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Triploid burbot, *Lota lota*, production: Optimization of thermal and hydrostatic parameters, tetraploid induction, and confirmation of triploid sterility

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Abstract

Experiments were conducted to optimize triploid induction parameters, and assess triploid sterility, in burbot. Duration and timing of shocks were based on degree minutes, temperature multiplied by time, denoted as °C min. Hydrostatic shock experiments investigated the duration of shock using 7500 or 8500 psi at 180°C min post-fertilization. Thermal shocks investigated duration of shock and post-fertilization shock timing using a shock of 16°C. Sterility experiments investigated egg survival when diploids were crossed with triploids. Hydrostatic shock of 7500 psi for 10 or 20°C min can induce triploidy ≥90% and exhibits survival that is statistically similar, $p \leq 0.05$, to controls. Hydrostatic shock of 8500 psi for 5 or 10°C min yielded triploid induction of 93% and 100%, respectively, with survival that is statistically similar to controls, $p \leq 0.05$. Thermal induction experiments indicated shocks at 120°C min post-fertilization, for durations between 350 and 450°C min, have potential to induce triploidy ≥90% while facilitating survival statistically similar to controls, $p \leq 0.05$. Induction of tetraploidy was observed. Sterility experiments determined that triploid burbot are

functionally sterile. These results may allow production of burbot where sterility is required.

KEYWORDS
burbot, sterility, tetraploid, triploid

1 | INTRODUCTION

Burbot, *Lota lota*, the only true freshwater gadoid-like fish species, are ideal candidates for intensive cool or coldwater aquaculture because of its high economic value, low temperature requirements, and fast growth rate (Palińska-Żarska et al., 2013). This species is acclaimed for its thick-flake white flesh fillets and provides value-added products as its roe and liver are both considered delicacies and the skin can be used as a leather product (Branion, 1930; Howell et al., 2020; Meeus et al., 2012). Burbot are tolerant of relatively low dissolved oxygen levels, grow well within the same water quality parameters that support ideal rainbow trout, *Oncorhynchus mykiss*, grow-out, and have been shown to be refractory to many salmonid pathogens (Bruce et al., 2020; Vaage & Myrick, 2022). Trout production facilities could, relatively cheaply, diversify production and increase value to an operation by incorporating burbot into last use water of raceways, ponds, or tanks. However, strict regulations against the introduction of invasive species in the United States may necessitate burbot production facilities to enact preemptive measures, induction of sterility, to reduce the risk of introduction of burbot into local ecosystems.

For many commercial fish species, it is observed that sexual maturation often results in reduction in somatic growth and flesh quality as fish redirect energy to production of eggs, milt, and spawning behavior (Hayashida et al., 2021). Additionally, production of reproductively viable fish species outside of their native distribution increases the risk of the foundation of an invasive population, potentially resulting in penalties or fines to the offending farm (Naylor et al., 2005; Taranger et al., 2010). The use of sterile fish in aquaculture has been shown to increase annual biomass production, maintain ideal flesh quality, and reduce the probability of escaped fish becoming self-sustaining outside of production facilities (Golpour et al., 2016; Taranger et al., 2010). Triploid fish are functionally sterile because of irregular division of chromosomes during meiosis, can be mass produced, and triploid induction in almost all species of fish results in functional sterility (Hayashida et al., 2021).

Triploid induction is accomplished by preventing the extrusion of the second maternal polar body, shortly after fertilization, through pressure or thermal shocks (Benfey, 2001). The three major variables important for the successful retention of the maternal polar body are timing, duration, and intensity of the post-fertilization shock (Maxime, 2008). Previous studies demonstrated that triploid induction in burbot is possible through manipulation of shock timing, duration, and intensity following either hydrostatic or thermal shocks (Oliver et al., 2020). A hydrostatic shock of 8500 psi at 180°C min post-fertilization for 10°C min yielded the highest percent triploid induction and survival relative to controls, 100% and 95%, respectively. Duration of shocks longer than 10°C min at 8500 psi and any shocks over 9500 psi resulted in 100% mortality of embryos before hatch. Additionally, a shock of 7500 psi resulted in 100% triploid induction but a survival of 65.5% at a shock duration of 30°C min. Thermal shocks were successful as well with exposure to 16°C at 120°C min post-fertilization for 500°C min yielding survival of 57.4% and a percent triploid induction of 96.6% relative to control groups. Shock temperatures above 16°C resulted in relatively higher percent triploid induction, but also generally resulted in lower percent survival. Following on from these studies, further optimization of hydrostatic and thermal shock parameters was evaluated to maximize triploid induction and survival. Further, progeny from initial induction trials were followed to maturity to determine whether functional gamete developed and whether sterility was induced.

