Notes

Nonlethal Tools to Identify Mass Ovarian Follicular Atresia in Burbot

Lauren M. McGarvey,* Jason E. Ilgen, Christopher S. Guy, Jason G. McLellan, Molly A.H. Webb

L.M. McGarvey
Montana Cooperative Fishery Research Unit, Department of Ecology, Montana State University, Bozeman, Montana 59717

Present address: Yellowstone Center for Resources, Yellowstone National Park, Wyoming 82190

J.E. Ilgen, M.A.H. Webb
U.S. Fish and Wildlife Service, Bozeman Fish Technology Center, Bozeman, Montana 59715

C.S. Guy
U.S. Geological Survey, Montana Cooperative Fishery Research Unit, Department of Ecology, Montana State University, Bozeman, Montana 59717

J.G. McLellan
Fish and Wildlife Department, Confederated Tribes of the Colville Reservation, Spokane, Washington 99201

Abstract

Skipped spawning occurs in many annual spawning fish species and should be evaluated to effectively manage their populations. We assessed plasma sex steroid concentrations and measured gonad size and ovarian follicle diameter as metrics to nonlethally identify mass ovarian follicular atresia (i.e., skipped spawning) in Burbot *Lota lota*. We maintained wild fish in captivity and exposed them to increasing water temperatures during a 3-wk period before the spawning season to induce mass ovarian follicular atresia. We collected ovarian follicles, blood plasma, and gonadal sonograms from fish weekly between January 28, 2018, and March 25, 2018. We histologically analyzed ovarian follicles to confirm stage of maturity. We measured concentrations of plasma sex steroids testosterone (T) and estradiol-17β (E2) by radioimmunoassay. We measured gonad diameter and circumference by ultrasonography and ovarian follicle diameter by image analysis. Mean plasma T concentration decreased from 8.94 ng/mL during late vitellogenesis to 1.83 ng/mL during atresia, suggesting that plasma T concentrations may be used to identify mass ovarian follicular atresia. We do not recommend using plasma E2 concentrations to identify mass ovarian follicular atresia because E2 concentrations rapidly decreased during the completion of vitellogenesis and the initiation of atresia in Burbot; therefore, plasma E2 may not accurately identify mass ovarian follicular atresia. Mean gonad diameter measured by ultrasonography decreased from 4.05 cm during late vitellogenesis to 3.65 cm during atresia. Mean diameter of ovarian follicles decreased during the final week of the study, suggesting that ovarian follicle diameter may be used to identify advanced mass ovarian follicular atresia. The nonlethal tools assessed—plasma sex steroid concentrations, ultrasonography, and ovarian follicle diameter—enable fisheries biologists to determine the occurrence and frequency of mass ovarian follicular atresia among Burbot in Lake Roosevelt and may be applied to other Burbot populations.

Keywords: reproductive physiology; nonlethal tools; plasma sex steroids; ultrasound; ovarian follicle diameter

Received: March 2022; Accepted: August 2022; Published Online Early: September 2022; Published: December 2022

Citation: McGarvey LM, Ilgen JE, Guy CS, McLellan JG, Webb MAH. 2022. Nonlethal tools to identify mass ovarian follicular atresia in Burbot. *Journal of Fish and Wildlife Management* 13(2):xx-xx; e1944-687X. https://doi.org/10.3996/JFWM-22-018

The findings and conclusions in this article are those of the author(s) and do not necessarily represent the views of the

U.S. Fish and Wildlife Service.

* Corresponding author: mcgarveylm@gmail.com

Introduction

Fisheries scientists have long recognized the importance of including indices of reproductive potential, such as sex ratio, population reproductive structure, and spawning periodicity, in modeling population growth (Trippel 1999; Jørgensen et al. 2006; Morgan 2008; Lowerre-Barbieri et al. 2011; Morgan et al. 2011; Maskill et al. 2022). Fisheries management routinely uses spawning stock biomass as an index of reproductive potential to predict population growth and inform management actions. However, spawning stock biomass assumes that all females of a certain size or age spawn at the most frequent interval (i.e., annual spawners spawn annually) and does not account for variation in spawning periodicity, first referred to as ''skipping reproduction'' (Trippel 1999) and now referred to as ''skipped spawning'' (Rideout et al. 2005). Skipped spawning has been documented in many fish species and is the failure of an individual to spawn during a reproductive cycle (Rideout et al. 2005; Rideout and Tomkiewicz 2011). Models using spawning stock biomass (i.e., not accounting for skipped spawning among females) may overestimate future population sizes and lead to ineffective management actions (Rideout and Tomkiewicz 2011).

