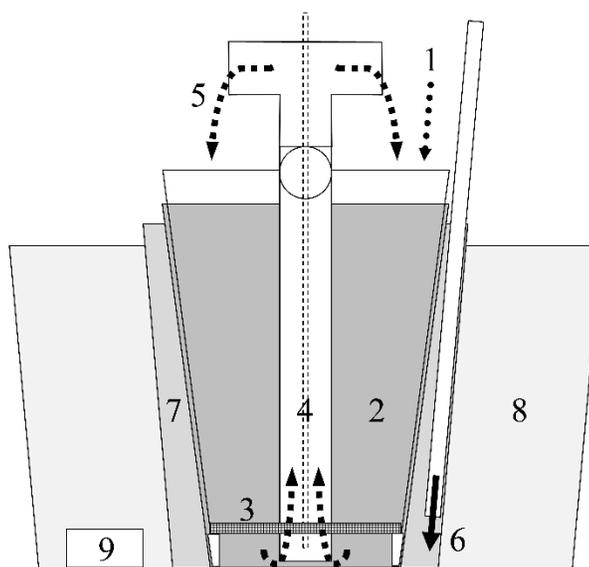
**Figure 4.0.1.** Illustration of a larviculture unit used in Trials 1−5 to test the effects of temperature on swim bladder inflation, feeding, growth, and survival of Burbot larvae. Numerical labels correspond to the following: 1) temperature-controlled inflow drip, 2) larval rearing zone, 3) bottom screen, 4) airlift pumping tube, 6) temperature-controlled inflow for inner water bath, 8) outer water bath, and 9) temperature data logger.

Larvae sampling at the end of each trial involved gently pouring fish from each tank into a 250-μm nylon net, rinsing larvae into a 500-mL white polystyrene tray, then carefully transferring larvae to a polystyrene 12-well cell culture plate (Corning®, Corning Incorporated, Corning, New York) using a 1-mL polyethylene transfer pipet (narrow tip removed). Trays and well plates were placed on an illuminated base for overhead imaging. Photos of larvae were then analyzed for total length, swim bladder inflation, feeding incidence, food ingestion, and survival using image analysis software (ImageJ, version 1.50b, National Institutes of Health, Bethesda, Maryland). Average total lengths were estimated from a random subsample of 25 larvae per rearing tank. All larvae were euthanized with an overdose of 300 mg/L tricaine methanesulfonate (Tricaine-S®, Syndel, Ferndale, Washington).

*4.2.3. Trial 1: Swim Bladder Inflation*

Yolk-sac larvae that hatched at 46 days postfertilization (dpf) were held in a 50-L tank at 3.5°C until the start of the experiment. At 4 days posthatch (dph), larvae were stocked at 206−329 fish per tank into temperature treatments of 3.8 ± 0.1, 5.3 ± 0.1, 7.3 ± 0.1, and 9.1 ± 0.1°C (mean ± SE). In all the trials of this study, larvae were allowed at least a 30-minute period to acclimate to different temperature treatments. Larvae were sampled at 12 dph for observations of swim bladder inflation which was determined by presence of a gas bubble in the bladder.

*4.2.4. Trial 2: First Feeding on Rotifers*

Yolk-sac larvae (46 dpf) hatched at 3.5°C were transferred to a shallow polypropylene tray (3 cm deep × 25 cm wide × 50 cm long) at 3 dph, then given 12 hours to gradually acclimate to 12.0°C. After completion of swim bladder inflation at 14 dph, larvae were acclimated to 3.5−4.0°C in a refrigerator overnight, then stocked at 121−159 fish per tank into temperatures treatments of 3.9 ± 0.1, 5.5 ± 0.1, 7.4 ± 0.1, and 9.3 ± 0.1°C. At 15 dph, the larvae were fed rotifers *Branchionus plicatilis*. Total length and width of rotifers ($n = 50$) was 174 ± 3 and 83 ± 2 μm, respectively. All larvae were sampled after an 8-hour feeding period for image analyses of ingested food as specific area of gut contents in the sagittal/lateral plane of the fish (units expressed as mm$^2$/larva).

*4.2.5. Trial 3: Feeding on Artemia Nauplii*

Larvae at 27 dph and previously fed rotifers ad libitum at 8−10°C in a 20-L black, polyethylene container (44 cm × 36 cm × 18 cm) were stocked at 85−124 fish per tank into temperature treatments of 5.6 ± 0.1, 7.4 ± 0.1, 9.3 ± 0.1, and 11.0 ± 0.1°C. At 28 dph, the larvae were fed *Artemia franciscana* nauplii. Mean ± SE total length and width of nauplii ($n = 50$) was 471 ± 5 and 154 ± 2 μm, respectively. All larvae were sampled after the 8-hour feeding period for image analyses

*4.2.6. Trial 4: Feeding on Artemia, Daphnia, and Rotifers*

A follow-up experiment at 30 dph tested three prey items (rotifers, *Artemia* nauplii, and *Daphnia* sp.) at two temperature treatments of 5.0 ± 0.1 and 7.6 ± 0.1°C. Similar to Trial 3, all larvae were fed rotifers prior to the start of the experiment. Larvae were then stocked into the array 24 hours before the start of the trial to allow for gut evacuation. Donner and Eckmann (2011) found that Burbot larvae completely evacuate their guts after 16−24 hours without food. The stocking density of larvae ranged from 96−131 fish per tank. *Daphnia* sp. were collected from an outdoor mesocosm, filtered through a 500-μm nylon sieve, and the filtrate was fed to larvae. Mean ± SE total length and width of *Daphnia* was 639 ± 20 and 346 ± 9 μm, respectively. All larvae were sampled after the 8-hour feeding period for image analyses.

*4.2.7. Trial 5: Growth and Survival of Larvae*

A 28-d trial was conducted in 2018 to evaluate the effects of temperature on growth and survival of larvae between hatch and late-preflexion development. Yolk-sac larvae (52 dpf) hatched at 3.5°C were stocked (189−297 fish per tank) into six temperature treatments. Each treatment warmed

differently over the trial duration from an initial temperature of 3.8−4.1°C (Figure 4.0.2). Rotifer feeding started at 10 dph for all treatments. *Nannochloropsis* sp. algae paste (Nanno 3600™, Reed Mariculture, Campbell, California) was added in 0.25-mL aliquots to each tank 30 minutes prior to each rotifer feeding. Digital images of the larviculture tanks were taken each week to document larvae behavior. Subsampling of larvae (*n* = 10) from each tank was done at 14 and 21 dph using a 1-mL polyethylene transfer pipet (narrow tip removed). All surviving larvae were sampled at 28 dph for image analyses, frozen at −20°C in 1.5-mL vials with distilled water and measured for dry weights after oven-drying at 60°C for 24 hours.
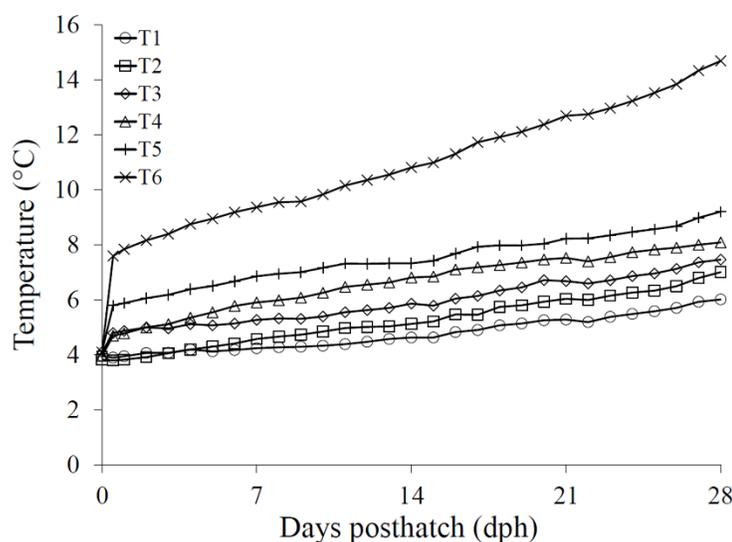


**Figure 4.0.2.** Trial 5 thermographs from six treatments (T1−T6) in a 28-d experiment of temperature effects on Burbot larvae development.

*4.2.8. Data Analyses*

Tested response variables (see Tables 4.0.1 and 4.0.2) included percent swim bladder inflation, feeding incidence, ingested food area, specific growth rate (Ricker 1975), survival, and an index of performance defined as the specific growth rate multiplied by probability of survival (Donner and Eckmann 2011). Generalized linear models (GLM) were used to evaluate proportional and continuous responses against discrete explanatory variables (binomial distribution/logit link and gaussian distribution/identity link, respectively). Some GLM analyses included a blocking factor and/or larvae stocking covariate when model fit was improved as determined by a likelihood-ratio test. Regressions of swim bladder inflation, feeding incidence, and ingested food area against degree-days posthatch used a nonlinear model based on the following 5-parameter logistic function (Gottschalk and Dunn 2005):

$$\hat{Y} = L_{\min} + \frac{L_{\max} - L_{\min}}{\left(1 + \left(\frac{D}{x}\right)^{s}\right)^{\theta}}$$

where,

$D = °C·dph$

$s$ = steepness of the curve

$L_{\max}$ = maximum value of the curve

$L_{\min}$ = minimum value of the curve

$\theta$ = asymmetry factor

$x$ = midpoint abscissa value of the curve

$\hat{Y}$ = predicted mean response

Second order polynomial regressions were used for analyses of dry weights against continuous explanatory variables (Steinarsson and Björnsson 1999). All models were validated with homoscedastic scatterplots of residuals versus fitted estimates. Assumptions of a normal error distribution for linearized models were verified with quantile–quantile plots. Power transformations determined by the Box−Cox method were used to normalize any nonnormal data. Instances of overdispersion were adjusted with a Pearson scale parameter. Type I error ($\alpha$) was defined at 0.05 for all statistical tests. Bonferroni and Tukey's honestly significant difference post hoc tests were performed to control the error rate in multiple pairwise comparisons between nonlinear parameter estimates and categorical treatments. Wald chi-square ($X^2$) and $F$-test statistics are presented with degrees of freedom in subscripts. All statistical analyses were performed in SAS version 9.4 (PROC GENMOD, PROC NLIN, PROC GLIMMIX; SAS Institute, Cary, North Carolina).

## 4.3. Results

### 4.3.1. Trail 1: Effects of Temperature on Swim Bladder Inflation

Swim bladder inflation (*SBI*; %) at 12 dph was reduced and/or delayed at 3.8°C compared to 5.3, 7.3, and 9.1°C ($X^2_{3, 8} = 143.67$, $P < 0.01$; Table 4.0.1). Nonlinear regression of *SBI* against °C·dph for pooled data estimated that mean ± SE swim bladder inflation increased rapidly from 11 ± 5% to 87 ± 3% between 40 and 60 °C·dph ($F_{3, 13} = 678.37$, $P < 0.01$; Figure 4.0.3). Larvae survival (*S*; %) was high in all treatments throughout the 9-day trial and total lengths (*TL*; mm) did not differ among treatments at 12 dph.
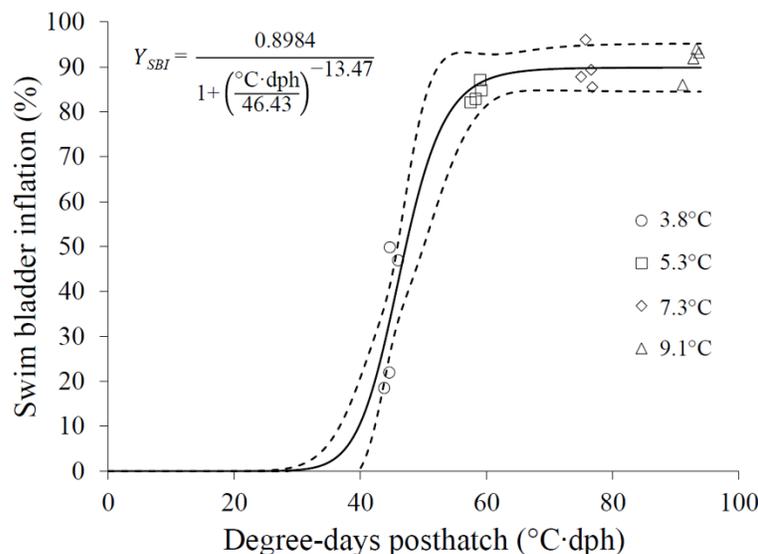
**Figure 4.0.3.** Nonlinear regression (equation is shown) of Burbot larvae swim bladder inflation (SBI) against degree-days posthatch (°C·dph) in Trial 1 (see Table 4.0.1 for trial details). The expected mean (solid line) and 95% confidence intervals (dashed lines) are estimated from pooled data (circles) of SBI observed in replicate ($n$ = 4) larviculture units for each temperature treatment.