2 | MATERIALS AND METHODS

2.1 | Broodstock management and gamete collection

All experiments were performed at the University of Idaho's Aquaculture Research institute (UI-ARI; Moscow, ID). The burbot broodstock used in this study originated from eggs and adults collected in British Columbia, Canada; the eggs were fertilized in Canada and both eggs and adults were transported to the UI-ARI for incubation and subsequent captive rearing in 2004. At the time of this study, the broodstock was composed of multiple generations, all descended from the founding population and raised in an aquaculture setting for their entire lives, the result of breeding between fish within the captive broodstock. The diploid population used for the optimization of triploidy and for assessment of triploid sterility averaged 1.46 kg and 57 cm in length and 4–12 years of age. The triploid population was generated at UI-ARI in 2019 using temperature or pressure shocks; triploid condition was confirmed by measurement of blood cell nuclei at 8 months of age via flow cytometry. The triploid population, used for assessment of triploid sterility, was composed of 4-year-old fish and exhibited an average weight and length of 0.56 kg and 45.3 cm, respectively. All fish, previously individually tagged, were housed in a recirculating aquaculture system and exposed to water quality and photoperiod regimes designed to mimic the natural conditions of the Kootenai River in Bonners Ferry, Idaho (Jensen et al., 2008). Fish were fed a sinking diet, 6 mm pellets, composed of Bio-Brood and Oncor (Skretting, Tooele, Utah) in a 1:1 mix at 0.3% of body weight per day. All fish reached sexual maturity naturally through exposure to low temperatures and increasing daylight after the winter solstice. Spawning condition of all fish was examined three times a week beginning at the first occurrence of eggs, females with free-flowing eggs were considered ripe, and males were considered ripe when they expressed milt when lightly stripped. For all replicates, one ripe male and female were manually stripped after anesthesia with buffered 150 ppm tricaine methanesulfonate (Syndel, Ferndale, WA), gametes remained separate, dry, and cold (~2°C) until fertilization. Broodstock pre- and post-spawn weights were collected in February and April, respectively.

2.2 | Optimization of triploid induction parameters

All experiments were replicated three times, and each of the replicates within an experiment utilized a distinct male and female. Milt was collected in a 10 mL glass vial, after assessing sperm motility of >80% with a microscope at 40×. Eggs were collected in a dry 3 L vessel incubated in an ~2°C water bath; five 30 mL glass vials were supplied with ~2 mL of eggs from the 3 L vessel immediately after collection. Vials containing the eggs and milt were promptly placed in a 2°C water bath after collection. Fertilization and incubation of eggs followed methods of Oliver et al. (2020).

All experiments were conducted within a week of each other, ensuring hatch would occur at the same general time for all experimental units. Fertilization of groups of eggs was staggered because of limited space in shocking devices, and to ensure the correct shocking time was achieved. The timing and duration of shocks were calculated based on degree minutes, time multiplied by temperature, and is denoted as °C min. A single vial of eggs was fertilized at the beginning and at the end of each experiment to account for any deleterious effects of eggs sitting in dry glass vials over the course of the trial. Controls were subjected to the same fertilization, water hardening, disinfection, and incubation methods as the corresponding experimental treatments.

At the conclusion of each experiment, each batch of eggs was volumetrically quantified using a graduated cylinder, after settling for 3 min, then transferred to a 1.27-cm diameter 10 mL experimental incubation chamber. Each chamber was placed, randomly, into a single mass pneumatic-drive incubator system using a shared water bath until hatch (Ashton et al., 2021). Incubation chambers were checked daily, dead eggs were removed, and temperature was monitored. Eggs were incubated at 1.7°C (±0.6) under a 24-h photoperiod and exposed to a daily 15-min 450 ppm dose of hydrogen peroxide. When ≥20% of the eggs within the experimental cylinders began to hatch, the incubation temperatures of all eggs were increased to 8°C to synchronize hatch. One day prior to initiation of hatch, all

dead eggs were removed, and egg numbers within experimental units were quantified as described earlier. Relative pre-hatch survival of each experimental unit was calculated based on the corresponding control group survival. The average pre-hatch egg volume of the two controls in each experiment was set to 100%, and the actual treatment survival was divided by the actual control survival.

