

ARTICLE

Temperature and Maternal Age Effects on Burbot Reproduction

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Abstract

River regulation alters the thermal regime of many freshwater ecosystems, and impoundments are known to negatively impact the 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 postdam regime. The river-adapted hatchery adults volitionally spawned in a broodstock facility at 2, 4, and 6°C. The spawning period spanned 18 d at 2°C but was shortened to 6–7 d at 4°C 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°C and 6°C. Young (age-3) and old (age-7 and older) females spawned 4–12 d 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 coldwater spawning habitat. Contraction of the spawning period by warmer temperatures, a truncated population age structure, or both could synchronize spawning to coincide with suboptimal conditions for normal embryo development.

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The Burbot *Lota lota* (Gadidae) is the only freshwater gadid that is widely distributed across the Holarctic ecozone at latitudes greater than 40°N (Van Houdt et al. 2003). Many southern populations of Burbot are imperiled as a result of manmade 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 extreme winter specialists (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 often 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 the 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 midwinter spawning at very cold temperatures (Evenson 2000; McPhail and Paragamian 2000; Cott et al. 2013a). However, there is some evidence of plastic life histories, with spawning times that extend from autumn through summer depending on the population and ecosystem, and most instances of non-winter spawning are 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-hr daylight). The timing and location of Burbot spawning in streams have been linked to the accessibility of cold water (Farkas 1993). Harrison et al. (2016b) found that Burbot actively selected for 4–6°C

habitat during the prespawning period but avoided temperatures >2°C between the spawning and postspawning periods. In contrast, Vught et al. (2008) reported spawning at water temperatures between 4°C and 6°C. Burbot have also been observed spawning in spring waters with a temperature 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 for spawning.

A more informative temperature threshold for Burbot reproduction might be extrapolated from embryo thermal tolerances. Fish often 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 et al. (1981) found that Burbot embryos survived at 1–7°C, but mortality increased sharply above or below 4°C. A similar trend was reported by Vught et al. (2008), except that 8°C was the upper lethal limit. In contrast, 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 after incubation at temperatures above 5.0°C 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 Myers 1993), size (Ouellet et al. 1997), 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 the 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 Eurasian Burbot *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 after 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, Idaho, during winter, showing substantial changes in weight and condition between January and 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 and 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 determine the impacts of postdam temperatures and posthatchery 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, the proportion of spent fish, spawning time, relative fecundity, egg size, embryo deformity, and embryo 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.

METHODS

Study area.—The Kootenai River (“Kootenay River” in Canada) is a tributary to the Columbia River and drains a 41,910-km² basin comprising parts of British Columbia, Montana, and Idaho (Burke et al. 2009). The present study focused 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 1).

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 and March at 38 different sites extending from Creston, British Columbia (rkm 144.5), to the Idaho–Montana border (rkm 276; Figure 1). Details of the hoopnetting methods are described by Ross et al. (2018). One of the sampling sites (i.e., Ambush Rock) is

near a putative main-stem 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 3 weeks of January in 2017 and 2018, the Kootenai Tribe of Idaho (KTOI) followed IDFG hoopnetting efforts and relocated river-adapted hatchery Burbot to the Twin Rivers Hatchery (Moyie Springs, Idaho) for use as broodstock. During transport, the adults were exposed to H₂O₂ at 25 mg/L and NaCl at 5 g/L for up to 60 min as a prophylactic treatment for external parasites.

Within 24 hr of arriving at the hatchery, fish were anesthetized in a buffered (equal parts NaHCO₃) 250-mg/L solution of tricaine methanesulfonate (Tricaine-S; Western Chemical, Ferndale, Washington) to allow determination of age (from PIT tag ID), 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 the sex and age of fish prior to the start of 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 the experiments. Preexperiment holding temperatures were similar to the river thermal regime until the last day of broodstock collection but were then maintained between 2°C and 5°C independent of river temperature until the start of the experiments (Figure 2).

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 were allowed to spawn 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; Frigid Units, Toledo, Ohio). Water was supplied in a flow-through arrangement with inflows of 7.5 L/min for each replicate broodstock tank. Dissolved oxygen levels were at least 9 mg/L according to a handheld meter (OxyGuard Handy Polaris 2; OxyGuard International, Farum, Denmark), and ammonia concentrations were below the detectable levels of a test kit (API; Mars, Inc., McLean, Virginia). Skylighting provided a natural photoperiod with a peak intensity no greater than 1,000 lx at the water surface based on handheld meter readings (MW700 portable lux meter; Milwaukee Instruments, Rocky Mount, North Carolina). Supplemental daylight-spectrum fluorescent lighting was used periodically for egg collection efforts.

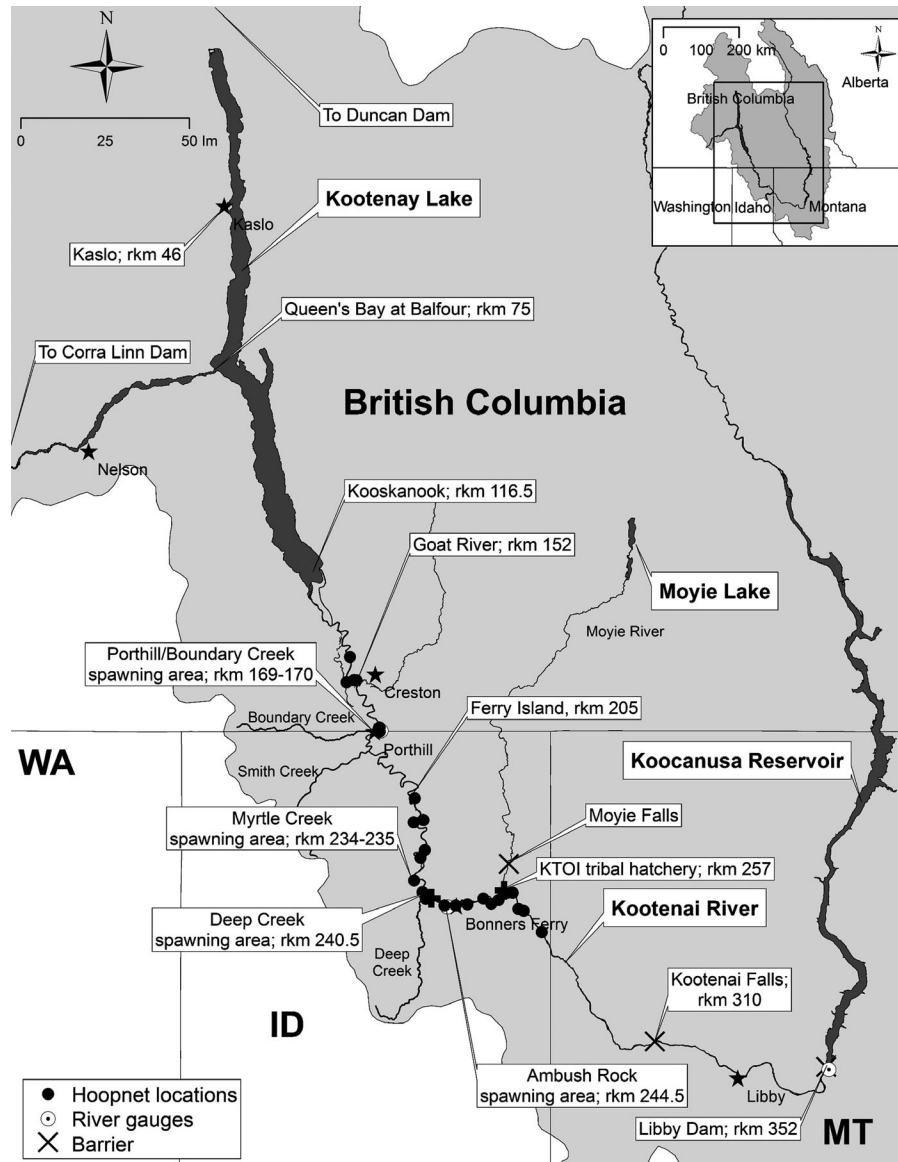


FIGURE 1. Map of the lower Kootenai River study area, with labels identifying donor population source (Moyie Lake), Twin Rivers Hatchery location (Kootenai Tribe of Idaho [KTOI]), hoop-net sampling sites, major tributaries, key river kilometers (rkm), temperature gauging stations, and significant barriers to migration.