**Table 4.0.1.** Measured responses of Burbot larvae in a series of larviculture trials with different temperature treatments and prey items. Mean values (with standard error [SE] in parentheses) of swim bladder inflation (*SBI*), feeding incidence ($F_i$), ingested food area ($I_a$), total length (*TL*), and survival (*S*) are shown between 12 to 30 days posthatch (dph). Lowercase letters next to column values indicate significant difference between treatments within a trial ($P \leq 0.05$).

| Trial | Treatment | $n$ | dph | °C·dph | *SBI* (%) | $F_i$ (%) | $I_a$ (mm²/larva) | *TL* (mm) | *S* (%) |
|---|---|---|---|---|---|---|---|---|---|
| 1. Swim | 3.8°C | 4 | 12 | 45 | 35 (8) y | | | 3.62 (0.03) | 99 (1) |
| bladder | 5.3°C | 4 | 12 | 58 | 85 (1) z | | | 3.68 (0.03) | 98 (1) |
| inflation | 7.3°C | 4 | 12 | 76 | 90 (2) z | | | 3.66 (0.03) | 99 (0) |
| | 9.1°C | 4 | 12 | 93 | 93 (1) z | | | 3.63 (0.03) | 99 (1) |
| | | | | | | | | | |
| 2. First | 3.9°C | 4 | 15 | 150 | | 16 (9) y | 0.01 (0.00) y | 3.84 (0.06) | |
| feeding | 5.5°C | 4 | 15 | 150 | | 26 (8) y | 0.01 (0.01) y | 3.76 (0.05) | |
| on | 7.4°C | 4 | 15 | 150 | | 55 (4) z | 0.04 (0.00) z | 3.81 (0.04) | |
| rotifers | 9.3°C | 4 | 15 | 150 | | 56 (4) z | 0.06 (0.01) z | 3.84 (0.04) | |
| | | | | | | | | | |
| 3. Feeding | 5.6°C | 4 | 28 | 250 | | 10 (5) x | 0.01 (0.01) x | 4.35 (0.04) | |
| on | 7.4°C | 4 | 28 | 250 | | 35 (8) y | 0.06 (0.02) y | 4.28 (0.03) | |
| *Artemia* | 9.3°C | 4 | 28 | 250 | | 64 (3) z | 0.13 (0.01) z | 4.36 (0.04) | |
| nauplii | 11.0°C | 4 | 28 | 250 | | 63 (3) z | 0.14 (0.02) z | 4.27 (0.03) | |
| | | | | | | | | | |
| 4. Feeding | 5.0°C/*Art* | 3 | 30 | 269 | | 29 (8) x | 0.03 (0.01) wx | 4.27 (0.05) | |
| on | 5.0°C/*Dap* | 3 | 30 | 269 | | 1 (0) w | 0.00 (0.00) w | 4.33 (0.04) | |
| *Artemia,* | 5.0°C/rot | 3 | 30 | 269 | | 77 (9) yz | 0.06 (0.02) xy | 4.22 (0.04) | |
| *Daphnia,* | 7.7°C/*Art* | 3 | 30 | 269 | | 62 (1) y | 0.10 (0.01) yz | 4.36 (0.04) | |
| and | 7.7°C/*Dap* | 3 | 30 | 269 | | 1 (0) w | 0.00 (0.00) w | 4.27 (0.04) | |
| rotifers | 7.7°C/rot | 3 | 30 | 269 | | 91 (5) z | 0.11 (0.03) z | 4.32 (0.04) | |

*4.3.2. Trials 2−4: Effects of Temperature and Prey Type on Larval Feeding*

Protolarvae at 150 °C·dph of development in Trial 2 transitioned to rotifer feeding at all the tested temperatures, however, feeding incidence ($F_i$; %) and ingested food area ($I_a$; mm$^2$/larva) were both reduced at 3.9°C and 5.5°C compared to 7.4°C and 9.3°C ($F_i$: $X^2_{3, 12}$ = 21.52, $P < 0.01$; $I_a$: $X^2_{3, 12}$ = 28.03, $P < 0.01$; Table 4.0.1). A similar trend was observed in larvae at 250 °C·dph in Trial 3 feeding on *Artemia* nauplii: both $F_i$ and $I_a$ were lower in the two coldest treatments ($F_i$: $X^2_{3, 12}$ = 54.59, $P < 0.01$; $I_a$: $X^2_{3, 12}$ = 30.18, $P < 0.01$). The *Artemia*-fed larvae in Trial 3 generally showed a greater $I_a$ than the rotifer-fed larvae in Trial 2, but only at temperatures >6°C; in contrast, a higher $F_i$ was observed in the rotifer-fed larvae between 5.5−7.4°C.

A follow-up experiment in Trial 4 tested the effects of two temperature treatments (5.0°C and 7.7°C) and three prey types (*Artemia*, *Daphnia*, and rotifers) on larvae feeding at 269 °C·dph of development. Temperature and prey type interacted to affect $F_i$ and $I_a$ ($F_i$: $X^2_{5, 12}$ = 193.42, $P < 0.01$; $I_a$: $X^2_{5, 12}$ = 31.33, $P < 0.01$; Table 4.0.1). Feeding incidence was greater with rotifer prey, and a similar but nonsignificant trend was also seen with ingested food area. Both $F_i$ and $I_a$ increased at 7.7°C with both rotifer and *Artemia* prey. There was no evidence of larvae feeding on *Daphnia* at either of the tested temperatures.

*4.3.3. Trial 5: Effects of Temperature on Growth and Survival of Larvae*

In Trial 5, larvae experienced rearing temperatures ranging from 3.8°C to 14.7°C across six temperature treatments (T1−T6) between 0 and 28 dph (Figure 4.0.2). General observations at 7 dph found that most larvae at <5.5°C (T1−T3; 33−40 °C·dph) were resting on the tank bottom and had not inflated their swim bladders (Figure 4.0.4A); conversely, many larvae at >6.0°C (T5−T6; 49−65 °C·dph) were neutrally buoyant and swimming (Figure 4.0.4B). We decided not to subsample at 7 dph to avoid damaging any non-inflated larvae. At 14 dph, very few larvae were seen on the bottom and nearly all subsampled larvae (>96%) across treatments had inflated swim bladders by 64−136 °C·dph (Table 4.0.2).

At 14 dph, no feeding incidence was observed in T1 and T2, whereas $F_i$ ranged from 13−100% in T3−T6. However, more than 86% of larvae from the coldest rearing temperatures (T1−T3) had food in their stomachs at 21 dph, and $F_i$ was high across all treatments at 28 dph (Table 4.0.2). Treatment and °C·dph interacted to affect $F_i$ ($X^2_{6, 65}$ = 9,036.09, $P < 0.01$) and $I_a$ ($X^2_{6, 65}$ = 83.31, $P < 0.01$). Nonlinear regressions of feeding incidence against °C·dph estimated that $F_i$ rose sharply after 78 °C·dph and reached 90% or more by 107 °C·dph (Figure 4.0.5A); T6 was not fitted to logistic analysis of $F_i$ because observed data did not coincide with any slope change on the curve. The two coldest treatments (T1−T2) had the lowest $F_i$ between 139−152°C·dph. Nonlinear regressions of

ingested food area found that T1−T2 had a lower $I_a$ than T4−T6 at 139°C·dph, and the highest $I_a$ occurred in the warmest treatment (T6; Figure 4.0.5B).
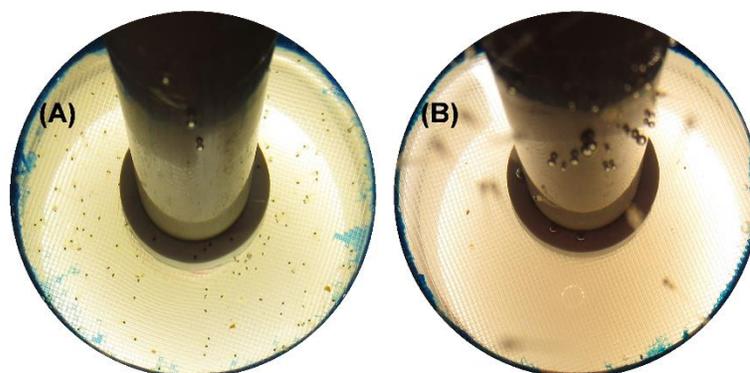


**Figure 4.0.4.** Images of Burbot protolarvae at 7 dph rearing in the coldest **(A)** and warmest **(B)** treatments (T1 and T6, respectively) of Trial 5 (see Figure 2 for treatment thermographs). Many larvae are resting on the bottom screen in **(A)**, whereas only swimming larvae can be seen in the foreground of **(B)**. The bottom screen area was briefly illuminated to aid visualization of larvae during imaging but otherwise not illuminated in the 28-d trial.

Average total lengths (*TL*; mm) of larvae increased in all treatments over the trial duration, whereas average dry weights (*W*; mg) decreased in the coldest treatments (T1−T3; Table 4.0.2). Average specific growth rate of *W* ($SGR_W$, %/d) was strongly correlated to rearing temperature ($X^2_{5, 10}$ = 88.23, $P < 0.01$), with larvae in coldest (T1) and warmest (T6) treatments having the lowest and highest $SGR_W$, respectively. A second order polynomial regression determined that *W* was affected by interaction between treatment and °C·dph ($X^2_{6, 54}$ = 26.68, $P < 0.01$; Figure 4.0.5C). At a covariate value of 139 °C·dph, estimates of *W* were lowest for T1 among treatments, and T2 was lower than T5−T6; however, there were no detectable difference in *TL*. Similar regression analysis of *W* against *TL* found that estimates of *W* from T1−T2 were significantly below T5−T6 at a covariate value of 4.06 mm TL. The covariates 139 °C·dph and 4.06 mm TL were averages for T1 at 28 dph and represented the largest values shared among all treatments during the trial.