An outline of the two hydrostatic (I and II) and thermal (III and IV) shock experiments, based on Oliver et al. (2020), can be found in Table 1. Experiments I and II examined the duration of hydrostatic shock, 180°C min post-fertilization, with applications of 7500 psi for 10, 20, or 30°C min as well as 8500 psi for 5, 10, and 15°C min, respectively. Experiment III investigated the optimal post-fertilization shock time using a thermal shock of 16°C at 60, 90, or 120°C min post-fertilization, for 400°C min. Experiment IV explored the optimal duration of shock using a thermal shock of 16°C for 350, 400, or 450°C min, 120°C min post-fertilization.

2.3 | Assessment of triploid sterility

Each experimental unit was an entire spawn from a randomly selected, ripe, anesthetized female from the 2018 burbot year-class. Each experimental unit was fertilized <1 min after spawning with ~2 mL of milt from a single randomly selected ripe male following confirmation of >80% sperm motility. The first three 2018-year class females, from the diploid and triploid populations, were selected for the three corresponding replicates of the three experiments. Briefly, females were gently patted dry and entire spawns were stripped into a dry 3 L vessel, milt was stripped directly onto the eggs, and 2 L of water at 2°C was used to mix and activate the gametes. At 2 min post-fertilization, the water and milt were decanted from the eggs, and eggs were quickly resuspended with 2 L of fresh 2°C water. Eggs were disinfected in a 25 ppm ovadine solution from 35 to 45 min post-fertilization, and then the disinfectant solution was decanted and water replaced as previously described. At 90 min post-fertilization, after water hardening, each experimental unit was volumetrically quantified as previously described in Section 2.2 and loaded into a 1 L upwelling Imhoff cone for the duration of the experiment. Eggs were incubated and hatch was synchronized using the methods and conditions described in Section 2.2. Absolute survival of eggs was calculated based on initial and final egg volumes. Finally, three samples of eggs, ~100 eggs per sample, were photographed weekly using a digital camera (PowerShot SX620 HS).

An outline of the experimental design can be found in Table 2. In experiment A, diploid female eggs were fertilized with diploid milt. In experiment B, triploid female eggs were fertilized with diploid milt. Milt from a triploid male was used to fertilize the eggs of a diploid female, in experiment C. All experiments had three replicates, males and females were used for a single experimental unit, thus only spawned once.

2.4 | Flow cytometry data collection and analysis

Flow cytometry for DNA content was conducted on 3–7 days post-hatch larvae as well as milt collected through the spawning season. Ten larvae were randomly collected from each surviving replicate in the triploid optimization

TABLE 1 Summary of triploid induction experiments.

Experiment	Shock type	Post-fertilization time (°C·min)	Duration of shock (°C·min)	Shock intensity/ temperature
I	Hydrostatic	180	10, 20, 30	7500 psi
II	Hydrostatic	180	5, 10, 15	8500 psi
III	Thermal	60, 90, 120	400	16°C
IV	Thermal	120	350, 400, 450	16°C

TABLE 2 Summary of crosses to assess triploid sterility.

Experiment	Male ploidy	Female ploidy
A	Diploid	Diploid
B	Diploid	Triploid
C	Triploid	Diploid

Note: Full female spawn was fertilized with ~2 mL of milt from respective male.