Egg collection and incubation.—Eggs were passively collected from the effluent of broodstock tanks via submerged nylon-screen containers with 400- μ m 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 min each day as a

prophylactic treatment against fungal infection (Polinski et al. 2010).

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) on broodstock- and embryo-related response variables (Table 1). Fish were not randomly selected for the experiment because research efforts were linked to hatchery production goals; therefore, only individuals with satisfactory health, Fulton's condition factor ($K \geq 0.50$; Ricker 1975), and size ($TL \geq 397$ mm) were chosen as broodstock. In

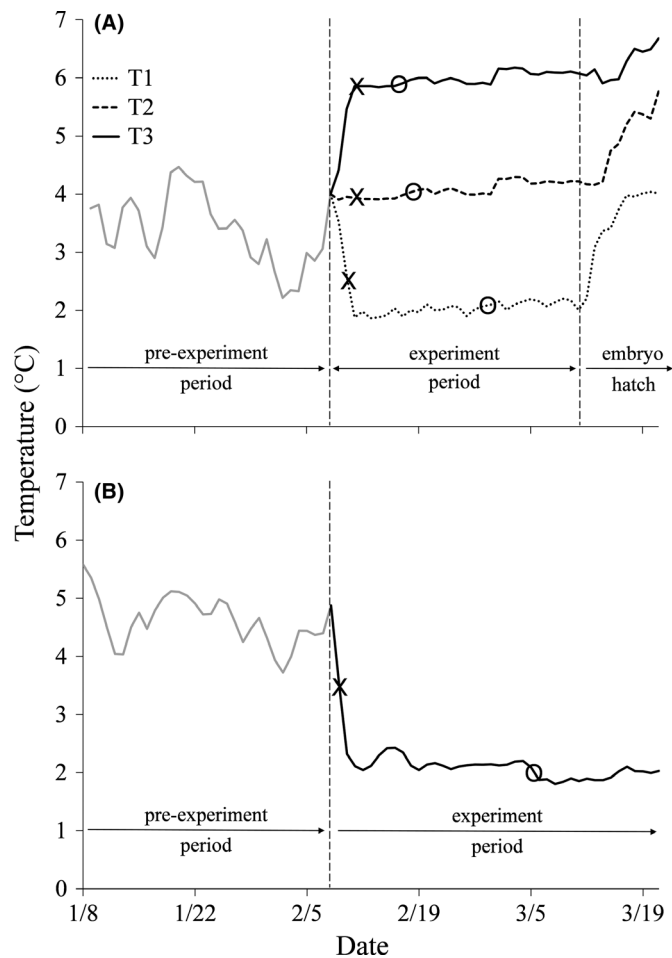


FIGURE 2. Thermographs for Burbot broodstock and egg incubation experiments at Twin Rivers Hatchery in 2017 and 2018. The pre-experiment period spanned the time from broodstock capture in the lower Kootenai River to the start of experiments at the hatchery. (A) In 2017, three temperature treatments (T1, T2, and T3) targeted 2, 4, and 6°C, respectively. (B) A profile of approximately 2°C was used in 2018 during an evaluation of maternal age-groups (X = start of observed spawning; circle = end of observed spawning).

total, 45 males and 36 females were selected for the experiment (Table 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 and 6 years old. Each treatment included three replicate 1,700-L, flat-bottom, circular tanks, with four females and five males per tank. Fish were stocked into the experiment at 4°C and were gradually acclimated to the different temperature treatments over a 2-d period (Figure 2). Observations of volitional spawning and egg collections ended on February 28, 2017. Each adult was anesthetized and measured for postexperiment morphometrics and spawning condition. Males were

checked for the 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). 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 d postspawning (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 embryo survival (AmScope SM-1TZ-RL-10MA trinocular microscope with MT1000-CK 10.7-megapixel digital camera; AmScope, Irvine, California). The percentage of living embryos in the subsamples was 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, Maryland).

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 (ages 3, 5, 6, and 7 and older [7+]) on broodstock- and embryo-related response variables (Table 1). In total, 64 males and 32 females were selected for the experiment (Table 2). Each treatment included two replicate 1,700-L, flat-bottom, circular broodstock tanks with four females and eight males per tank. Male broodstock ranging in age from 3 to 7 years were evenly distributed into the tanks. Female broodstock were segregated into tanks according to the four different maternal age-groups. The age-7+ females consisted of 2009 and 2011 year-classes; two females were of unknown age, but their large size and weight (i.e., 796 and 840 mm TL; $W = 5.1$ 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 were gradually acclimated to 2.0–2.4°C (Figure 2). Spawning observations and egg collections were terminated on March 5, 2018. Each adult was anesthetized, measured for postexperiment 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 stage (Figure 2). The previously described methods of sampling and imaging were used for estimating egg

TABLE 1. A list of covariate, response, and treatment variables used in statistical analyses of results for Burbot from study years 2017 and 2018 (pre = preexperiment; post = postexperiment).

Variable	Description	Abbreviation	Definition or formula	Units
Covariate	Preexperiment 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	Number of days into February
Spawning period		t_p	Number of days between the first and last spawn	d
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	[(number of spent females)/(number of females)]·100	%
Percent weight loss		WL	$ [(W_{post} - W_{pre})/W_{pre}] \cdot 100 $	%
Specific weight loss		SWL	$ [\log_e(W_{post}/W_{pre})/\text{days}] \cdot 100 $	%/d
Egg diameter		E_d	Cross-sectional distance of a live egg	mm
Change in condition		K	$ K_{pre} - K_{post} $	g/mm^3
Embryo deformity		D	[(number of deformed embryos)/(number of live embryos)]·100	%
Treatment	Embryo survival	S	[(mL of live embryos)/(mL of spawned eggs)]·100	%
	Temperature	T	Constant 2, 4, and 6°C	°C
	Maternal age	A_f	3, 5, 6, and 7+ years old	years

^aSee Methods section for a definition of standard weight (W_s) used to calculate W_r .

diameters, embryo deformity, and embryo survival. Observations of egg incubation ended on March 12, 2018.

Data analyses.—The experimental unit was the subpopulation of fish in each tank. Observational units included individual fish, eggs, and spawning events. Table 1 lists the various covariate, response, and treatment variables included in statistical analyses. Fish robustness was defined by K (Ricker 1975) and relative weight (W_r ; Wege and Anderson 1978) calculated from the standard weight (W_s) equation for North American Burbot *Lota lota maculosa* ($\log_{10}W_s = 2.898 \cdot \log_{10}TL - 4.868$; Fisher et al. 1996). Spearman's rank correlation coefficient (ρ) was used to examine relationships between covariate or response pairs. A Bonferroni correction was used to control the 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 a normal error distribution for simple linear models were verified with quantile–quantile plots. Power transformations determined by the Box–Cox method were used to normalize any non-normal data. Instances of overdispersion were adjusted with a Pearson scale parameter. Type I error (α) 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 (χ^2) are presented with degrees of freedom in subscripts. All statistical analyses were performed in SAS version 9.4 (PROC GENMOD; SAS Institute, Cary, North Carolina).