Larvae survival differed between treatments at 28 dph ($X^2_{5, 12}$ = 35.87, $P < 0.01$), with higher survival in T2 compared to T4−T6, and lower survival in T6 compared to T1−T3. A binomial regression determined that *S* was affected by interaction between treatment and °C·dph ($X^2_{6, 24}$ = 291.03, $P < 0.01$) but failed to detect any differences between treatments at a covariate value of 139 °C·dph. To clarify, survival trends differed between temperature treatments for larvae of the same age but were not significantly different at similar degree-days of larvae development. Larvae performance (*PF*) was strongly correlated to rearing temperature, with the lowest and highest index values corresponding to coldest (T1) and warmest (T6) treatments, respectively ($X^2_{5, 10}$ = 88.23, $P < 0.01$).
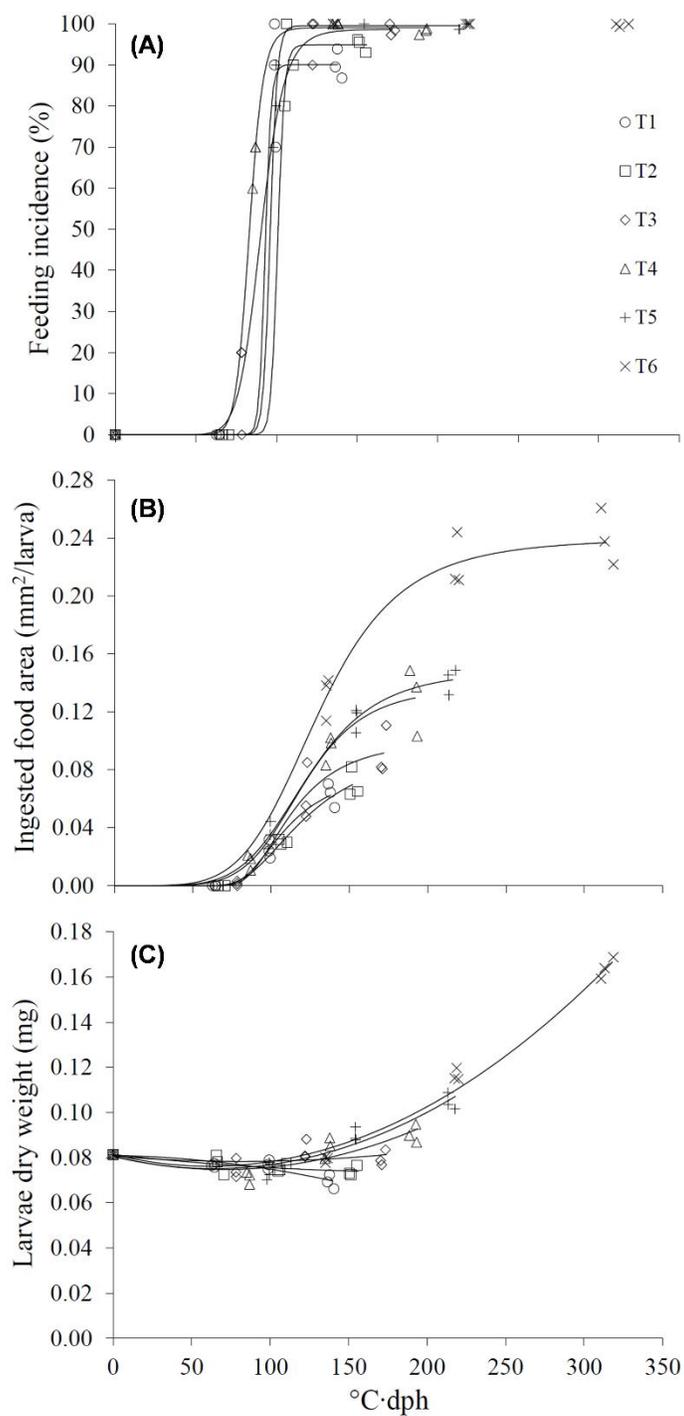
**Figure 4.0.5.** Regression models of **(A)** feeding incidence, **(B)** ingested food area and **(C)** larvae dry weight against degree−days of larvae development posthatch (°C·dph). Marker symbols represent observed data from replicate (*n* = 3) larviculture rearing units across six treatments (T1−T6; see Figure 4.0.2 for treatment thermographs) in a 28-d trial. Solid lines are the expected means predicted by modeling.

**Table 4.0.2.** Measured responses of Burbot larvae (means with standard errors [SE] in parentheses) across different temperatures treatments in a 28-d larviculture trial. Thermographs of each treatments are shown in Figure 4.0.2. Dry weights ($W$), specific growth rates in total length and dry weight ($SGR_{TL}$ and $SGR_W$, respectively), and performance ($PF$) are shown in addition to other response metrics defined in Table 4.0.1. Lowercase letters next to column values indicate significant difference between treatments at 28 days posthatch ($P \leq 0.05$).

| Treatment | $n$ | dph | °C·dph | $SBI$ (%) | $F_i$ (%) | $I_a$ (mm²/larva) | $TL$ (mm) | $W$ (mg) | $SGR_W$ (%/d) | $S$ (%) | $PF$ (d⁻¹) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| All | 3 | 0 | 0 | | | | 3.48 (0.04) | 0.081 (0.001) | | | |
| T1 | 3 | 14 | 64 | 100 (0) | 0 (0) | 0.00 (0.00) | 3.85 (0.06) | 0.077 (0.001) | -0.44 (0.04) | | |
| T2 | 3 | 14 | 67 | 97 (3) | 0 (0) | 0.00 (0.00) | 3.96 (0.07) | 0.078 (0.002) | -0.35 (0.22) | | |
| T3 | 3 | 14 | 78 | 100 (0) | 13 (7) | 0.00 (0.00) | 3.95 (0.03) | 0.075 (0.003) | -0.57 (0.24) | | |
| T4 | 3 | 14 | 86 | 100 (0) | 67 (3) | 0.02 (0.00) | 3.97 (0.06) | 0.071 (0.002) | -0.95 (0.17) | | |
| T5 | 3 | 14 | 99 | 100 (0) | 80 (6) | 0.04 (0.01) | 4.07 (0.07) | 0.074 (0.002) | -0.72 (0.22) | | |
| T6 | 3 | 14 | 136 | 100 (0) | 100 (0) | 0.13 (0.01) | 4.16 (0.05) | 0.079 (0.001) | -0.19 (0.07) | | |
| T1 | 3 | 21 | 99 | 100 (0) | 86 (9) | 0.02 (0.00) | 3.86 (0.03) | 0.077 (0.001) | -0.26 (0.07) | | |
| T2 | 3 | 21 | 107 | 100 (0) | 90 (6) | 0.03 (0.00) | 3.80 (0.04) | 0.075 (0.001) | -0.38 (0.06) | | |
| T3 | 3 | 21 | 122 | 100 (0) | 97 (3) | 0.06 (0.01) | 3.94 (0.02) | 0.083 (0.003) | 0.10 (0.14) | | |
| T4 | 3 | 21 | 137 | 100 (0) | 100 (0) | 0.10 (0.01) | 3.99 (0.04) | 0.085 (0.003) | 0.19 (0.15) | | |
| T5 | 3 | 21 | 154 | 100 (0) | 100 (0) | 0.12 (0.00) | 4.04 (0.03) | 0.090 (0.002) | 0.48 (0.09) | | |
| T6 | 3 | 21 | 219 | 100 (0) | 100 (0) | 0.22 (0.01) | 4.61 (0.05) | 0.117 (0.002) | 1.71 (0.07) | | |
| T1 | 3 | 28 | 139 | 99 (0) | 90 (2) x | 0.06 (0.00) w | 4.06 (0.02) u | 0.069 (0.002) u | -0.59 (0.09) u | 95 (1) wx | -0.56 (0.09) u |
| T2 | 3 | 28 | 152 | 99 (0) | 95 (1) y | 0.07 (0.01) w | 4.16 (0.01) v | 0.074 (0.001) v | -0.35 (0.06) v | 96 (1) w | -0.33 (0.06) v |
| T3 | 3 | 28 | 172 | 99 (1) | 98 (1) yz | 0.09 (0.01) x | 4.29 (0.05) w | 0.080 (0.002) w | -0.07 (0.09) w | 91 (3) wxy | -0.07 (0.09) w |
| T4 | 3 | 28 | 192 | 99 (0) | 98 (0) yz | 0.13 (0.01) y | 4.40 (0.01) x | 0.091 (0.002) x | 0.38 (0.09) x | 87 (3) xyz | 0.34 (0.09) x |
| T5 | 3 | 28 | 215 | 100 (0) | 99 (0) z | 0.14 (0.01) y | 4.62 (0.05) y | 0.105 (0.002) y | 0.90 (0.07) y | 85 (3) yz | 0.77 (0.04) y |
| T6 | 3 | 28 | 314 | 100 (0) | 100 (0) z | 0.24 (0.01) z | 5.47 (0.01) z | 0.164 (0.003) z | 2.50 (0.06) z | 81 (1) z | 2.03 (0.03) z |

**4.4. Discussion**

Water temperatures below 6°C prolonged yolk-sac larvae resting behavior, delayed swim bladder inflation, and reduced Burbot larvae first-feeding activity, growth, and performance up to 28 dph. We did not detect a definitive thermal threshold for larvae feeding between 3.9−14.7°C; however, larvae averaging 4.3 mm TL did show greater feeding incidence and ingestion at 5°C on slow, small rotifers as opposed to faster, larger *Artemia* nauplii and *Daphnia*. We concluded that the previous report of Burbot larvae starvation at <8°C by Jäger et al. (1981)—a finding expounded to several ecological studies (Kjellman et al. 2000; Miler and Fischer 2004; Donner and Eckmann 2011; Sandberg 2015)—is likely specific to laboratory conditions involving *Artemia* nauplii as a first-feeding prey item in cold water. This conclusion is also supported by other evidence of exogenous nutrition at 6°C in Burbot larvae measuring ~4 mm TL (Müller 1960; Ghan and Sprules 1991; 1993). To our knowledge, the present study is the first empirical account of high survival through swim bladder inflation, first feeding, and preflexion stages of Burbot larvae development. Other studies have associated this "critical period" with mass mortality of Burbot (Schram 2000; Ghan and Sprules 1991; Taylor and McPhail 2000; Jensen et al. 2008; Żarski et al. 2009b; Palińska-Żarska et al. 2014) or reported high survival from larviculture experiments that started after swim bladder inflation and/or yolk absorption (Wolnicki et al. 2002; Shiri Harzevili et al. 2003; 2004; Donner and Eckmann 2011; Wocher et al. 2011; Barron et al. 2012; Palińska-Żarska et al. 2015).

Cold regimes could be a risk to Burbot larvae emergence from substrates. Generally, the limited mobility of fish protolarvae increases their risk of suffocation in silt (Bailey and Boyd 1972), and our results showed that cold water prolongs the resting phase of yolk-sac larvae. Taylor and McPhail (2000) surmised that positive phototaxis induces swim up of Burbot larvae and reduces the risk of suffocation in fine substrates; we further emphasize that the positive effects of warming are important to emergence of protolarvae. Hatching can occur in thermal regimes that range from near freezing to temperate conditions (McPhail and Paragamian 2000). Swimming or "wriggling" behavior associated with hatching quickly subsides (0−1 dph at 3.5°C in the present study), and the resting phase of yolk-sac larvae may last a few days or several weeks depending upon temperature (Taylor and McPhail 2000; Vught et al. 2008; Palińska-Żarska et al. 2014). Previous hatchery work at UI−ARI saw high mortality of yolk-sac larvae after prolonged resting on the tank bottom at cold temperatures; much of the death was ascribed to protozoan infections, unsuccessful inflation, and starvation (unpublished data). The impact of cold hydropower discharges in spring that spatiotemporally overlap with emergence require greater consideration, especially given the history of hydroelectric dams negatively impacting Burbot populations (Paragamian and Wakkinen 2008; Stapanian et al. 2010; Hardy and Paragamian 2013; Hardy et al. 2015; Harrison et al. 2016a; Blabolil

et al. 2018; Pandakov et al. 2020; Ross et al. 2020). As an example, the endemic Burbot population in the lower Kootenai River (Idaho) is subjected to cold discharges from Libby Dam that slow the natural process of river warming in April; post-dam temperatures average 1−3°C colder than pre-dam conditions (Ashton et al. 2019, 2021). Our study results suggest that such examples of artificial cooling might increase Burbot protolarvae resting behavior on the bottom, which in the lower Kootenai River mostly consists of sand/silt/clay unsuitable for early life stages of a co-existing endangered population of White Sturgeon *Acipenser transmontanus* (Hardy et al. 2020). We are not aware of any studies on substrate types that support Burbot larvae emergence and more research is needed in this area, particularly with respect to the effects of different thermal regimes.