experiments; larvae were stored in water at 4°C until analysis. A single drop of milt was collected and fixed in 75% alcohol from all diploid and triploid fish used in the triploid sterility assessment. Nuclei staining, sample composition, and preparation, as well as flow cytometry analysis, were carried out as described by Oliver et al. (2020). Briefly, for each sample a single larva or 4 μL of fixed milt was deposited in a microcentrifuge tube with 800 μL of staining solution (50 mg L^{-1} propidium iodide [VWR, Wayne PA, USA], 10 mg L^{-1} RNase A [VWR, Wayne PA, USA] in Isoton II solution [VWR, Wayne PA, USA]). All ploidy analysis included a single internal standard, 1 μL of a 0.05x dilution of diploid burbot blood, collected from 3-year-old diploid fish and diluted in 75% alcohol. Samples were incubated in the staining solution in the dark at 4°C for ~24 h, then vortexed and aspirated through a 26 G needle to break up samples into individual cells. After sample was sufficiently fragmented, samples were drawn into a 1 mL syringe and then dispensed from the syringe through a 60-μm mesh (Ablazecustom, Hong Kong, China) and clean 26 G needle into a sterile 1.5 mL microcentrifuge tube. A Beckman Coulter Cytoflex S flow cytometer (Beckman Coulter Inc., Brea CA, USA) was used to record 50,000 events per sample. The ratio of the mean florescence intensity (PerCP-A) between diploid sample peak and the sample peak was used to determine the ploidy of the sample (Figure 1). A triploid larva was confirmed when the sample mean fluorescence was 1.5 times higher than the mean fluorescence of the diploid burbot blood sample peak; aneuploidy of triploid sperm was confirmed when the mean fluorescence of the sample was something other than 0.5 times the diploid burbot blood sample peak.

2.5 | Data analysis

Weight loss over the spawn season was calculated $(((W_{pre} - W_{post})/W_{pre}) \times 100)$, where W_{pre} is pre-spawn weight and W_{post} is post-spawn weight (Table 3). Condition factor (K; Ricker, 1975) and relative weight (W_r; Wege & Anderson, 1978; Table 3) were used to determine broodstock vigor and were calculated using the standard weight equation for North American burbot *Lota lota* maculosa ($\log10Ws = 2.898 \cdot \log10TL - 4.868$; Fisher et al., 1996). The equation utilized is $10^5 \times (W/TL^3)$, where 10^5 is the standard condition variable for burbot, W is the pre-spawn weight, and TL is total length (Ashton et al., 2019; Table 3). Total volume of eggs stripped from each female was used to determine apparent fecundity using E/W_r, where E is the total volume of eggs and W_r is the pre-spawn weight of the corresponding female. Volumetric quantification of eggs was determined by multiplying the volume of eggs, in milliliters, by 1000 (Jensen et al., 2008). The spawn volume was collected after egg hardening, 24 h post-fertilization, and again 1 day prior to hatch initiation. Total incubation time was calculated in degree-days, days of incubation multiplied by average incubation temperature, monitoring of degree-days was arrested 1 day prior to hatch or at time of entire spawn death. Dead burbot eggs are removed from incubators via hydrogen peroxide, thus spawn death was determined when all eggs had been removed from incubators. Three samples of 100 eggs, at 24 h post-fertilization, were collected from each replicate in the sterility experiments and analyzed for fertilization, and egg diameter with ImageJ software (version 1.50b; National Institutes of Health, Bethesda, Maryland).