RESULTS

Temperature Effects on Broodstock Spawning and Embryo Development

Broodstock collection and spawning.—Temperatures in the lower Kootenai River between December 2016 and January 2017 were 3–6°C warmer than pre-dam levels

TABLE 2. Pre- and postexperiment morphometrics (symbols are defined in Table 1) of Burbot broodstock in experiments with temperature and maternal age as treatments. Means (SE in parentheses) are shown for male and female individuals (*N*) within a treatment subpopulation.

Study period and year	Sex	Treatment	<i>N</i>	<i>A</i> (years)	TL (mm)	<i>W</i> (g)	<i>K</i> (g/mm ³)	<i>W_r</i> (%)
Preexperiment, 2017	Female	2°C	12	5.2 (0.3)	562 (24)	1,431 (241)	0.73 (0.03)	103 (5)
	Female	4°C	12	5.4 (0.3)	550 (25)	1,301 (208)	0.72 (0.03)	100 (4)
	Female	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)
	Male	4°C	15	5.1 (0.2)	494 (14)	866 (77)	0.69 (0.02)	96 (3)
	Male	6°C	15	4.9 (0.3)	512 (16)	931 (84)	0.67 (0.02)	93 (2)
Postexperiment, 2017	Female	2°C	12		561 (25)	1,084 (186)	0.55 (0.02)	78 (3)
	Female	4°C	12		551 (25)	1,006 (154)	0.55 (0.02)	78 (2)
	Female	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)
	Male	4°C	15		495 (14)	721 (65)	0.57 (0.02)	79 (2)
	Male	6°C	15		511 (16)	793 (71)	0.57 (0.01)	80 (1)
Preexperiment, 2018	Female	Age 3	8	3.0 (0.0)	471 (14)	834 (78)	0.79 (0.03)	109 (4)
	Female	Age 5	8	5.0 (0.0)	555 (14)	1,216 (107)	0.70 (0.02)	99 (3)
	Female	Age 6	8	6.0 (0.0)	676 (30)	2,411 (308)	0.75 (0.02)	108 (3)
	Female	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)
	Male	Age 5	16	4.6 (0.4)	501 (24)	873 (107)	0.65 (0.02)	90 (2)
	Male	Age 6	16	4.6 (0.4)	482 (22)	837 (119)	0.67 (0.02)	93 (3)
	Male	Age 7+	16	4.5 (0.4)	483 (25)	849 (121)	0.69 (0.02)	95 (2)
Postexperiment, 2018	Female	Age 3	8		472 (13)	667 (63)	0.63 (0.03)	87 (5)
	Female	Age 5	8		552 (15)	939 (87)	0.55 (0.02)	77 (3)
	Female	Age 6	7		670 (32)	1,816 (267)	0.58 (0.02)	83 (3)
	Female	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)
	Male	Age 5	16		495 (23)	745 (95)	0.57 (0.01)	79 (2)
	Male	Age 6	15		488 (23)	756 (104)	0.60 (0.02)	82 (3)
	Male	Age 7+	14		500 (24)	780 (105)	0.58 (0.01)	81 (2)

(Figure 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 preexperiment period, and 11% of males failed to express milt upon capture; however, only one of these individuals was not spermiating in postexperiment examinations. Broodstock survival was 100% for the duration of the 21-d experiment.

The spawning period occurred between February 10 and 28 at 2°C but was between February 11 and 18 at 4°C and 6°C; thus, the total range of spawning events was extended by 11 d at 2°C. A regression analysis estimated that mean \pm SE spawning time (t_s) was delayed by 0.7 ± 0.2 d per 1°C decrease in temperature ($\chi^2_{1,16} = 13.49$, $P < 0.01$). A similar result was found for the mean \pm SE spawning period (t_p), which increased by 1.4 ± 0.1 d per 1°C decrease in temperature ($\chi^2_{1,16} = 18.51$, $P < 0.01$). Temperature did not have a significant effect on collected egg volumes (E_v), relative fecundity (F_r), or egg diameter (E_d ; Table 3). Both E_v and F_r failed to correlate with other

response variables (i.e., t_s , percent weight loss [WL], specific weight loss [SWL], ΔK , and ΔW_r).

Covariates among male and female subpopulations (i.e., upriver distance of fish capture [RKM], preexperiment period [t_{pre}], fish age [A], TL, W , K , and W_r) did not differ between treatments at the start of the experiment (Table 2). Female W_r values were close to average for North American Burbot, while male W_r values were slightly lower than average (Table 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 (i.e., WL and SWL) and diminishing condition (ΔK) than males ($\chi^2_{1,16} = 31.05$, $P < 0.01$), but opposing trends in ΔK between sexes were observed at 4°C (Table 3).

Postexperiment examinations determined that one age-4 female at 4°C potentially skipped spawning; the female showed no urogenital inflammation and had a WL of 8%, which was much lower than average for the subpopulation (Table 3). Males at 2°C readily expressed viscous milt at the end of the experiment; however, at 4°C the milt was

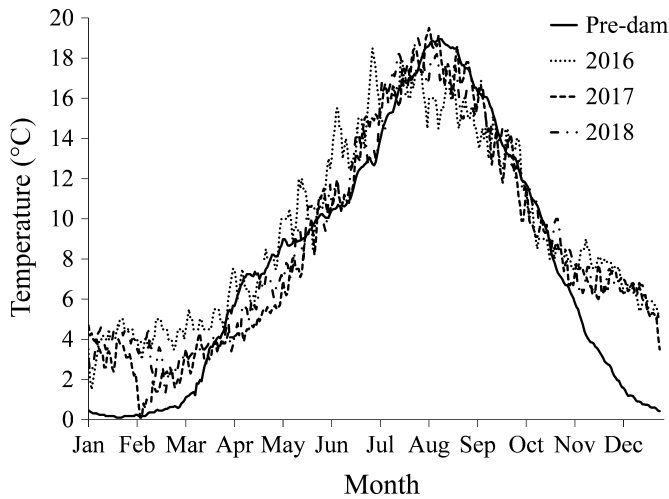


FIGURE 3. A comparison of average daily temperatures in the main channel of the lower Kootenai River for compiled pre-dam years (1963–1971) and three recent postdam years (2016–2018). Temperature data for pre-dam years were sourced from the U.S. Geological Survey (USGS station 12322000 at Porthill, Idaho), whereas thermographs for 2016–2018 were sourced from a nearby station (USGS station 12309500 at Bonners Ferry, Idaho).

less copious and at 6°C only small droplets of milt were observed in clear fluid. Despite postexperiment 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).