Cold water increases the number of days required for Burbot protolarvae to inflate their swim bladders, however, the number of degree-days to inflation seems consistent across different rearing temperatures. We found that most larvae showed signs of inflation by 60 °C·dph in waters between 4−9°C; nearly all the larvae were swimming horizontally with inflated swim bladders by 70°C·dph. Similarly, Palińska-Żarska et al. (2014) observed >50% inflation by 60 °C·dph in larvae reared at a constant 12°C, and Vught et al. (2008) reported that larvae transition from resting behavior to swimming by 72 °C·dph at a constant 4°C. High mortality associated with hyperinflation of the swim bladder around the time of first feeding has occurred under mass rearing efforts at the UI−ARI hatchery (unpublished data). A common symptom is "rafts" of overly buoyant larvae trapped on the water surface; this condition appears to be irreversible and leads to starvation. The excessive ingestion of air, which presumably underlies hyperinflation in protolarvae, may be a result of yolk absorption, empty gut, and a persistent oil globule increasing larvae buoyancy; in freshwater, the proteinaceous yolk of embryos is negatively buoyant, and the lipid or oil globule provides hydrostatic lift (Craik and Harvey 1987; Sundby and Kristiansen 2015; Xu et al. 2017). Some investigators have postulated that Burbot protolarvae must inhabit shallow waters to successfully inflate due to weak and sporadic swimming bursts to the surface (Harsányi and Aschenbrenner 1992; Palińska-Żarska et al. 2014; Brackwehr et al. 2016). Palińska-Żarska et al. (2014) recommended ≤10 cm water depth for successful inflation in a laboratory setting. Koporikov and Bogdanov (2011) assumed that larvae passively migrate downstream from spawning areas to shallow floodplains in the lower Ob River. In Lake Michigan, high densities of newly hatched larvae within the 3-m bottom contour were attributed to passive dispersal by currents from nearshore spawning habitats (Mansfield et al. 1983). It is unclear how these examples of shallow-water spawning and larvae distribution compare with inflation success in other life history patterns that involve spawning in deep water, as reported in several populations inhabiting dimictic/oligomictic lakes of North America and Europe (Fischer 1999; Donner and Eckmann 2011; Jude et al. 2013; Robichaud et al. 2014). In contrast to findings by

Palińska-Żarska et al. (2014), Fischer (1999) presumed that yolk-sac larvae are able to vertically migrate from the cold, deep profundal zone to the surface to inflate their swim bladders; yolk-sac larvae have also been sampled throughout the water column to a depth of 10 m in other studies (Clady 1976; Hudd et al. 1983; Eloranta 1985; Ghan and Sprules 1991, 1993; Wang and Appenzeller 1998). Since Burbot protolarvae can initiate feeding before total resorption of their yolk sac (Taylor and McPhail 2000; Palińska-Żarska et al. 2014), there may be interactions between intrinsic larvae buoyancy and food ingestion that play a critical role in emergence, vertical migration, and inflation success for deep-spawning populations. Given that little is known about the effects of spawning depth on Burbot recruitment in lakes and rivers, further investigation is needed to reveal how bathymetric distribution of eggs and migration patterns of protolarvae impact inflation success under different temperature and hydrodynamic regimes.

Cold water also reduces Burbot larvae first feeding on rotifers and suppresses consumption of larger, faster, and potentially more nutritious/energy dense prey such as *Artemia* nauplii. We observed larvae at 150 °C·dph of development exhibiting lower feeding incidence and ingestion of rotifers at 3.9−5.5°C compared to 7.4−9.3°C. In a closely related species, Atlantic Cod *Gadus morhua*, larvae also reduce feeding on rotifers at <8°C (Steinarsson and Björnsson 1999; Jordaan and Kling 2003). There are many reports of larval Burbot at 90−150 °C·dph that first feed on *Artemia* nauplii at temperatures ≥10°C (Wolnicki et al. 2001, 2002; Donner and Eckmann 2011; Wocher et al. 2011; Trabelsi et al. 2011; Furgala-Selezniow 2014; Kupren et al. 2014; Trejchel et al. 2014; Palińska-Żarska et al. 2015); however, Jäger et al. (1981) found that hatchery-reared larvae died of starvation when temperatures were <8°C because of unsuccessful first feeding on *Artemia* nauplii. We also found that larvae at 250 °C·dph exhibited poor feeding on *Artemia* nauplii at 5.6−7.4°C compared to 9.3−11.0°C. Interestingly, Donner and Eckmann (2011) documented high 15-d survival of larger larvae that fed and grew on *Artemia* nauplii at 5°C, and we recorded improved feeding on *Artemia* nauplii at 5°C once larvae reached 269 °C·dph. These results suggest that feeding success of Burbot larvae on larger prey items in cold water may quickly improve with more degree-days of development. Comparatively, larvae feeding on rotifers in this study exceed that of *Artemia* nauplii at both 5.0 and 7.7°C, and there were negligible signs of *Daphnia* consumption. The transition to exogenous feeding on rotifers occurred between 78−107 °C·dph at rearing temperature between 5−7°C, which is similar to results from Vught et al. (2008), but later than the 60−72 °C·dph transition to *Artemia* nauplii at 12°C recorded by Palińska-Żarska et al. (2014). Ghan and Sprules (1993) found that the earliest larval stages of Burbot selectively feed on globular rotifers over other prey that may exhibit faster swimming and/or protuberances that hinder capture and ingestion. Field studies have also shown that *Daphnia* only make up a substantial portion of the Burbot larvae diet in later stages of

development (Wang and Appenzeller 1998; George et al. 2013). We saw algae in the esophagus, stomach, and hindgut of first-feeding larvae at rearing temperatures between 5−10°C. Vanheule (2012) reviewed the topic of first feeding in Burbot larvae and emphasized the importance of phytoplankton as (1) a nutritional supplement, (2) augmenter of gut microflora, and (3) stimulatory precursor to digestion of essential small zooplankton such as rotifers and copepods. In laboratory studies, Vachta (1990) observed larvae first ingesting phytoplankton before transitioning to copepod nauplii, and Shiri Harzevili et al. (2003) found that the addition of *Chlorella* sp. algae greatly improved larvae survival up to 350 °C·dph. Our inclusion of *Nannochloropsis* sp. algae may partly explain high larvae survivals through the critical period. The ease at which algae is ingested during the earliest stages of larvae development could be especially important in cold waters that suppress larvae feeding on zooplankton. More research is needed regarding phytoplankton importance, especially given the potential implications of reduced primary productivity in cold river regimes downstream of hydropower operations in spring (see Hardy et al. 2008; Hardy and Paragamian 2013). Recently, artificial nutrient additions were shown to increase periphyton, phytoplankton, and zooplankton communities in the lower Kootenai River (Kruse et al. 2019) and Kootenay Lake Schindler et al. 2020), and possibly improving nursery habitats for Burbot larvae in those systems.

We found that growth, condition, and performance of Burbot larvae increases with warming in a controlled environment at rearing temperatures between 3.9−14.7°C up to 28 dph. These results provide an important benchmark of vital rates during a critical period of larvae development across a range of temperatures typical of many boreal waters in spring. Other Burbot larviculture studies involving warmer regimes showed that 12−16°C was optimal with respect to these same vital rates (Wolnicki et al. 2002; Shiri Harzevili et al. 2004; Donner and Eckmann 2011; Barron et al. 2012). Taylor and McPhail (2000) cultured Burbot larvae up to 27 dph (~214 °C·dph) on pond zooplankton and reported larvae growth to 4.25 mm TL in tanks that warmed from 4.0 to 9.5°C (~7.5°C average); in the present study, there was slightly faster growth to 4.62 mm TL at 28 dph (215 °C·dph). Taylor and McPhail (2000) reported mortality approaching 75% and estimated that Burbot larvae reach a starvation-induced "point of no return" (PNR) at ~4.6 mm TL. Point of no return is a reference to irreversible starvation effects that cause death even if feeding is resumed (Blaxter and Hempel 1963; Blaxter and Ehrlich 1974; Miller et al. 1988). Deterioration of the larval gut and organs important to digestion (i.e. liver and pancreas) during starvation is a potential causative factor of PNR in some fishes (see Garcia et al. 2020 and references therein). We saw no clear evidence of PNR based on common methods of detection (see Ríos-Durán et al. 2016), however, our results did show that larvae reared below 7.5°C failed to gain weight by 28 dph, and those reared below 6.0°C were on a trajectory of continued weight loss despite high feeding incidence and survival; additionally, the

coldest treatments produced larvae with the poorest relative condition at 28 dph as determined by lowest estimated dry weight for a given covariate value of °C·dph and total length. Collectively, our results suggest that cold regimes potentially increase the risk of PNR in Burbot larvae under conditions of high rotifer prey availability. Kucharczyk et al. (2004) found that time to PNR was inversely related to water temperature, whereas Lahnsteiner et al. (2012) reported that temperature had no effect on the timing of starvation-related mortality occurring between 12−18 dph at 2, 6, and 9°C. We suggest that 4.6 mm TL is probably a good estimate for a PNR threshold because Lahnsteiner et al. (2012) and Palińska-Żarska et al. (2014) both observed mortality spikes at 4.6 mm TL in cold and warm regimes, respectively. In our study, larvae in the coldest regimes had not grown to 4.6 mm TL by 28 dph, which might explain the lack of any obvious PNR indicators like a mortality spike and/or reduced feeding. Temporal discrepancies of estimated PNR between cold-water studies are probably due to differences in larvae size at hatching, which was 3.5 mm TL in Taylor and McPhail (2000), but 4.5 mm TL in Lahnsteiner et al. (2012). Very cold embryo incubation temperatures cause a delay in hatching and increase larvae size and development at emergence (McCrimmon 1959; Meshkov 1967; Muth and Smith 1974); concomitantly, embryos were incubated in a colder regime (i.e., ≤2°C) by Lahnsteiner et al. (2012). We concluded that (1) cold regimes during the first month of Burbot larvae development put them at higher risk of lower performance and being underweight due to limitations in growth and feeding activity, and (2) total length is a better threshold estimate of PNR—as opposed to temporal metrics like dph or °C·dph—mainly because of potential variability in larvae size at hatch. These conclusions are especially poignant to case-study populations such as the lower Kootenai River where spawning and embryo incubation may be in ice-covered tributaries, but larval rearing likely occurs in the main stem impacted by artificially cold water and low productivity as a result of the upstream hydroelectric dam. We recommend that any evaluations of Burbot larvae recruitment in similar ecosystems incorporate larvae sampling in tributaries for estimates of total length and weight at hatch, yolk-sac size, water temperature, and plankton abundance/diversity in littorals and epilimnion (see various larvae sampling methods used by Mansfield et al. 1983; Fisher 2000; Kjellman et al. 2000; Koporikov and Bogdanov 2011, 2019; Saunders et al. 2014) to monitor PNR risks in relation to spatiotemporal aspects of larvae dispersal and food availability. It may also be possible to compare larvae traits to maternal/paternal effects via metadata collected during parentage-based genetic tagging efforts and/or from hatchery broodstock collection and embryo/larvae stocking efforts.

### 4.5. Conclusions

This study is the first to examine the effects of artificially cold regimes in spring on early development of Burbot larvae. Previous research has focused on the effects of artificially warm regimes in fall/winter on spawning migration (Paragamian et al. 2005; Paragamian and Wakkinen 2008), final maturation and spawning time (Ashton et al. 2019), and embryo development (Ashton et al. 2019, 2021). We found that Burbot larvae can inflate their swim bladders, prey on rotifers, and exhibit high survival through a critical period of development in cold water. However, there was also prolonged resting behavior of protolarvae, delayed inflation, reduced feeding activity, slower growth, and diminished performance of larvae in cold regimes, which could increase the risk of cumulative mortality and negatively impact Burbot recruitment.

Houde (1989) reviewed the interactions between temperature, larvae growth, and survival in relation to recruitment and spatiotemporal patterns of larval distribution; an important takeaway is that ecological variability and small decreases in larvae growth and/or survival can accumulate throughout an extended larval stage to become a recruitment bottleneck. When our results are framed within the context of Houde's (1989) synopsis, the potential linkage between Burbot larvae recruitment failures and cold, nutrient-poor waters discharged by hydroelectric dams in spring becomes a major concern. We demonstrated that waters <6°C present significant inefficiencies in activity and growth during the critical period of Burbot larvae development.