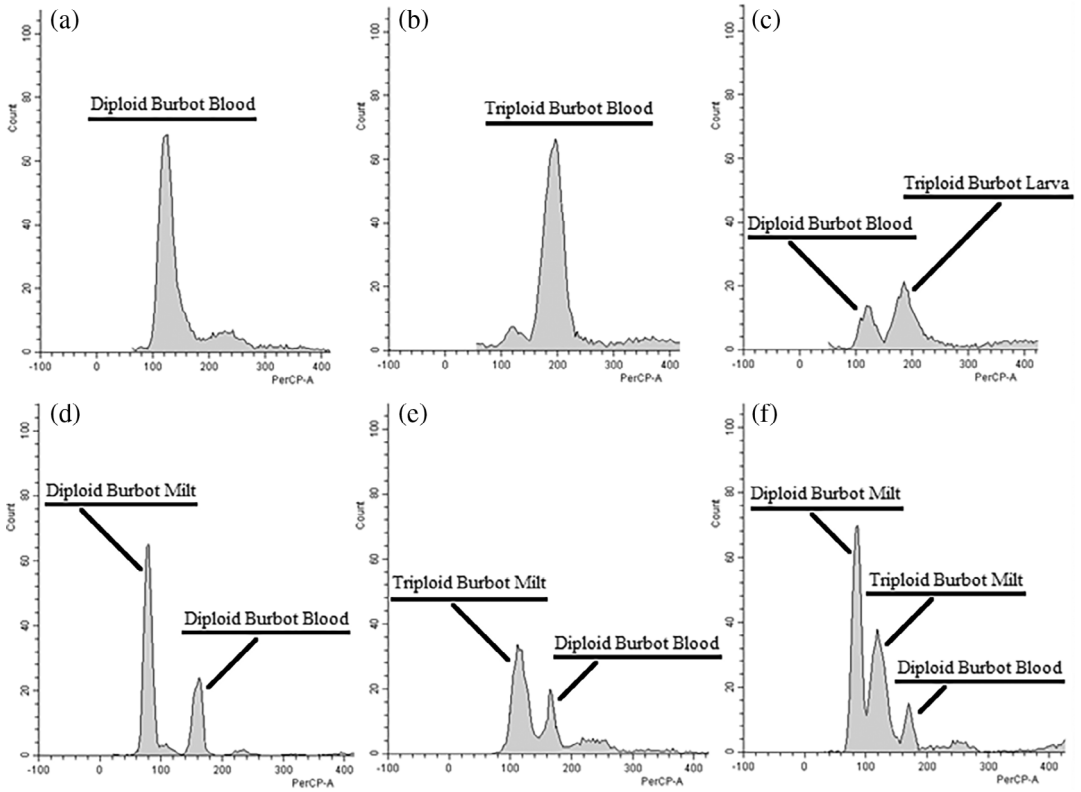

FIGURE 1 Illustration of flow cytometry analysis of nucleic DNA from burbot, *Lota lota*, samples; peak fluorescent intensity (PerCP-A) corresponds to diploid, triploid, haploid, or aneuploid cells. Along the *x*-axis, nuclear DNA values are reported in arbitrary units of fluorescence intensity (PerCP-A), and count of events is displayed on the y axis. Relative difference of fluorescence intensity between samples of the same type, in different graphs, is attributed to deviation in flow cytometry calibration between experiments. A total of 50,000 events were recorded for each sample, and peaks within individual graphs with multiple peaks are described from left to right.
(a) represents diploid burbot blood, (b) is triploid burbot blood, (c) displays diploid burbot blood and a triploid burbot larva, (d) displays diploid burbot milt and blood, (e) displays triploid burbot milt and diploid blood, and (f) displays diploid burbot milt, triploid burbot milt, and diploid burbot blood.

TABLE 3 List of formulas used prior to statistical analysis of broodstock and eggs.

Variable	Definition or formula	Units
Total length (TL)	Distance from tip of snout to end of tail	mm
Wet weight (W)	Mass of a fish	kg
Condition factor (K)	$10^5 \times (W/TL^3)$	g/mm^3
Apparent fecundity (F)	E/W_r	Eggs/g
Percent weight loss (WL)	$(((W_{pre} - W_{post})/W_{pre}) \times 100)$	%
Egg diameter (D)	Cross sectional distance of a live egg	mm
Absolute survival (AS)	Initial egg volume/pre-hatch egg volume	%
Relative survival (RS)	Average control egg volume/experimental unit egg volume	%

2.6 | Statistical analysis

Data were analyzed after normality and homogeneity of variance was determined using a Shapiro–Wilk and a Bartlett test, respectively. Parametric data with two treatments were analyzed using a Welch two-sample t-test. Parametric data with more than two treatments were analyzed via ANOVA; if significance was observed, a Tukey's post-hoc test was employed to determine significances between treatments. Nonparametric data with two treatments were analyzed with Wilcoxon signed-rank test. Finally, Kruskal–Wallis one-way analysis of variance was used to analyze nonparametric data with more than two treatments; post hoc analysis was performed using a Dunn's test. All analysis was performed with Rstudio software, significance was noted when $p \leq 0.05$.