Embryo development.—Embryos incubating at 2°C showed the lowest D and highest S up to the eyed stage (D : $\chi^2_{2,6} = 42.56$, $P < 0.01$; S : $\chi^2_{2,6} = 202.08$, $P < 0.01$; Table 3). Embryo deformity at the eyed stage was extremely high at 6°C, less severe at 4°C, and rarely observed at 2°C (Figure 4). The odds of deformity increased by a factor of 3.78 ± 0.86 per 1°C increase between the range of 2°C and 6°C ($\log_temperature = 1.33 \pm 0.23$; $\chi^2 = 34.46$, $P < 0.01$); concomitantly, the odds of normal embryo survival decreased by a factor of 0.27 ± 0.05 ($\log_temperature = -1.31 \pm 0.19$; $\chi^2 = 47.49$, $P < 0.01$; Figure 5). A repeated-measures analysis detected differences in embryo survival between treatments by 4 dps ($\chi^2_{2,6} = 265.32$, $P < 0.01$), and mortality primarily occurred between 0 and 9 dps (Figure 6). Generally, there was more variability in survival estimates at 4°C compared to 2°C 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 million embryos hatched from the 2°C treatment and 1.4 million embryos hatched from the 4°C treatment. Spawning temperature had no detectable effect on egg size, and E_d did not change significantly between the one-cell stage and the eyed stage (Table 3).

Maternal Age Effects on Broodstock Spawning and Embryo Development

Broodstock collection and spawning.—Temperatures were again elevated in the lower Kootenai River between December 2017 and January 2018 relative to average pre-dam levels (Figure 3). Broodstock were primarily captured during mid-January 2018 in the meander reach

TABLE 3. A summary of measured responses (symbols are defined in Table 1) for Burbot broodstock and embryos in experiments with temperature and maternal age as treatments. Means (SE in parentheses) are shown for replicate broodstock subpopulations (n) within each treatment. Lowercase letters next to column values indicate significance differences between treatments within a study year ($P \leq 0.05$).

Study year	Treatment	n	Sex	WL (%)	t_s (d)	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)	One-cell	1.21 (0.02)		
	4°C	3	Female	21.6 (1.0) x	13.1 (0.8) yx	0.41 (0.10)	One-cell	1.20 (0.01)		
	6°C	3	Female	24.0 (0.7) w	12.5 (0.5) y	0.44 (0.09)	One-cell	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	Male	17.0 (0.6) y			Eyed	1.21 (0.01)	15.7 (3.9) y	47.9 (7.3) y
	6°C	3	Male	14.5 (0.4) zy			Eyed	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)	One-cell			
	Age 5	2	Female	22.9 (0.6)	13.3 (0.2) y	0.45 (0.05)	One-cell			
	Age 6	2	Female	22.2 (0.9)	17.2 (1.2) z	0.40 (0.18)	One-cell			
	Age 7+	2	Female	15.3 (4.2)	21.3 (0.2) x	0.21 (0.13)	One-cell			
	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	Male	15.4 (0.2)			Eyed	1.25 (0.01) z	1.7 (0.2)	71.1 (5.2)
	Age 6	2	Male	12.9 (0.2)			Eyed	1.24 (0.01) z	3.1 (2.3)	69.5 (23.6)
	Age 7+	2	Male	15.2 (0.9)			Eyed	1.24 (0.02) z	3.4 (0.5)	71.1 (6.2)

downstream of Bonners Ferry (<250 rkm). The majority of males expressed milt during the preexperiment period, and only two individuals were not spermiating in postexperiment 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 t_s ($\chi^2_{3,4} = 26.93$, $P < 0.01$). Young (age-3) and old (age-7+) females had a t_s that was 4–12 d later than that of 5- and 6-year-old females (Table 3). Generally, spawning events in 2018 were more dispersed and occurred later in February compared to 2017. The mean \pm SE percentage of spent females (SP_f) was $75 \pm 25\%$ for the age-3 treatment, $100 \pm 0\%$ for the age-5 treatment, $75 \pm 25\%$ for the age-6 treatment, and $53 \pm 28\%$ for the age-7+ treatment; 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 F_r . Maternal age did not significantly affect the E_v or F_r (Table 3). Variability in E_v was high (except for age-5 females), and E_v (mean \pm SE) across treatments was $1,357 \pm 550$ mL (age 3), $2,179 \pm 214$ mL (age 5), $3,050 \pm 1,010$ mL (age 6), and $2,908 \pm 2,043$ mL (age 7+). Interestingly, subpopulations with larger, older 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). Similar to the previous year, a higher preexperiment K was observed in females than in males ($\chi^2_{1,4} = 12.25$, $P < 0.01$). Females were older ($\chi^2_{7,8} = 60.01$, $P < 0.01$) and weighed more ($\chi^2_{7,8} = 62.40$, $P < 0.01$) than males in the age-6 and age-7+ treatments (Table 2). The mean \pm SE female biomass (B) was $3,338 \pm 78$ kg for the age-3 treatment, $4,862 \pm 115$ kg for the age-5 treatment, $9,645 \pm 335$ kg for the age-6 treatment, and $12,635 \pm 1,938$ kg for the age-7+ treatment;

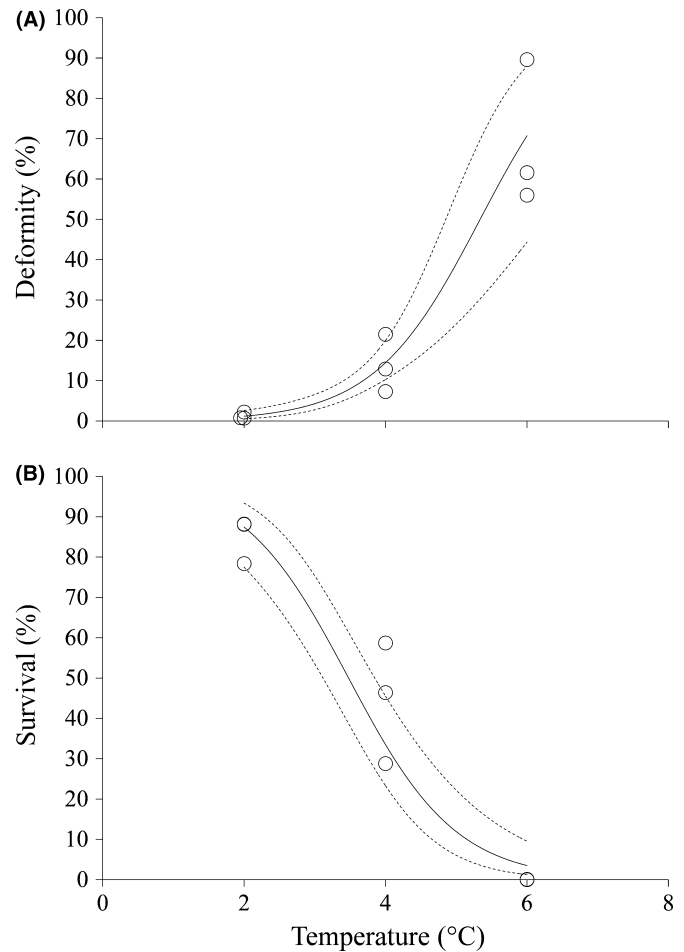


FIGURE 5. Logistic regression models of (A) Burbot embryo deformity and (B) normal embryo survival against incubation temperatures of 2, 4, and 6°C. Circles represent observed data from replicate ($n=3$) broodstock subpopulations. Solid lines are the expected means predicted by modeling. Dotted lines represent 95% confidence intervals for the predicted means.

male B and age did not differ among treatments and averaged $6,905 \pm 154$ kg and 4.53 ± 0.04 years, respectively. Female broodstock showed greater changes in