Lastly, the high survival observed in our study highlights the feasibility of improving aquaculture techniques for Burbot larvae. Interest in Burbot aquaculture has grown in North America and Europe over the last decade with various projects in areas of stock enhancement and commercialization, however, mortality associated with swim bladder inflation and first feeding have been major obstacles to overcome for hatcheries (Jensen et al. 2008; Żarski et al. 2009; Trabelsi et al. 2011; Wocher et al. 2011; Barron et al. 2012; Palińska-Żarska et al. 2014, 2015). In the present study, there was no mass mortality of protolarvae associated with prolonged resting on the bottom, non-inflation, hyperinflation, or transitioning to exogenous food, and we attribute that successful outcome to greater control over larviculture parameters in the experimental design. As a final point of emphasis, we strongly advocate for more field research on the ecophysiology of Burbot larvae between hatching and first feeding to better understand how different abiotic and biotic factors like substrate type, hydrodynamics, plankton productivity, PNR, and predation interact with temperature-dependent ontogeny to influence recruitment in both altered and natural ecosystems.

## 4.6. References

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# Chapter 5: Modeling the Winter/Spring Post-dam Thermal Regime of the Lower Kootenai River for Burbot Conservation

## Abstract

An imperiled population of endemic North American Burbot (*Lota lota maculosa*) inhabiting the lower Kootenai River in a transboundary region of Idaho and British Columbia struggles with recruitment failures linked to incompatibility between early life history patterns and upstream hydropower operations at Libby Dam in Montana. Loss of natural river ice during winter due to discharge from Koocanusa Reservoir above the dam has led to unnatural warming and thermal flux in historical downstream spawning habitats of Burbot. Multiple linear regression modeling determined the influence of different factors on river temperature including Koocausa storage, reservoir discharge, tailwater temperature, tributary flow, regional air temperature, and winter year. It was concluded that daily changes in air temperature predominately explain variance in river temperature in the modeled post-dam regime; other factors in the model were comparatively minor. An exception is during December, when high reservoir discharges potentially have equal or more impact than climate on river temperature. This finding suggests that December discharge and concomitant river warming precludes the formation of ice later in winter. In contrast, the winter pre-dam regime was characterized by ice cover that dampened the thermal impact of typical daily fluctuations in climate and river flow. No solution could be resolved from modeling that returns ice cover to the river without major conflict with existing regulatory commitments to flood control, mitigation for other endangered fish, and hydroelectric power generation. It is recommended that existing resources for Burbot conservation be committed to restoring, monitoring, and evaluating tributary and side-channel habitats that may prove to be more gainful for supporting natural reproduction and recruitment.

## 5.1. Introduction

Operation of Libby Dam (Libby, Montana) by the U.S. Army Corps of Engineers (USACE), in conjunction with hydroelectric power management by Bonneville Power Administration (BPA), has been implicated as one of several factors causing the imperilment of North American Burbot (*Lota lota maculosa*) in the lower Kootenai River (Hardy and Paragamian 2013; BC Hydro 2015). Since the dam's completion in 1972, river regulation has altered the natural hydrograph and thermograph, resulting in higher flows in fall−winter and lower flows in spring−summer; concomitantly, downstream temperatures are warmer between October−March, slightly cooler in April, and colder in late-summer. The main channel no longer has extensive ice cover after

construction of the dam, and this loss of ice has led to warming and more thermal flux in possible winter spawning habitats of Burbot in the main channel (Ashton et al. 2021). Beginning in 2003, the dam has operated under guidelines of Variable-Flow Flood Control (VARQ) to provide greater storage in Koocanusa Reservoir for flood control and increased springtime flows for White Sturgeon (*Acipenser transmontanus*) protected under the Endangered Species Act (ESA) (USFWS 2006). VARQ has generally resulted in lower discharge in winter and higher discharge in spring/summer compared to previous phases of dam operation. The effect of VARQ on the average post-dam thermal regime has been subtle, with slightly cooler winter/spring temperatures in the meander reach over the last two decades (see Chapter 1, subheading 1.3 River Regulation).

Modeling of different factors (e.g. discharge, air temperature, tributary flow, etc.) potentially affecting the post-dam thermal regime has been done for spring−summer because it is linked to the U.S. Fish and Wildlife Service (USFWS) Biological Opinion (BIOP) recommendation to maintain river temperatures ≥10°C for sturgeon spawning and natural in-river reproduction (Easthouse 2009). Libby Dam operations incorporate a selective withdrawal system that enables discharge of water from different reservoir depths to better mimic the pre-dam thermograph downstream (Hoffman et al. 2002). There is a paucity of longitudinal river temperature data prior to 2003 because it predates the BIOP and VARQ, with only a single long-term thermographic recording from 1963−present at Porthill (Idaho) located 183 river kilometers (rkm) downstream from Libby Dam (U.S. Geological Survey [USGS] station 12322000). With the onset of VARQ, thermographic records from 1999−present also became available at Bonners Ferry (Idaho) near historical White Sturgeon and Burbot spawning grounds located ~95 rkm downstream of Libby Dam (USACE station BFEI [1999-present]; USGS station 12309500 [2011−present]). Records of tributary temperatures are mostly associated with autumn because of monitoring and evaluation for Bull Trout (*Salvelinus confluentus*) spawning, and only in recent years have winter thermographs been recorded for several tributaries with historical Burbot spawning (S. P. Young, Kootenai Tribe of Idaho [KTOI], personal communication).

The main objective of this study is to follow-up modeling efforts by Easthouse (2009) and evaluate similar factors potentially influencing Kootenai River temperatures for the winter−spring regime near historic spawning habitat of Burbot. Comparisons are made with results from Easthouse (2009) to elucidate any winter-specific pattern changes. Findings from the present study will supplant missing information about qualitative and quantitative mechanisms impacting the post-dam thermal regime during a relatively unstudied winter period of VARQ operations at Libby Dam.

## 5.2. Methods

### 5.2.1. Study Area

Background information about the lower Kootenai River watershed, river regulation, and Burbot conservation has been reviewed in Chapter 1 of this dissertation. Additionally, an in-depth detail of the Kootenai River Subbasin is covered by the Kootenai Tribe of Idaho and Montana Fish, Wildlife & Parks (2004). Easthouse (2009) also provides a brief synopsis of the study area drainage, dam operations, and regional climate.

### 5.2.2. Modeled Parameters and Data Sources

Data were sourced between November–April for years 2002–2021. Modeling parameters included a single dependent response (Kootenai River temperature at Bonners Ferry) and five explanatory effects: 1) Koocanusa Reservoir storage, 2) ambient air temperature at Libby, 3) river discharge at Libby, 4) river temperature at Libby, and 5) cumulative tributary flow between Bonners Ferry and Libby.

Temperatures of the Kootenai River at Bonners Ferry were sourced from USACE station BFEI (2002–2011) and USGS station 12309500 (2011–2021). Koocanusa Reservoir storage estimates and air temperatures were obtained from USACE station LIB. River discharges at Libby came from USGS station 12301933. River temperatures at Libby came from USACE station LIBM. Cumulative tributary flows between Bonners Ferry and Libby were estimated by the difference between discharge at Bonners Ferry (USGS station 12310100) and the previous day of discharge at Libby. Easthouse (2009) estimated the time lag of discharge traveling between these two locations was approximately 24 hours. The same time lag was applied to other upstream effects including river temperature at Libby and Koocanusa Reservoir storage. River data were sourced from web databases for USACE (Dataquery 2.0; https://www.nwd-wc.usace.army.mil/dd/common/dataquery/www/) and USGS (National Water Information System (https://waterdata.usgs.gov/nwis/). Winter temperature records between 2014–2016 were also sources from lowland reaches of nine creeks or tributaries downstream of Bonners Ferry including Canyon, Caribou, Corn, Cow, Deep, Fisher, Goat, Myrtle, and Trout (K. Greenleaf and G. Hoyle, KTOI, personal communication).

### 5.2.3. Data Analyses

Following methods from Easthouse (2009), multiple linear regression (MLR) was used to estimate the proportion of variance (adjusted $R^2$) and the influence of parameters estimates (β) from multiple effects on river temperature at Bonners Ferry. Type III analysis of variance (ANOVA) *F*-tests summarized the significance of each effect in the model. Auto-correlation between regression

residuals was removed by randomizing rows of variables in the dataset with respect to time. A Durbin-Watson test and plot of residuals against time verified that serial correlation was not significant in each model analysis (Easthouse 2009). All generalized linear model analyses were validated with homoscedastic scatterplots of residuals versus fitted estimates. Assumptions of normal error distribution were verified with quantile–quantile plots. Type I error ($\alpha$) was defined at 0.05 for all statistical tests. Separate regression plots of river temperature at Bonners Ferry against river discharge and air temperature at Libby were produced for December, January, February, March, and April to visualize any change in trends during the winter−spring season. Average daily mainstem temperatures at Bonners Ferry were compared with daily temperatures averaged across nine different downstream tributaries between February−April for years 2014−2016. All statistical analyses were performed in SAS (Statistical Analysis Software program, PROC REG, version 9.4; SAS Institute, Cary, NC, USA).

## 5.3. Results

A comparison of main channel thermographs from Libby and Bonners Ferry between 2003−2021 shows greater temperature fluctuation, faster cooling in December−January, colder temperatures in February, and faster warming in March−April at the Bonners Ferry reach (Figure 5.0.1).
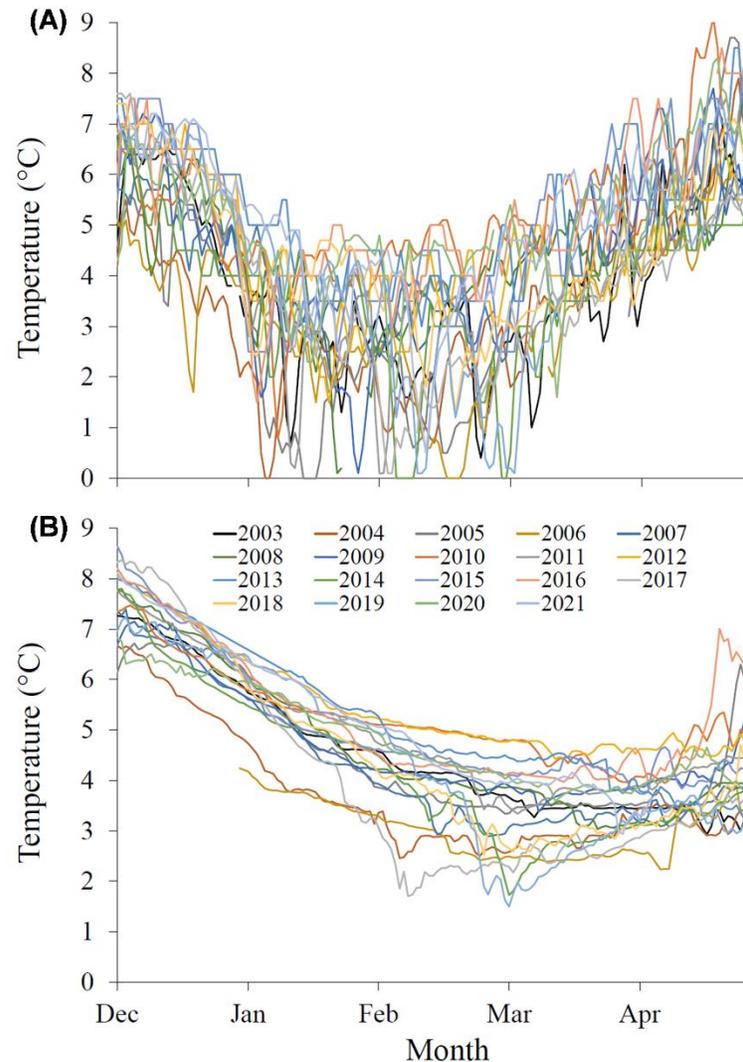
**Figure 5.0.1.** Average daily temperatures of the mainstem Kootenai River at Bonners Ferry (Idaho; panel A) and Libby (Montana; panel B) between December−April for years 2002−2021. Temperature data for years 2002−2011 and 2011−2021 at Bonners Ferry were sourced from the U.S. Army Corps of Engineers (Dataquery 2.0, USACE station BFEI) and U.S. Geological Survey (USGS station 12309500), respectively. Temperature data for years 2003−2021 at Libby were sourced from the U.S. Army Corps of Engineers (Dataquery 2.0, USACE station LIBM). Winter years shown in the graph include December from the previous year.