3 | RESULTS

3.1 | Triploid induction optimization

Triploid and tetraploid induction in larvae from experiment I is based on a single surviving replicate; because of a malfunction during incubation for this treatment, statistical analysis was not possible. The single surviving replicate demonstrates that a shock of 7500 psi at 180°C min post-fertilization for 10 or 20°C min induced triploidy at or over 90%; furthermore, tetraploid induction of 10% was observed with a shock duration of 20°C min. Relative pre-hatch survival in experiment I is based on three surviving replicates; no significant differences in survival were found between the control, 10, and 20°C min shock durations; however, survival for the 30°C min shock duration was significantly lower when compared with all other groups (Table 4). In experiment II, triploid induction was statistically similar for all durations of shock; the 15°C min shock duration was the only experimental treatment that was statistically similar to the control group. Generation of tetraploids was observed in the 5°C min shock duration group. Relative survival of pre-hatch embryos in experiment II was significantly lower for the 15°C min treatment group relative to the control and other treatment groups (Table 5).

In experiment III, percent triploid induction was statistically similar for all treatments, and tetraploids were observed at a post-fertilization shock time of 120°C min. Relative survival for all experimental treatments was statistically similar, but all statistically lower relative to the control group (Table 6). Triploid and tetraploid induction in larvae from experiment IV is based on a single surviving replicate because of incubator malfunctions, and therefore, statistical analysis was not possible for this experiment. The single surviving replicate demonstrates that a duration of shock of 16°C at 120°C min post-fertilization for 350, 400, or 450°C min has the potential to induce triploidy at or over 90%. Relative pre-hatch survival in experiment IV is based on three surviving replicates; no significant differences in survival were found between the control and any of the experimental treatments (Table 7).

TABLE 4 Experiment I: Shock of 7500 psi at 180°C min post-fertilization with different duration of shock.

Duration of shock (°C·min)	Triploid induction (%)	Tetraploid induction (%)	Relative pre-hatch embryo survival (%)
None	0.00	0.00	100.00 (±0.00)^{z}
10	100.00	0.00	72.22 (±14.70)^{z}
20	90.00	10.00	43.46 (±5.14)^{z}
30	-	-	20.59 (±15.09)^{x}

Note: Ploidy induction values obtained from a single replicate; relative pre-hatch survival based on three replicates. Superscript letters denote statistical differences observed between treatment groups ($p \leq 0.05$). Dashes indicate eggs larvae did not hatch and thus could not be analyzed for ploidy.

TABLE 5 Experiment II: Shock of 8500 psi at 180°C min post-fertilization with different duration of shock.

Duration of shock (°C·min)	Triploid induction (%)	Tetraploid induction (%)	Relative pre-hatch embryo survival (%)
None	-	-	100.00 (±0.00)^{z}
5	93.33 (±6.67)^{a}	6.67 (±6.67)	51.53 (±32.04)^{z}
10	100.00 (±0.00)^{a}	-	41.10 (±29.29)^{z}
15	33.33 (±33.33)^{ab}	-	0.10 (±0.10)^{x}

Note: Superscript letters denote statistical differences observed between treatment groups ($p \leq 0.05$). Dashes denote treatment groups with no triploid or tetraploid individuals.

TABLE 6 Experiment III: Shock of 16°C for 400°C min at a different post-fertilization time.

Post-fertilization shock time (°C·min)	Triploid induction (%)	Tetraploid induction (%)	Relative pre-hatch embryo survival (%)
Control	-	-	100.00 (±0.00)^{z}
60	86.67 (±8.82)^{a}	-	32.23 (±36.13)^{x}
90	76.67 (±14.53)^{a}	-	36.13 (±18.19)^{x}
120	76.67 (±8.82)^{a}	3.33 (±3.33)	37.47 (±7.79)^{x}

Note: Superscript letters denote statistical differences observed between treatment groups ($p \leq 0.05$). Dashes denote treatment groups with no triploid or tetraploid individuals.

TABLE 7 Experiment IV: Shock of 16°C at 120°C min post-fertilization for different shock durations.

Duration of shock (°C·min)	Triploid induction (%)	Relative pre-hatch embryo survival (%)
None	0.00	100.00 (±0.00)^{z}
350	90.00	74.79 (±17.14)^{z}
400	90.00	45.08 (±16.10)^{z}
450	100.00	32.26 (±3.92)^{z}

Note: Ploidy induction values obtained from a single replicate; relative pre-hatch survival based on three replicates. Superscript letters denote statistical differences observed between treatment groups ($p \leq 0.05$).