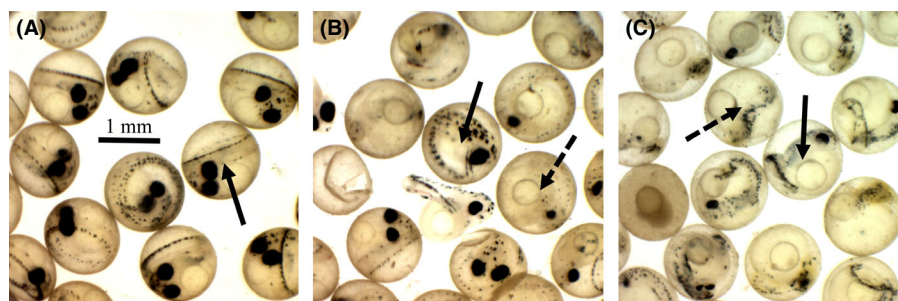


FIGURE 4. Images of Burbot embryos from the lower Kootenai River incubating at (A) 2°C, (B) 4°C, and (C) 6°C. The scale bar for all images is shown in panel A. Solid arrows indicate embryos that were categorized as normal and without overt signs of deformity. Dashed arrows point to examples of deformed embryos with patent eye malformation (panel B) or arrested development (panel C). [Color figure can be viewed at afs-journals.org.]

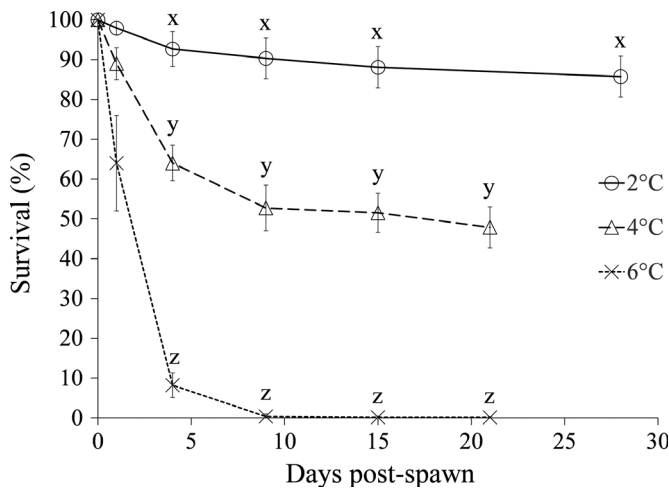


FIGURE 6. Cumulative survival of Burbot embryos from spawning to the eyed stage at incubation temperatures of 2, 4, and 6°C. Circles, triangles, and x-symbols represent the estimated mean (\pm SE) embryo survival from replicate ($n=3$) broodstock subpopulations at the respective temperatures. Lowercase letters indicate significant differences in embryo survival between the temperature treatments ($P < 0.05$).

morphometrics (i.e., WL, SWL, and ΔK) over the experiment period relative to males (Table 3). Grouping by maternal age had no detectable effect on body metric changes in either sex.

Embryo development.—Age-3 females spawned eggs with smaller diameters ($\chi^2_{3,4} = 16.04$, $P < 0.01$; Table 3). No correlation was found between E_d and D or S . Generally, the youngest and oldest females spawned eggs with the highest and lowest percentage of normal embryos, respectively (Table 3).

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 postdam 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+ years) after relocation to a broodstock facility. Adults exhibited rate functions (i.e., size at maturity and age at maturity) and W_r values similar to those of 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 for wild fish (Kainz and Gollman 1996; Arndt and Hutchinson 2000). Live eggs were homogeneous with respect to yolk transparency and oil globule polarization—two critical ovum

maturation criteria for Burbot (Foltz et al. 2012). Egg size and embryo survival to the eyed stage were also within reported ranges from other investigations (Jäger et al. 1981; Kainz and Gollman 1996; Kujawa et al. 1999; Taylor and McPhail 2000; Vught et al. 2008; 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°C and 6°C also spawned, but the average spawning time and overall spawning period were shortened compared to those of adults held at 2°C. Egg incubation at 4°C 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 springwaters, 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 reported for other wild Burbot populations. Żarski et al. (2010) observed a 17-d spawning period at temperatures $< 2^\circ\text{C}$, but only 10 d of spawning occurred in a warming episode from 2°C to 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 survival rates 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 (Vught et al. 2008; Lahnsteiner et al. 2012). In the present study, 4°C and 6°C did not inhibit spawning but further synchronized gamete release to coincide with thermal conditions that were suboptimal for normal embryo development. Recruitment failures among Burbot populations affected by impoundments, climate change, or both could be tied to limited access to spawning habitats that do not have ice cover in midwinter and are consistently warmer than 4°C.

The approach of winter stimulates reproduction in many coldwater 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 to

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°C higher than pre-dam levels during November and December. Temperatures also fluctuated between 2°C and 5°C during the preexperiment 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 the installation of Libby Dam (Partridge 1983; Paragamian et al. 2000). Although the extent of broodstock exposure to main-stem 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 monthlong temperatures of 6–7°C. Their findings support the results herein, which show that a warmer postdam regime from autumn to 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 et al. 2010); therefore, a warmer autumn–winter regime could lead to earlier spawning of Burbot. However, data from the present study do not support this inference. Hatchery adults recaptured from the warmer postdam regime initiated spawning in mid-February, coinciding with the spawning time of the donor population inhabiting Moyie Lake, which is often ice-covered and colder in midwinter (Stephenson, personal communication). Further research is needed to (1) determine whether elevated prespawning temperature alters Burbot spawning time and (2) establish a thermal threshold (or thresholds) for inhibition of gametogenesis, final maturation, and spawning. Testing the effects of constant 2, 4, and 6°C from December to 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-old females. Unlike the other tested age-groups, 100% of the age-5 females spawned during 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 1-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 1990; Pulliainen and Korhonen 1993; Cott et al. 2013a). Similar patterns of delayed or skipped spawning among the youngest and oldest adults and more consistent reproduction from middle-aged individuals have been reported in other marine gadids (Hutchings and Myers 1993; Trippel 1998; Wieland et al. 2000; Wright and Gibb 2005; Scott et al. 2006; 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 *Gadus morhua*, 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 Myers 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 flavescens*, Northern Pike *Esox lucius*, and Walleye *Sander vitreus*) is often driven by match–mismatch dynamics impacting early life stages (Jolley et al. 2010; Hühn et al. 2014; Boehm 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 Gollman (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 larval 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 also did not include evaluations of larvae. Further research on the topic of maternal effects on offspring fitness is needed for Burbot.