Graphical analysis of extreme examples of cold and warm winters (2016 and 2017, respectively) illustrate that upward and downward spikes in river temperature at Bonners Ferry are synchronized with similar trends in regional air temperature but not discharge immediately below Libby Dam (Figure 5.0.2).
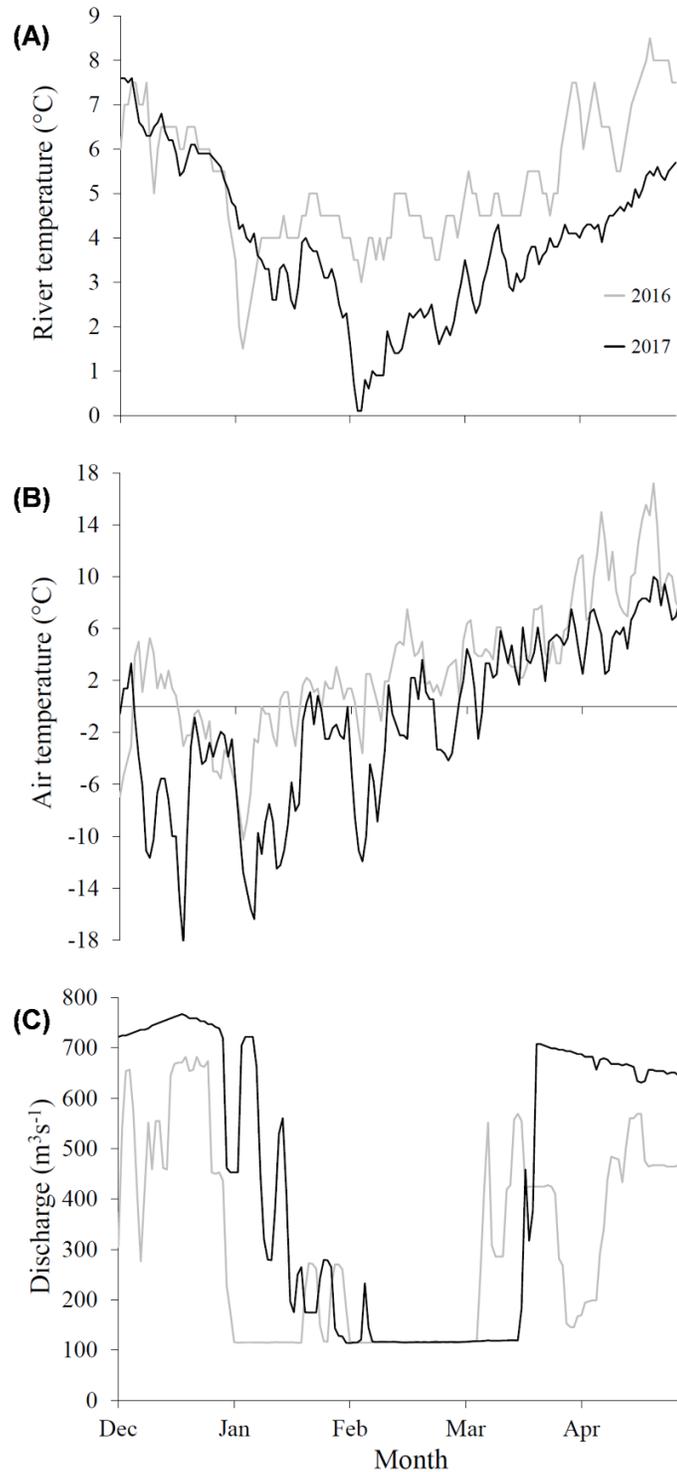
**Figure 5.0.2.** A comparison of average daily temperatures of the mainstem Kootenai River at Bonners Ferry (Idaho; panel A) between December–April for years 2016–2017 with average air temperature and river discharge at Libby (Montana; panels B and C, respectively). River temperature data were sourced from USGS station 12309500. Air temperature data were obtained from the U.S. Army Corps of Engineers (Dataquery 2.0, USACE station LIB). Discharge data came from USGS station 12301933. Winter years shown in the graph include December from the previous year.

Regressions of river temperature at Bonners Ferry against Libby Dam discharge and regional air temperature for each month between December−April indicate changes in the correlation and proportion of variance explained by air temperature and discharge (Figure 5.0.3).
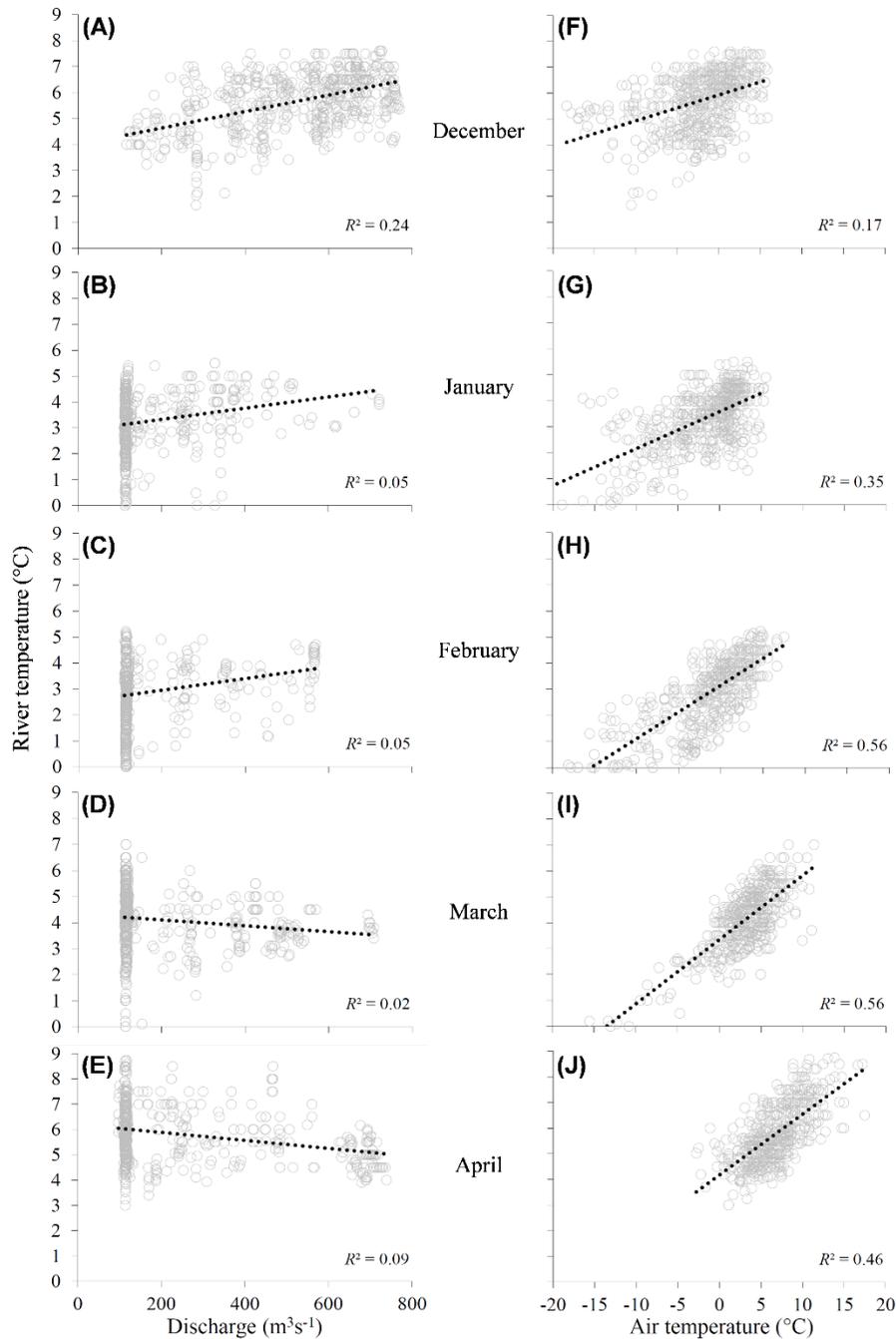


**Figure 5.0.3.** Regressions of average daily temperatures of the Kootenai River at Bonners Ferry (Idaho) against average discharge (panels A−E) and air temperature (panels F−J) at Libby (Montana) between December−April for winter years 2003−2021. See Figures 5.0.1 and 5.0.2. for details about the data sources.

Discharge initially has a positive effect on river temperature in early winter, but then transitions to a negative effect in early spring. Conversely, air temperature consistently has a positive correlation with river temperature. In December, discharge explains a higher proportion of the variance, but thereafter air temperature is a more influential and explains nearly half or more of the variance in February, March, and April. Interestingly, the highest and lowest river temperatures in mid-winter and early spring coincide with the lowest discharge (Figure 5.3C, D, E).

When other effects are considered in multiple linear regression (MLR) analyses, air temperature remains the most influential factor that explains modeled variance in river temperature at Bonners Ferry between 2002−2021 for the months of December−April (Table 5.0.1). Over the last 19 years, there has been a significant warming trend, with an estimated yearly increase of $0.0402 \pm .0034$ °C (mean $\pm$ SE) or ~1°C over the last 25 years. Tributary flow may have a small cooling effect in the months of January−April. The river temperature at Bonners Ferry is estimated to change $0.5804 \pm .0034$°C for each 1°C change immediately below the dam, with a thermal impact of Koocanusa Reservoir possibly being greater during in December and February. Likewise, each 1°C change in air temperature is estimated to change the river temperature at Bonners Ferry by $0.2234 \pm .0040$°C, with regional climate impacts possibly being greater in March and April, but weakest during December. Discharge at Libby Dam has a warming effect between December−February but cooling effect from March−April. However, unlike previous graphical analyses, the MLR model estimates the strongest effect of discharge in January. Storage in Koocanusa Reservoir was not a significant effect in January and March and otherwise not very influential in the model. Overall, the order of effect significance on Bonners Ferry river temperature from highest to lowest is consist across December−April, and is as follows: 1) air temperature, 2) river temperature at Libby, 3) discharge at Libby, 4) winter year, 5) tributary flows, and 6) Koocanusa storage. There are minor deviations from this pattern for certain months and in terms of proportional strength for each effect.