3.2 | Assessment of triploid sterility

All milt from triploid fish was aneuploid; eggs from triploid fish lacked a single oil globule and were white and opaque instead of transparent (Figure 2). The pre-hatch survival of eggs from experiments B and C, the triploid x diploid crosses, was 0.0 (±0.0) %, and was significantly lower relative to experiment A, the diploid x diploid crosses, 37.25 (±10.78) %. Fertilization was statistically similar for the two experiments that used eggs from diploid fish, but was significantly lower for the experiment utilizing eggs from triploid burbot (Table 8). The total incubation time was significantly lower for eggs from triploid females relative to the diploid x diploid crosses, while incubation time for diploid eggs fertilized with triploid sperm did not significantly differ from either treatment. The diploid fish used for the sterility assessment were significantly longer and heaver, relative to the fish utilized from the triploid population. Spawn volume was significantly lower for triploid females, 64.00 (±48.12) mL, relative to diploid females, 365.83 (±36.10) mL. However, eggs/g^{-1} were not significantly different between diploid and triploid females, 198.21 (±34.43) and 111.33 (±82.85), respectively. Condition factor between diploids and triploids were not significantly different prior

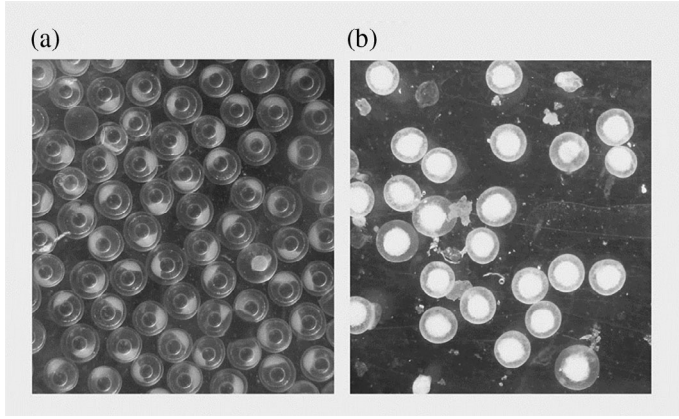

FIGURE 2 Photographs of burbot eggs, 24 h post-fertilization. (a) is a photograph of diploid burbot eggs, and (b) is a photograph of triploid burbot eggs.

TABLE 8 Sterility experiments, male and female ploidy indicate parental crosses for each experiment.

Experiment	Male ploidy	Female ploidy	Pre-hatch survival (%)	Fertilization (%)	Incubation time (°C × days)
A	Diploid	Diploid	37.25 (±10.78)	85.67 (±6.43)^{m}	104.00 (±8.73)^{x}
B	Diploid	Triploid	-	-	13.90 (±10.94)^{y}
C	Triploid	Diploid	-	93.00 (±0.00)^{m}	56.80 (±16.39)^{xy}

Note: Incubation time ended at hatch or when all eggs had died; dead eggs are removed from incubators via hydrogen peroxide treatments. Superscript letters denote statistical differences observed between treatment groups ($p \leq 0.05$). Dashes denote treatment groups with no survival or no observed fertilization.

to or after the spawning season. Weight loss over the spawning season was significantly lower for triploid male and females relative to diploid males and females. Despite displaying differences in structure, eggs from diploids and triploids were not significantly different in regard to diameter (Table 9).

4 | DISCUSSION

Shock results are consistent with the first experiments investigating triploid induction in burbot, with application of 7500 or 8500 psi observed to induce up to 100% triploid induction, and thermal shocks at 16°C inducing survival at or above 90% (Oliver et al., 2020). Increase in shock duration, intensity, or both was found to significantly reduce survival of eggs (Oliver et al., 2020). Therefore, it was important to optimize burbot triploid induction while reducing these stressors to maximize survival while maintaining high triploid induction. Experiments I and II confirm that shorter shock durations have the potential to induce 100% triploid induction while significantly improving survival (Oliver et al., 2020). However, unlike the hydrostatic trials, experiment IV exhibited no improvements in survival with reduced duration of shock. Shorter shocks may be preferable to aquaculturists, as less time is required to perform the induction and, in the case of hydrostatic shock, expensive shocking equipment is unnecessary (Maxime, 2008).