In conclusion, 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 postdam 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 the 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 age 3 to age 7+ was important for 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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REFERENCES

- Abdulfatah, A., P. Fontaine, P. Kestemont, S. Mila, and M. Marie. 2013. Effects of the thermal threshold and the timing of temperature reduction on the initiation and course of oocyte development in cultured female of Eurasian Perch *Perca fluviatilis*. *Aquaculture* 376:90–96.
- Anguis, V., and J. P. Cañavate. 2005. Spawning of captive Senegal Sole (*Solea senegalensis*) under a naturally fluctuating temperature regime. *Aquaculture* 243:133–145.
- Arndt, S. K. A., and J. Hutchinson. 2000. Characteristics of a tributary-spawning population of Burbot from Columbia Lake, British Columbia. Pages 48–60 in V. L. Paragamian and D. W. Willis, editors. *Burbot: biology, ecology and management*. American Fisheries Society, Fisheries Management Section, Publication 1, Bethesda, Maryland.
- Atse, C., C. Audet, and J. de la Noüe. 2002. Effects of temperature and salinity on the reproductive success of Arctic Charr, *Salvelinus alpinus* (L.): egg composition, milt characteristics and fry survival. *Aquaculture Research* 33:299–309.
- Bailey, M. M. 1972. Age, growth, reproduction, and food of the Burbot, *Lota lota* (Linnaeus), in southwestern Lake Superior. *Transactions of the American Fisheries Society* 4:667–674.
- Berkeley, S. A., M. A. Hixon, R. J. Larson, and M. S. Love. 2004. Fisheries sustainability via protection of age structure and spatial distribution of fish populations. *Fisheries* 29(8):23–32.
- Blabolil, P., J. Duras, T. Jůza, L. Kočvara, J. Matěna, M. Muška, M. Řiha, L. Vejřík, M. Holubová, and J. Peterka. 2018. Assessment of Burbot *Lota lota* (L. 1758) population sustainability in central European reservoirs. *Journal of Fish Biology* 92:1545–1559.
- Boehm, H. I. A. 2016. Identifying recruitment bottlenecks for age-0 Walleye *Sander vitreus* in northern Wisconsin lakes. Master's thesis. University of Wisconsin, Stevens Point.
- Bonar, S. A., L. G. Brown, P. E. Mongillo, and K. Williams. 2000. Biology, distribution, and management of Burbot (*Lota lota*) in Washington State. *Northwest Science* 74:87–96.
- Bosveld, J., J. Kranenbarg, H. J. R. Lenders, and A. J. Hendriks. 2015. Historic decline and recent increase of Burbot (*Lota lota*) in the Netherlands. *Hydrobiologia* 757:49–60.
- Brackwehr, L., M. Bunzel-Drücke, U. Detering, G. Jacobs, M. Kühlmann, S. Kuss, K. P. Lampert, M. Möhlenkamp, B. Peinert, A. Petruck, M. Scharf, V. Schulz, T. Seume, and O. Zimball. 2016. Die Quappe (*Lota lota*) im Einzugsgebiet der Lippe: Ökologie, Schutzmaßnahmen, Zucht und Wiederansiedlung. [The Burbot (*Lota lota*) in the catchment area of the Lippe: ecology, protective measures, breeding and resettlement.] Landesfischereiverband Westfalen und Lippe, Münster, Germany.
- Brown, J. A., M. Helm, and J. Moir. 1995. New species for aquaculture. Pages 341–362 in A. D. Boghen, editor. *Coldwater aquaculture in Atlantic Canada*, 2nd edition. Tribune Press, Sackville, New Brunswick.
- Burke, M., K. Jorde, and J. M. Buffington. 2009. Application of a hierarchical framework for assessing environmental impacts of dam operation: changes in stream flow, bed mobility and recruitment of riparian trees in a western North American river. *Journal of Environmental Management* 90:224–236.
- Chambers, R. C., and K. G. Waiwood. 1996. Maternal and seasonal differences in egg sizes and spawning characteristics of captive Atlantic Cod, *Gadus morhua*. *Canadian Journal of Fisheries and Aquatic Sciences* 53:1986–2003.
- Chen, L. C. 1969. The biology and taxonomy of the Burbot, *Lota lota leptura*, in interior Alaska. *Biological Papers of the University of Alaska* 11.
- Clemens, H. P. 1951. The growth of the Burbot, *Lota lota maculosa* (LeSueur), in Lake Erie. *Transactions of the American Fisheries Society* 80:163–173.
- Cott, P. A., T. A. Johnston, and J. M. Gunn. 2013a. Stability in life history characteristics among Burbot populations across environmental gradients. *Transactions of the American Fisheries Society* 142:1746–1756.
- Cott, P. A., T. A. Johnston, and J. M. Gunn. 2013b. Sexual dimorphism in an under-ice spawning fish: the Burbot (*Lota lota*). *Canadian Journal of Zoology* 91:732–740.
- Cushing, D. H. 1990. Plankton production and year-class strength in fish populations: an update of the match/mismatch hypothesis. *Advances in Marine Biology* 26:249–293.
- Cushing, D. H., and J. W. Horwood. 1994. The growth and death of fish larvae. *Journal of Plankton Research* 16:291–300.
- Dembkowski, D. J., M. J. Weber, and M. R. Wuellner. 2017. Factors influencing recruitment and growth of age-0 Yellow Perch in eastern