**Table 5.0.1.** Summary statistics from a multiple linear regression model with the mainstem Kootenai River average daily temperature between December−April for years 2002−2021 as the single response variable dependent on six other effects. Winter years include December from the previous year.

| Model Effects | Month | $N$ | $R^2$ | β Estimate | β SE | $F$ | $P$ |
|---|---|---|---|---|---|---|---|
| All | Dec−Apr | 2741 | 0.72 | | | 1061 | <.0001 |
| Winter Year | Dec−Apr | 2741 | | 0.0402 | 0.0034 | 142 | <.0001 |
| Libby Air Temperature | Dec−Apr | 2741 | | 0.2234 | 0.0040 | 3182 | <.0001 |
| Tributary Flow | Dec−Apr | 2741 | | 0.0002 | 0.0002 | 1 | 0.2809 |
| Libby Water Temperature | Dec−Apr | 2741 | | 0.5804 | 0.0186 | 978 | <.0001 |
| Libby Discharge | Dec−Apr | 2741 | | 0.0018 | 0.0001 | 293 | <.0001 |
| Koocanusa Storage | Dec−Apr | 2741 | | 0.0002 | 0.0000 | 13 | 0.0003 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| All | Dec | 588 | 0.85 | | | 465 | <.0001 |
| Winter Year | Dec | 588 | | 0.0461 | 0.0037 | 156 | <.0001 |
| Libby Air Temperature | Dec | 588 | | 0.0896 | 0.0043 | 444 | <.0001 |
| Tributary Flow | Dec | 588 | | -0.0027 | 0.0004 | 60 | <.0001 |
| Libby Water Temperature | Dec | 588 | | 0.8259 | 0.0421 | 386 | <.0001 |
| Libby Discharge | Dec | 588 | | 0.0018 | 0.0001 | 206 | <.0001 |
| Koocanusa Storage | Dec | 588 | | 0.0002 | 0.0001 | 9 | 0.0034 |
| | | | | | | | |
| All | Jan | 584 | 0.83 | | | 441 | <.0001 |
| Winter Year | Jan | 584 | | 0.0577 | 0.0041 | 202 | <.0001 |
| Libby Air Temperature | Jan | 584 | | 0.1817 | 0.0047 | 1501 | <.0001 |
| Tributary Flow | Jan | 584 | | -0.0053 | 0.0006 | 85 | <.0001 |
| Libby Water Temperature | Jan | 584 | | 0.5216 | 0.0304 | 294 | <.0001 |
| Libby Discharge | Jan | 584 | | 0.0043 | 0.0002 | 454 | <.0001 |
| Koocanusa Storage | Jan | 584 | | 0.0000 | 0.0001 | 0 | 0.7673 |
| | | | | | | | |
| All | Feb | 521 | 0.84 | | | 429 | <.0001 |
| Winter Year | Feb | 521 | | 0.0544 | 0.0047 | 136 | <.0001 |
| Libby Air Temperature | Feb | 521 | | 0.1908 | 0.0055 | 1185 | <.0001 |
| Tributary Flow | Feb | 521 | | -0.0017 | 0.0005 | 13 | 0.0003 |
| Libby Water Temperature | Feb | 521 | | 0.6242 | 0.0326 | 366 | <.0001 |
| Libby Discharge | Feb | 521 | | 0.0019 | 0.0002 | 98 | <.0001 |
| Koocanusa Storage | Feb | 521 | | -0.0004 | 0.0001 | 31 | <.0001 |
| | | | | | | | |
| All | Mar | 521 | 0.75 | | | 244 | <.0001 |
| Winter Year | Mar | 521 | | 0.0494 | 0.0052 | 89 | <.0001 |
| Libby Air Temperature | Mar | 521 | | 0.2255 | 0.0084 | 729 | <.0001 |
| Tributary Flow | Mar | 521 | | -0.0011 | 0.0002 | 20 | <.0001 |
| Libby Water Temperature | Mar | 521 | | 0.5402 | 0.0375 | 208 | <.0001 |
| Libby Discharge | Mar | 521 | | -0.0007 | 0.0002 | 12 | 0.0006 |
| Koocanusa Storage | Mar | 521 | | 0.0000 | 0.0001 | 0 | 0.8208 |
| | | | | | | | |
| All | Apr | 527 | 0.75 | | | 252 | <.0001 |
| Winter Year | Apr | 527 | | 0.0436 | 0.0052 | 72 | <.0001 |
| Libby Air Temperature | Apr | 527 | | 0.2335 | 0.0089 | 686 | <.0001 |
| Tributary Flow | Apr | 527 | | -0.0015 | 0.0003 | 34 | <.0001 |
| Libby Water Temperature | Apr | 527 | | 0.5210 | 0.0392 | 177 | <.0001 |
| Libby Discharge | Apr | 527 | | -0.0017 | 0.0001 | 168 | <.0001 |
| Koocanusa Storage | Apr | 527 | | 0.0003 | 0.0001 | 25 | <.0001 |

A comparison of average daily river temperatures at Bonners Ferry with water temperatures averaged across nine different regional downstream tributaries between February−April for years 2014, 2015, and 2016 shows that tributaries have consistently colder water temperatures throughout the typical spawning and egg incubation period of Burbot (Figure 5.0.4). First hatch of embryos, spawned at an assumed peak spawning time of February 15th, is estimated to be delayed by at least one week in tributaries using embryo development models developed by Ashton et al. (2021).
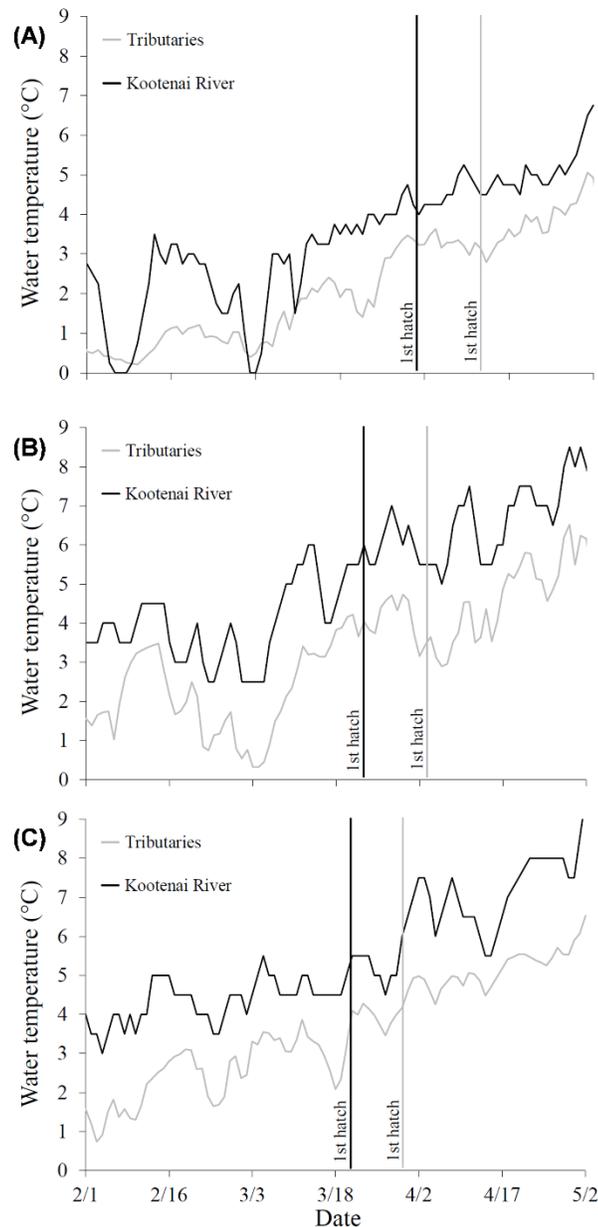


**Figure 5.0.4.** A comparison of average daily temperatures of the mainstem Kootenai River at Bonners Ferry with daily water temperatures averaged across nine different regional downstream tributaries between February−April for years 2014, 2015, and 2016 (panels A, B, and C, respectively). Vertical lines represent demarcations of estimated first hatch of Burbot embryos spawned at an assumed peak spawning time of February 15th. Sources of temperature data are detailed in the Methods under section 5.2.2.

It is also evident from Figure 5.0.4. that thermal fluctuations are synchronous between the Kootenai River at Bonners Ferry and the sampled downstream tributaries, despite being hydrologically independent from each other. This finding confirms that the common factor of climate or air temperature has a major influence on the fluvial thermal regimes of the subbasin in winter−spring.

## 5.4. Discussion

Regulation of Kootenai River discharge and storage in Koocanusa Reservoir are the only controllable effects modeled in this study, but neither factor explains most of the variance in river temperatures near historic spawning/egg incubation habitats of Burbot between December−April for the VARQ phase of the post-dam regime since 2003. Daily changes in air temperature have the strongest effect on post-dam river temperatures in winter, which parallels findings by Easthouse (2009) for spring. River temperatures immediately below Libby Dam were the second strongest effect in the present winter model, as well as the spring model by Easthouse (2009). That investigation also concluded that Libby Dam discharge and tributary flows had a small negative correlation with river temperatures near Bonners Ferry in spring. This agrees with the present model results for March and April, however, discharge in early winter months has a stronger influence, particularly in January and December. The effect of Libby Dam discharge on downstream river temperatures is generally weak during February, but this is likely due to more consistent regulation of minimum flows compared to other months.

Colder and more stable river temperature characteristic of the predam regime during winter were a result of ice cover that shielded or insulated the river from more erratic changes in air temperature (Partridge 1983). Thus, loss of river ice due to hydropower operations at Libby Dam is the primary cause for warming and flux in the post-dam regime during winter, and fluctuations in daily air temperature is really a secondary effect that now predominates without the tempering effects of ice cover. Cott et al. (2013) emphasized the importance of winter ice to provide a cold, stable, and austere environment for successful Burbot reproduction. Ashton et al. (2019, 2021) demonstrated that warming above 3°C during the first two weeks of embryo development leads to increased deformity and mortality, and fluctuations to 5°C has severe adverse effects during that sensitive period of embryogenesis. Episodic climate warming in some winters (e.g. 2016) may cause the post-dam regime to become inhospitable for successful reproduction of Burbot.

The warmest and coldest river temperatures during the peak of Burbot spawning in February coincide with the minimum regulatory discharge of 113 m³/s or 4,000 ft³/s (see USWFS 2006 for a review of minimum flows). Contemporary post-dam flows in January−February are relatively close to pre-dam levels (see Chapter 1: Figure 1.0.3), and it is possible that absence of thick ice formation in

the lower Kootenai River is mostly due to high reservoir discharges in December. Reduced discharge throughout December might improve the likelihood of ice cover downstream. However, implementing such a change in Libby Dam operations contradicts established power generation and flood management goals for the Kootenai River Subbasin and larger Columbia River Basin. Conversely, higher discharges in February might lead to more thermal stability downstream, but it would probably be near 4°C based on results shown herein, which is above the thermal preference for normal Burbot embryo incubation (Ashton et al. 2019, 2021). There are also potential negative impacts of higher discharge on Burbot spawning migration upstream (Paragamian et al. 2005; Paragamian and Wakkinen 2008), and unknown consequence of higher current flows on the habitat of incubating embryos. Additionally, implementing higher winter discharges for Burbot management contradicts VARQ operations at Libby Dam specifically managed for increasing winter reservoir storage to support higher spring−summer flows for endangered White Sturgeon.

A less contentious management plan for improving the success of Burbot reproduction in the lower Kootenai River watershed may be to focus on habitat restoration in spawning tributaries and side channels of the mainstem. Recent data shows that some tributaries are colder than the mainstem between February−April, plus these tributaries appear to remain <4°C throughout winter even during warm climatic episodes (e.g. 2016). Additionally, the timing of first hatch in tributaries could be later in the spring, and this may be advantageous for larval recruitment that potentially hinges upon increased temperatures and productivity in the mainstem (see Chapter 4: section 4.5 Discussion). Ross et al. (2020) reported that river-adapted hatchery fish are migrating to tributaries during the winter and releasing gametes, but more research is needed to determine if these spawning events support substantial natural recruitment. Reconstruction of side-channel habitats in the lower Kootenai River might also benefit Burbot reproduction by providing different thermal exposures, spawning substrates, hydrodynamics, and predation levels compared to existing conditions in the main channel of the post-dam regime—which are known to be unsupportive of consistent natural recruitment. Overall, more spatiotemporal evaluations of water temperature and other environmental attributes are needed to better gauge the suitability of different Burbot spawning habitats throughout the lower Kootenai River watershed.

Lastly, the mainstem of the Kootenai River near Bonner Ferry between December−April potentially warmed ~0.8°C over the last 19 years of regulation under VARQ. This trend coincides with significant evidence of global warming over the same period (IPCC 2018). If similar levels of warming continue throughout the 21st century, then the contemporary post-dam thermal regime may cease to support functional reproduction of Burbot within 100 years due to the lethal effects of elevated water temperatures on early-stage embryos. Further investment in mitigation strategies

involving hatcheries, habitat restoration, and hydropower management will likely be critical for preserving this imperiled population.