The timing of shock after fertilization is critical; if shocks are applied shortly after the second meiotic division has occurred, then the resulting embryo will likely develop as a diploid with one set of chromosomes from each parent, as the second female chromosome set has been extruded at this time. Experiment III, investigation of post-fertilization thermal shock time, showed no significant difference in triploid induction among shock treatments,

TABLE 9 Experimental parameters analyzed for sterility experiments.

Experimental parameter	Diploid	Triploid
Male pre-spawn weight (kg)	1.17 (±0.15)*	0.58 (±0.02)
Female pre-spawn weight (kg)	1.83 (±0.23)*	0.55 (±0.30)
Male length (cm)	54.22 (±1.82)*	44.67 (±0.33)
Female length (cm)	61.67 (±2.49)*	44.67 (±0.88)
Spawn volume (mL)	365.83 (±36.10)*	64.00 (±48.12)
Eggs per gram of female body weight	198.21 (±34.43)	111.33 (±82.85)
Male pre-spawn condition factor	0.71 (±0.04)	0.65 (±0.00)
Female pre-spawn condition factor	0.71 (±0.07)	0.62 (±0.03)
Male post-spawn condition factor	0.65 (±0.04)	0.65 (±0.00)
Female post-spawn condition factor	0.75 (±0.05)	0.62 (±0.03)
Male spawn season weight loss (%)	10.55 (±0.03)	-
Female spawn season weight loss (%)	34.17 (±0.09)	-
Egg diameter (mm)	1.16 (±0.04)	1.04 (±0.06)

Note: Asterisks denote statistical differences observed between treatment groups ($p \leq 0.05$). Dashes denote where weight loss was 0.0%.

indicating that the second meiotic division does not occur before 120°C min post-fertilization (Wu et al., 2019). After completion of meiosis in the egg, the zygote initiates mitotic division, an application of shock after the duplication of the zygote chromosomes will prevent the first cellular cleavage and the one cell zygote retains the duplicated set of two parental chromosomes, the zygote is tetraploid. After the shock, mitosis and cellular cleavage continue as normal but all cells remain tetraploid after each mitotic cycle. Tetraploids were produced in three of the optimization experiments, indicating that the first cleavage occurs at some point between 120 and 180°C min post-fertilization; this is the first documentation of tetraploid burbot. Tetraploids are useful in aquaculture as diploid gametes from tetraploids pair properly with haploid gametes from diploids and can generate entire cohorts of triploids through breeding and without the use of shocking procedures or equipment (Wu et al., 2019).

The sterility experiments demonstrate that gametes produced by triploids were not viable and unable to combine with respective diploid gametes to generate viable larvae. Eggs from triploid burbot did not appear to fully mature, and no fertilization was observed. Conversely, milt from triploid burbot was functional and successfully fertilized diploid eggs, but its aneuploid genetic composition led to errors in mitosis and fertilized eggs aborted approximately half-way through incubation. Triploid burbot were not crossed with other triploids because of lack of spawning triploid female; however, the literature suggests that these crosses would not produce viable larvae (Allen & Wattendorf, 1987).

In some cases, triploid fish have been reported to reach a larger size, relative to their diploid peers (Maxime, 2008), but this was not part of the analysis in this study. The triploid population used in this study was younger, significantly lighter, and shorter relative to the diploid population utilized. Furthermore, growth of the triploid population used here was likely stunted as the fish were held in 10 gal aquarium during the first year of development (Huntingford et al., 2006). Despite these issues, biometric analysis of the triploid and diploid populations through the spawning season demonstrated that triploid fish lost less weight through the reproductive season relative to the diploid populations. This may indicate that triploid burbot devote less energy to generation of gametes, and this may allow a greater rate of growth of adult triploids relative to adult diploids.

Optimum conditions for triploid induction and egg survival in burbot are outlined in this study. The sterility of triploid burbot was confirmed and initial biometric information suggests that triploid burbot devote fewer resources to spawning and gamete generation. This is the first report of tetraploid induction in burbot. This could have

significant implications for future research and aquaculture production in general. If tetraploid burbot could be crossed with triploid burbot, it could eliminate the use of shocking methods and materials altogether. The use of sterile burbot for aquaculture is considered a key manipulation and may allow producers to culture the species in regions where escapement may be considered a risk.

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CONFLICT OF INTEREST STATEMENT

No conflicts of interest are declared by the authors.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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