- South Dakota glacial lakes. *Fisheries Management and Ecology* 24:372–381.
- Donner, M. T., and R. Eckmann. 2011. Diel vertical migration of larval and early-juvenile Burbot optimizes survival and growth in a deep, pre-alpine lake. *Freshwater Biology* 56:916–925.
- Dorts, J., G. Grenouillet, J. Douxfils, S. N. M. Mandiki, S. Milla, F. Silvestre, and P. Kestemont. 2012. Evidence that elevated water temperature affects the reproductive physiology of the European Bullhead *Cottus gobio*. *Fish Physiology and Biochemistry* 38:389–399.
- Evenson, M. J. 1990. Age and length at sexual maturity of Burbot in the Tanana River, Alaska. Alaska Department of Fish and Game, Fishery Manuscript 90-2, Juneau.
- Evenson, M. J. 2000. Reproductive traits of Burbot in the Tanana River, Alaska. Pages 61–70 in V. L. Paragamian and D. W. Willis, editors. *Burbot: biology, ecology and management*. American Fisheries Society, Fisheries Management Section, Publication 1, Bethesda, Maryland.
- Farkas, J. 1993. Zur biologie der Aalrutte in der oberen Drau und ihren Nebengewässern. [Biology of the Burbot in the upper Drau and its backwaters.] *Carinthia II* 183/103:593–612.
- Fischer, P. 1999. Otolith microstructure during the pelagic, settlement, and benthic phases in Burbot. *Journal of Fish Biology* 54:1231–1243.
- Fisher, S. J., D. W. Willis, and K. L. Pope. 1996. An assessment of Burbot (*Lota lota*) weight–length data from North American populations. *Canadian Journal of Zoology* 74:570–575.
- Foltz, J. R., N. J. Jensen, M. P. Polinski, S. C. Ireland, and K. D. Cain. 2012. Characterization of oocyte development in hatchery-reared Burbot. *North American Journal of Aquaculture* 74:408–412.
- Fortier, L., D. Ponton, and M. Gilbert. 1995. The match/mismatch hypothesis and the feeding success of fish larvae in ice-covered southeastern Hudson Bay. *Marine Ecology Progress Series* 120:11–27.
- Geraudie, P., M. Gerbron, E. Hill, and C. Minier. 2010. Roach (*Rutilus rutilus*) reproductive cycle: a study of biochemical and histological parameters in a low contaminated site. *Fish Physiology and Biochemistry* 36:767–777.
- Gotceitas, V., V. Puvanendran, L. L. Leader, and J. A. Brown. 1996. An experimental investigation of the “match/mismatch” hypothesis using larval Atlantic Cod. *Marine Ecology Progress Series* 130:29–37.
- Hardardottir, K., O. S. Kjesbu, and G. Marteinsdottir. 2001. Relationship between atresia, fish size and condition in Icelandic cod (*Gadus morhua* L.). *International Council for the Exploration of the Sea, Copenhagen*.
- Hardy, R. S., S. M. Stephenson, M. D. Neufeld, and S. P. Young. 2015. Adaptation of lake-origin Burbot stocked into a large river environment. *Hydrobiologia* 757:35–47.
- Harrison, P. M., L. F. G. Gutowsky, E. G. Martins, D. A. Patterson, S. J. Cooke, and M. Power. 2016a. Burbot and large hydropower in North America: benefits, threats and research needs for mitigation. *Fisheries Management and Ecology* 23:335–349.
- Harrison, P. M., L. F. G. Gutowsky, E. G. Martins, D. A. Patterson, S. J. Cooke, and M. Power. 2016b. Temporal plasticity in thermal-habitat selection of Burbot *Lota lota* a diel-migrating winter-specialist. *Journal of Fish Biology* 88:2111–2129.
- Hixon, M. A., D. W. Johnson, and S. W. Sogard. 2013. BOFFFFF: on the importance of conserving old-growth age structure in fishery populations. *ICES (International Council for the Exploration of the Sea). Journal of Marine Science* 71:2171–2185.
- Hu, F., F. Gao, Y. Jian, X. Wang, L. Li, S. Zhang, and W. Guo. 2017. Effect of temperature on incubation period and hatching success of Fat Greenling (*Hexagrammos otakii* Jordan and Starks) eggs. *Aquaculture Research* 48:361–365.
- Hühn, D., K. Lübke, C. Skov, R. Arlinghaus, and E. Taylor. 2014. Natural recruitment, density-dependent juvenile survival, and the potential for additive effects of stock enhancement: an experimental evaluation of stocking Northern Pike (*Esox lucius*) fry. *Canadian Journal of Fisheries and Aquatic Sciences* 71:1508–1519.
- Hutchings, J. A., and W. A. Myers. 1993. Effect of age on the seasonality of maturation and spawning of Atlantic Cod, *Gadus morhua*, in the Northwest Atlantic. *Canadian Journal of Fisheries and Aquatic Sciences* 50:2468–2474.
- Jäger, T., W. Nellen, W. Schöfer, and F. Shodjal. 1981. Influence of salinity and temperature on early life stages of *Coregonus albula*, *C. lavaretus*, *R. rutilus*, *L. lota*. *Rapports et Procès-Verbaux des Reunions Conseil International pour l'Exploration de la Mer* 178:345–348.
- Jensen, N. R., S. C. Ireland, J. T. Siple, S. R. Williams, and K. D. Cain. 2008. Evaluation of egg incubation methods and larval feeding regimes for North American Burbot. *North American Journal of Aquaculture* 70:162–170.
- Jolley, J. C., D. W. Willis, and R. S. Holland. 2010. Match–mismatch regulation for Bluegill and Yellow Perch larvae and their prey in Sandhill lakes. *Journal of Fish and Wildlife Management* 1:73–85.
- Jude, D. J., Y. Wang, S. R. Hensler, and J. Janssen. 2013. Burbot early life history strategies in the Great Lakes. *Transactions of the American Fisheries Society* 142:1733–1745.
- Kainz, E., and H. P. Gollman. 1996. Artificial hatching and first rearing experiments with Burbot (*Lota lota*). *Österreichs Fischerei* 49:154–160.
- Katzman, L. M., and A. V. Zale. 2000. Age and growth of an exploited Burbot population in Upper Red Rock Lake, Montana. Pages 139–146 in V. L. Paragamian and D. W. Willis, editors. *Burbot: biology, ecology and management*. American Fisheries Society, Fisheries Management Section, Publication 1, Bethesda, Maryland.
- Kjesbu, O. S. 1994. Time of start of spawning in Atlantic Cod (*Gadus morhua*) females in relation to vitellogenic oocyte diameter, temperature, fish length and condition. *Journal of Fish Biology* 45:719–735.
- Kjesbu, O. S., D. Righton, M. Krüger-Johnson, A. Thorsen, K. Michalson, M. Fonn, and P. R. Witthames. 2010. Thermal dynamics of ovarian maturation in Atlantic Cod (*Gadus morhua*). *Canadian Journal of Fisheries and Aquatic Sciences* 67:605–625.
- Kujawa, R., D. Kucharczyk, and A. Mamcarz. 1999. The influence of temperature on embryonic development of Burbot (*Lota lota* L.). Pages 133–134 in *Towards predictable quality: International Conference Aquaculture Europe '99, Trondheim, Norway*. European Aquaculture Society, Special Publication 27, Ostende, Belgium.
- Kurita, Y., S. Meier, and O. S. Kjesbu. 2003. Oocyte growth and fecundity regulation by atresia of Atlantic Herring (*Clupea harengus*) in relation to body condition throughout the maturation cycle. *Journal of Sea Research* 49:203–219.
- Lahnsteiner, F., M. Kletzl, and T. Weismann. 2012. The effect of temperature on embryonic and yolk-sac larval development in the Burbot *Lota lota*. *Journal of Fish Biology* 81:977–986.
- McPhail, J. D., and V. L. Paragamian. 2000. Burbot biology and life history. Pages 11–23 in V. L. Paragamian and D. W. Willis, editors. *Burbot: biology, ecology and management*. American Fisheries Society, Fisheries Management Section, Publication 1, Bethesda, Maryland.
- Mustonen, A. M., P. Nieminen, and H. Hyvärinen. 2002. Liver and plasma lipids of spawning Burbot. *Journal of Fish Biology* 61:1318–1322.
- Muth, K. M., and L. L. Smith. 1974. *The Burbot fishery in Lake of the Woods*. University of Minnesota, Agricultural Experimental Station, Technical Bulletin 296, St. Paul.
- Ouellet, P., Y. Lambert, and M. Castonguay. 1997. Spawning of Atlantic Cod (*Gadus morhua*) in the northern Gulf of St. Lawrence: a study of adult and egg distributions and characteristics. *Canadian Journal of Fisheries and Aquatic Sciences* 54:198–210.