## 5.5. Conclusions

A multiple linear regression model of mainstem Kootenai River temperatures near Bonners Ferry during contemporary VARQ regulation at Libby Dam found that regional air temperature explains most of the variance in river temperature during winter. This conclusion parallels findings from a study by Easthouse (2009) that incorporated similar modeling efforts for April and May. Loss of natural ice cover in the meander reach due to upstream discharge from Koocanusa Reservoir between December−March underlies river sensitivity to daily air temperature changes that produce thermal flux and warming in the typical winter post-dam regime. No recommendations for dam operations could be resolved without presenting unreasonable conflict with existing flood management, power generation, and mitigation efforts for other fisheries protected under ESA. Instead, resources devoted to conversation of imperiled Burbot in the lower Kootenai River should focus on restoring, monitoring, and evaluating habitats in tributaries, side-channels, and other off-channel areas that are potentially supportive of natural reproduction and recruitment.

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# Conclusions

The most important finding of this dissertation was confirming that river-adapted hatchery Burbot mature and volitionally release viable gametes following multiple years of exposure to the post-dam regime of the lower Kootenai River. Up until that collaborative experiment with KTOI and IDFG in 2017, it was unknown if all the work and expense committed toward,

- a conservation strategy initiated in 2005 by KTOI and KVRI stakeholders
- decades of monitoring and evaluation work by IDFG
- years of research by B.C. Ministry on lake and river populations in the subbasin
- more than a decade of experimental hatchery research by UI−ARI
- a multi-million-dollar Twin Rivers Hatchery completed in 2015

would ultimately lead to a successful conservation program that delivered on the goal of restoring a viable and harvestable Burbot population. It is important to reemphasize that the post-dam thermal regime is often 3−6°C warmer than pre-dam levels just prior to peak spawning in February, and Burbot are an extreme winter specialist uniquely adapted to cold water. The ability of 3-month-old juvenile hatchery fish to survive, grow, mature, and reproduce in the drainage is fortuitous for the population, management program, and more specifically to my dissertation—because it narrows the timeframe of suspected recruitment failure to embryonic and early larval ontogeny.

The post-dam thermal regime of the lower Kootenai River presents two major obstacles for embryo incubation: 1) reservoir discharge precludes ice formation in the mainstem and subsequent river warming potentially shortens the spawning period to coincide with suboptimal temperatures for embryo development, and 2) fluctuations in river temperature coinciding with regional climate changes and discharge likely cause more embryo deformity and mortality when waters warm above 3°C during the first two weeks of embryogenesis. For certain climate anomalies causing river temperature spikes to 5°C in February, it is probably almost lethal for embryos and severely limits reproduction from spawning habitats in the main channel. This latter scenario is not yet a common occurrence, but evidence of a ~0.8°C warming trend in the meander reach near Bonners Ferry over the last 19 years—along with global climate predictions of further warming in the 21st century— foreshadow an unfavorable future for Burbot reproduction from main-channel spawning habitats. Further reductions in discharge between December−March to encourage ice formation in the mainstem conflicts with societal needs for flood control and electrical power generation. Therefore,

my recommendation is that Burbot conservation efforts focus on restoring tributary and side-channel habitats that may provide better spawning refuge from mainstem warming compounded by hydropower and climate change. There is recent evidence of river-adapted hatchery Burbot migrating into tributaries and releasing gametes (T. J. Ross, IDFG, personal communication), and restoration work may be an important tool for mitigating the impacts of Libby Dam without complicating hydropower management.

Colder incubation temperatures in tributaries should improve Burbot embryo survival and delay hatching. This assumption needs to be confirmed with field studies because it is critical to the calculus of recruitment. Incubation temperature is not the only determinant of embryo survival to hatch, and this research must consider other factors such as hydrodynamics, substrate, predation, and disease. Subsequent transport of larvae from tributaries to mainstem nursery habitats should coincide with warmer river temperatures later in spring. Biologists need to verify that the meander reach is the common sink for passive downstream migration of protolarvae. Spatiotemporal and ecophysiological mechanisms of protolarvae migration are key factors to our understanding of recruitment. Experiments herein determined that yolk-sac larvae transitioned to exogenous feeding on rotifers within the typical range (4−7°C) of early-spring temperatures in the lower Kootenai River. These larvae also exhibited high survival up to four weeks after hatch in the laboratory. Previously, it was unclear if larvae could thrive in a post-dam thermal regime between March−April because the mainstem is often <8°C—a former misconstrued thermal threshold for first feeding by other investigators. However, larvae will likely exhibit accelerated swim bladder inflation and improved feeding, growth, and performance in waters >6°C. It is also possible that larval rearing at <6°C increases the risk of "point of no return" or PNR after larvae size exceeds 4.6 mm TL. Overall, my research suggests that newly hatched larvae can persist in cold regimes if other rearing factors like hydrodynamics, substrate, predation, disease, and phytoplankton/zooplankton availability match with larvae requirements for swim bladder inflation and first feeding.

Cumulative mortality between swim-up and first feeding is the greatest unknown component of Burbot recruitment in the lower Kootenai River watershed. This phase of ontogeny is called the "critical period" for many fishes with indirect early life histories with larval stages. High mortality of Burbot can occur during the critical period in a hatchery setting, and it is reasonable to assume that it may be a recruitment bottleneck in nature when nursery environments are suboptimal for growth and survival. Future recruitment research should be committed to identifying mortality estimates for swim-up and first feeding across the system including main channel, off channel, tributary, and lake habitats. This will probably be the most difficult aspect of monitoring and evaluation for the Burbot conservation program, and it will take years of work to determine when, where, and how larvae exist

through the critical period. There should be at least a month-long period to monitor Burbot larvae abundance in the mainstem before PNR risks become widespread. Assuming a peak spawning time of mid-February and given accurate modeling results from this dissertation, larvae monitoring in the meander reach and South Arm of Kootenay Lake should begin the third week of March and continue until at least the end of May. Millions of yolk-sac larvae are now being stocked by KTOI annually in various habitats of the system and the outlook for natural reproduction is improving. It should be possible to capture substantial numbers of larvae and to get estimates of abundance and vital rates for recruitment modeling. If proven sampling techniques fail to capture larvae in the river or lake in spring, then it is reasonable to hypothesize that system-wide challenges exist for embryo rearing and/or swim-up.

Incompatibilities between early life history and anthropogenic changes to the ecosystem underlie successive recruitment failures of wild Burbot in the lower Kootenai River. The predominate causality remains uncertain, but the following sequence of winter−spring events related to hydropower operations at Libby Dam likely have a role:

1) High discharge from Koocansua Reservoir between December−January possibly discourages upriver spawning migrations and prevents formation of thick ice cover in the main channel during peak spawning.

2) River temperatures in winter fluctuate with air temperature and discharge around a baseline post-dam temperature that averages 3°C warmer than pre-dam levels.

3) Embryos incubating in the post-dam regime experience warming and thermal flux that increases deformity and mortality but shortens the incubation period.

4) Embryos in the mainstem hatch in March and larvae are exposed to a post-dam regime characterized by higher flows and colder temperatures compared to pre-dam levels.

5) Cold, nutrient-poor discharges from Koocanusa Reservoir between March−April reduce the rate of river warming and suppress productivity in the food web.

6) Colder river temperatures ~~and diminished plankton abundance~~ in early spring reduces swimming activity, feeding, growth, and performance of Burbot larvae.

Evidence herein suggests that colder post-dam regimes in spring and loss of nursery habitats in the meander reach best explains successive recruitment failures of Burbot in the lower Kootenai River. An alternative explanation could be that mass mortality occurs during swim-up in tributaries and/or the mainstem due to unknown challenges related to hydrology and/or ecology. A more distant theory

for recruitment failure is wide-spread mortality of embryos, but this seems unlikely given the number of tributaries that should support some level of embryo rearing to hatch, even when thermal conditions in the mainstem are too warm during extreme winter climate episodes.

Recommendations herein subscribe to mitigation strategies that do not require drastic changes in hydropower operations. A return of winter ice cover to the mainstem and a natural flood pulse in spring to the meander reach is not a societal reality for the 21st century. A more popular solution could be to focus on restoring off-channel habitats and floodplain lakes as nurseries for larvae. There are early indications that yolk-sac larvae stocked into inundated floodplain habitat can recruit to the Burbot fishery (S. P. Young, KTOI, personal communication). The conservation program still has work to do if it hopes to deliver on the overarching goal of restoring natural reproduction of Burbot in the lower Kootenai River. Identifying and remediating causalities of recruitment failure will be huge undertakings that likely require decades of mitigation work. The short-term outlook for natural reproduction is hopeful as the stocking program rebuilds the population and more information is gained about ecophysiology of early life stages. The long-term outlook is more foreboding if global warming trends continue through this century. One of the greatest existential threats to southern Burbot populations throughout the Holarctic ecozone is warming of embryo rearing habitat. This unilateral externality will impact altered and natural ecosystems with few options for refuge. The onus of conserving these populations may largely fall on aquaculture programs to maintain stocks and possibly even select for genetic adaptations that improve the thermal tolerance of embryos.

# Appendix A - Permission to Reuse Published Article

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**Appendix B - Institutional Animal Care and Use Committee Permissions**

# Institutional Animal Care and Use Committee

**Date:** September 22, 2017
**To:** Kenneth D. Cain
**From:** University of Idaho
Institutional Animal Care and Use Committee
**Re:** Protocol IACUC-2016-54 *Temperature Effects on Early Life Stages of Burbot*

Your requested renewal of the animal care and use protocol listed above was reviewed and approved by the Institutional Animal Care and Use Committee on 09/21/2017.

This renewal was originally submitted for review on: 08/21/2017 02:58:56 PM PDT
The original approval date for this protocol was: 09/29/2016
This approval will remain in effect until: 09/21/2018
The protocol may be continued by annual updates until: 09/29/2019

Federal laws and guidelines require that institutional animal care and use committees review ongoing projects annually. For the first two years after initial approval of the protocol you will be asked to submit an annual update form describing any changes in procedures or personnel. The committee may, at its discretion, extend approval for the project in yearly increments until the third anniversary of the original approval of the project. At that time, the protocol must be replaced by an entirely new submission.

_____
Craig McGowan, IACUC Chair

# University of Idaho
# Institutional Animal Care and Use Committee

**Date:** Thursday, September 29, 2016
**To:** Kenneth D. Cain
**From:** University of Idaho
Institutional Animal Care and Use Committee
**Re:** Protocol 2016-54
Temperature Effects on Early Life Stages of Burbot

Your animal care and use protocol for the project shown above was reviewed and approved by the Institutional Animal Care and Use Committee on Thursday, September 29, 2016.

This protocol was originally submitted for review on: Wednesday, September 7, 2016
The original approval date for this protocol is: Thursday, September 29, 2016
This approval will remain in affect until: Friday, September 29, 2017
The protocol may be continued by annual updates until: Sunday, September 29, 2019

Federal laws and guidelines require that institutional animal care and use committees review ongoing projects annually. For the first two years after initial approval of the protocol you will be asked to submit an annual update form describing any changes in procedures or personnel. The committee may, at its discretion, extend approval for the project in yearly increments until the third anniversary of the original approval of the project. At that time, the protocol must be replaced by an entirely new submission.

_____
Craig McGowan,

# University of Idaho
## Institutional Animal Care and Use Committee

**Date:** Tuesday, September 15, 2015

**To:**   Kenneth D. Cain

**From:** University of Idaho
Institutional Animal Care and Use Committee

**Re:**   Protocol 2013-97
Development of conservation aquaculture techniques for Burbot: continued maintenance of broodstock and progeny

Your requested renewal of the animal care and use protocol shown above was reviewed and approved by the Institutional Animal Care and Use Committee on Tuesday, September 15, 2015.
This protocol was originally submitted for review on: Tuesday, October 15, 2013
The original approval date for this protocol is: Friday, January 31, 2014
This approval will remain in effect until: Thursday, September 15, 2016
The protocol may be continued by annual updates until: Tuesday, January 31, 2017
Federal laws and guidelines require that institutional animal care and use committees review ongoing projects annually. For the first two years after initial approval of the protocol you will be asked to submit an annual update form describing any changes in procedures or personnel. The committee may, at its discretion, extend approval for the project in yearly increments until the third anniversary of the original approval of the project. At that time, the protocol must be replaced by an entirely new submission.

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Barrie Robison, IACUC Chair