- Pankhurst, N. W., G. J. Purser, G. Van Der Kraak, P. M. Thomas, and G. N. R. Forteach. 1996. Effect of holding temperature on ovulation, egg fertility, plasma levels of reproductive hormones and in vitro ovarian steroidogenesis in the Rainbow Trout *Oncorhynchus mykiss*. *Aquaculture* 146:277–290.
- Paragamian, V. L., and M. J. Hansen. 2011. Stocking for rehabilitation of Burbot in the Kootenai River, Idaho, USA and British Columbia, Canada. *Journal of Applied Ichthyology* 27:22–26.
- Paragamian, V. L., B. J. Pyper, M. J. Daigneault, and R. C. P. Beamesderfer. 2008. Population dynamics and extinction risk of Burbot in the Kootenai River, Idaho, USA, and British Columbia, Canada. Pages 213–234 in V. L. Paragamian and D. Bennett, editors. *Burbot: ecology, management, and culture*. American Fisheries Society, Symposium 59, Bethesda, Maryland.
- Paragamian, V. L., and V. D. Wakkinen. 2008. Seasonal movement of Burbot in relation to temperature and discharge in the Kootenai River, Idaho, USA and British Columbia, Canada. Pages 55–77 in V. L. Paragamian and D. H. Bennett, editors. *Burbot: ecology, management, and culture*. American Fisheries Society, Symposium 59, Bethesda, Maryland.
- Paragamian, V. L., V. Whitman, J. Hammond, and H. Andrusak. 2000. Collapse of the Burbot fisheries in Kootenay Lake, British Columbia, Canada, and the Kootenai River, Idaho, USA, post-Libby Dam. Pages 155–164 in V. L. Paragamian and D. W. Willis, editors. *Burbot: biology, ecology and management*. American Fisheries Society, Fisheries Management Section, Publication 1, Bethesda, Maryland.
- Partridge, F. 1983. Kootenai River fisheries investigations. Idaho Department of Fish and Game, Federal Aid to Fish and Wildlife Restoration, Project F-73-R-5, Job Completion Report, Boise.
- Polinski, M. P., N. R. Jensen, K. D. Cain, K. A. Johnson, and S. C. Ireland. 2010. Assessment of formalin and hydrogen peroxide use during egg incubation of North American Burbot. *North American Journal of Aquaculture* 72:111–117.
- Pörtner, H., and M. Peck. 2010. Climate change effects on fishes and fisheries: towards a cause-and-effect understanding. *Journal of Fish Biology* 77:1745–1779.
- Probst, W. N., and R. Eckmann. 2009. Diet overlap between young-of-the-year Perch, *Perca fluviatilis* (L.) and Burbot, *Lota lota* (L.), during early life-history stages. *Ecology of Freshwater Fish* 18: 527–537.
- Pulliaainen, E. P., and K. Korhonen. 1990. Seasonal changes in condition indices in adult mature and non-maturing Burbot, *Lota lota* (L.), in northeastern Bothnian bay, northern Finland. *Journal of Fish Biology* 36:251–259.
- Pulliaainen, E. P., and K. Korhonen. 1993. Does the Burbot, *Lota lota*, have rest years between normal spawning seasons? *Journal of Fish Biology* 43:355–362.
- Ricker, W. E. 1975. Computation and interpretation of biological statistics of fish populations. *Bulletin of the Fisheries Research Board of Canada* 191.
- Rombough, P. J. 1997. The effects of temperature on embryonic and larval development. Pages 177–224 in C. M. Wood and G. D. McDonald, editors. *Global warming: implications for freshwater and marine fish*. Cambridge University Press, Cambridge, UK.
- Ross, T. J., K. N. McDonnell, and R. S. Hardy. 2018. Kootenai River resident fish mitigation: White Sturgeon, Burbot, and native salmonid monitoring and evaluation. Idaho Department of Fish and Game, Boise.
- Scott, B. E., G. Marteinsdottir, G. A. Begg, P. J. Wright, and O. S. Kjesbu. 2006. Effects of population size/age structure, condition and temporal dynamics of spawning on reproductive output in Atlantic Cod (*Gadus morhua*). *Ecological Modelling* 191:383–415.
- Shuter, B. J., A. G. Finstad, I. P. Helland, I. Zweimüller, and F. Hölker. 2012. The role of winter phenology in shaping the ecology of freshwater fish and their sensitivities to climate change. *Aquatic Sciences* 74:637–657.
- Skjæraasen, J. E., R. D. M. Nash, K. Korsbrekke, M. Fonn, T. Nilssen, J. Kennedy, K. H. Nedreaas, A. Thorsen, P. R. Witthames, A. J. Geffen, H. Hoie, and O. S. Kjesbu. 2012. Frequent skipped spawning in the world's largest cod population. *Proceedings of the National Academy of Sciences of the USA* 109:8995–8999.
- Stapanian, M. A., V. L. Paragamian, C. P. Madenjian, J. R. Jackson, J. Lappalainen, M. J. Evenson, and M. D. Neufeld. 2010. Worldwide status of Burbot and conservation measures. *Fish and Fisheries* 11:34–56.
- Taranger, G. L., and T. Hansen. 1993. Ovulation and egg survival following exposure of Atlantic Salmon, *Salmo salar* L., broodstock to different water temperatures. *Aquatic Fisheries Management* 24:151–156.
- Targońska, K., D. Żarski, K. Kupren, K. Palińska-Żarska, A. Mamacz, R. Kujawa, A. Skrzypczak, G. Furgala-Selezniowa, T. K. Czarkowski, A. Hakuc-Błażowska, and D. Kucharczyk. 2014. Influence of temperature during four following spawning seasons on the spawning effectiveness of Common Bream, *Abramis brama* (L.) under natural and controlled conditions. *Journal of Thermal Biology* 39:17–23.
- Taylor, J. L., and J. D. McPhail. 2000. Temperature, development, and behavior in the early life history of Burbot from Columbia Lake, British Columbia. Pages 30–37 in V. L. Paragamian and D. W. Willis, editors. *Burbot biology, ecology, and management*. American Fisheries Society, Fisheries Management Section, Publication 1, Bethesda, Maryland.
- Teletchea, F., J. N. Gardeur, E. Kamler, and P. Fontaine. 2009. The relationship of oocyte diameter and incubation temperature to incubation time in temperate freshwater fish species. *Journal of Fish Biology* 74:652–668.
- Trippel, E. A. 1998. Egg size and viability and seasonal offspring production of young Atlantic Cod. *Transactions of the American Fisheries Society* 127:339–359.
- Tveiten, H., S. E. Solevag, and H. K. Johnsen. 2001. Holding temperature during the breeding season influences final maturation and egg quality in Common Wolffish. *Journal of Fish Biology* 58:374–385.
- Van Der Kraak, G., and N. W. Pankhurst. 1997. *Temperature effects on the reproductive performance of fish*. Cambridge University Press, Cambridge, UK.
- Van Houdt, J. K., B. Hellemans, and F. A. M. Volckaert. 2003. Phylogenetic relationships among Palearctic and Nearctic Burbot (*Lota lota*): Pleistocene extinctions and recolonization. *Molecular Phylogenetics and Evolution* 29:599–612.
- Vught, I., A. S. Harzevili, J. Auwerx, and D. De Charleroy. 2008. Aspects of reproduction and larviculture of Burbot under hatchery conditions. Pages 167–178 in V. L. Paragamian and D. Bennett, editors. *Burbot: ecology, management, and culture*. American Fisheries Society, Symposium 59, Bethesda, Maryland.
- Wang, N., F. Teletchea, P. Kestemont, S. Milla, and P. Fontaine. 2010. Photothermal control of the reproductive cycle in temperate fishes. *Reviews in Aquaculture* 2:209–222.
- Wege, G. J., and R. O. Anderson. 1978. Relative weight (W_r): a new index of condition for Largemouth Bass. Pages 79–91 in G. D. Novinger and J. G. Dillard, editors. *New approaches to the management of small impoundments*. American Fisheries Society, North Central Division, Special Publication 5, Bethesda, Maryland.
- Wieland, K., A. Jarre-Teichmann, and K. Horbowa. 2000. Changes in the timing of spawning of Baltic Cod: possible causes and implications for recruitment. *ICES (International Council for*

- the Exploration of the Sea) *Journal of Marine Science* 57:452–464.
- Wright, P. J., and F. M. Gibb. 2005. Selection of birth date in North Sea Haddock and its relation to maternal age. *Journal of Animal Ecology* 74:303–312.
- Wright, P. J., and E. A. Trippel. 2009. Fishery-induced demographic changes in the timing of spawning: consequences for reproductive success. *Fish and Fisheries* 10:283–304.
- Żarski, D., D. Kucharczyk, W. Sasinowski, K. Targońska, and A. Mamarz. 2010. The influence of temperature on successful reproductions of Burbot, *Lota lota* (L.) under hatchery conditions. *Polish Journal of Natural Sciences* 25:93–105.
- Żarski, D., K. Palińska-Żarska, S. Krejszeff, and D. Kucharczyk. 2014. A first successful induction of spawning of the hatchery reared Burbot, *Lota lota* L. in *Aquaculture Europe 2014*, San Sebastián, Spain. European Aquaculture Society, Oostende, Belgium.