Previous research has described three forms of skipped spawning in female fish: resting, retaining, and reabsorbing (Rideout et al. 2005; Rideout and Tomkiewicz 2011). Resting refers to mature (i.e., capable of spawning) females that do not enter the reproductive cycle by maintaining all ovarian follicles in the primary growth stage (i.e., previtellogenic), whereas retaining refers to mature females that produce fully developed ovarian follicles that are never released (Rideout and Tomkiewicz 2011). Reabsorbing refers to mature females that enter the reproductive phase (i.e., begin vitellogenesis), but ovarian follicles ultimately undergo mass atresia (Rideout and Tomkiewicz 2011).

Ovarian follicular atresia is the breakdown and removal of ovarian follicles from the ovary to recover and recycle the materials (Grier et al. 2009; Wootton and Smith 2014). Ovarian follicular atresia is common in female teleosts and is thought to maintain ovarian homeostasis by controlling the number of developing ovarian follicles (Tyler and Sumpter 1996; Lubzens et al. 2010). However, increased levels of ovarian follicular atresia can be initiated by stress, fasting, environmental contaminants, light, temperature, confinement, or inadequate hormone levels (Miranda et al. 1999; Lubzens et al. 2010). Mass ovarian follicular atresia (i.e., the majority of ovarian follicles undergoing atresia) results in skipped spawning during a reproductive cycle (Rideout et al. 2000, 2005).

Skipped spawning is difficult to detect, often requiring invasive or lethal sampling methods. Gonadal histology is the most accurate method to identify mass ovarian follicular atresia in fishes, but requires sacrificing the fish (Rideout et al. 2005). It is possible to visualize atretic follicles in stained histological sections (Corriero et al. 2021) or in unstained histological sections under fluorescence microscopy highlighting lipofuscins (Medina et al. 2021). Talbott et al. (2011) used plasma sex steroid concentrations to nonlethally identify mass ovarian follicular atresia in White Sturgeon *Acipenser transmontanus*. Ovarian follicles undergoing atresia cease to synthesize sex steroids, resulting in decreased plasma sex steroid concentrations (Linares-Casenave et al. 2002; Webb et al. 2002; Talbott et al. 2011) and decreased size due to the yolk being phagocytized and resorbed (Miranda et al. 1999).

The purpose of this study was to assess nonlethal tools to identify mass ovarian follicular atresia in Burbot *Lota lota*, a determinate and total spawning teleost. Previous studies have documented variation in spawning periodicity in several Burbot populations: approximately 15% of adult female Burbot in the Tanana River, Alaska, and 30% of adult female Burbot in the Bothnian Bay, Finland, were thought to skip spawning during the annual reproductive cycle (Pulliainen and Korhonen 1990; Evenson 2000); however, it is unknown whether these females initiated mass ovarian follicular atresia. Assessing the frequency of skipped spawning via mass ovarian follicular atresia among female Burbot enables fisheries biologists to monitor for nonoptimal environmental conditions that are known to cause skipped spawning, such as temperature alterations and environmental pollution (Corriero et al. 2021). In addition, accounting for the decreases in a population's reproductive potential via mass ovarian follicular atresia among females enables fisheries biologists to more accurately model population growth and establish effective harvest regulations.

Study Area

Lake Roosevelt is located in northeastern Washington and formed after the construction of Grand Coulee Dam on the Columbia River. It is 1–3 km wide, with a maximum depth of 122 m, and extends 241 km upstream from Grand Coulee Dam to the Canadian border (Polacek et al. 2006). Lake Roosevelt supports many recreational fisheries, including a Burbot fishery. The Burbot fishery is relatively small, with approximately 485 angler trips (2,942 angler h) and 1,325 harvests per y, as estimated from creel survey data (Spokane Tribe of Indians, unpublished data). However, estimates may be low because the creel survey design does not adequately capture the Burbot fishery, which often occurs at nonsurvey shoreline locations and at night. Thus, the

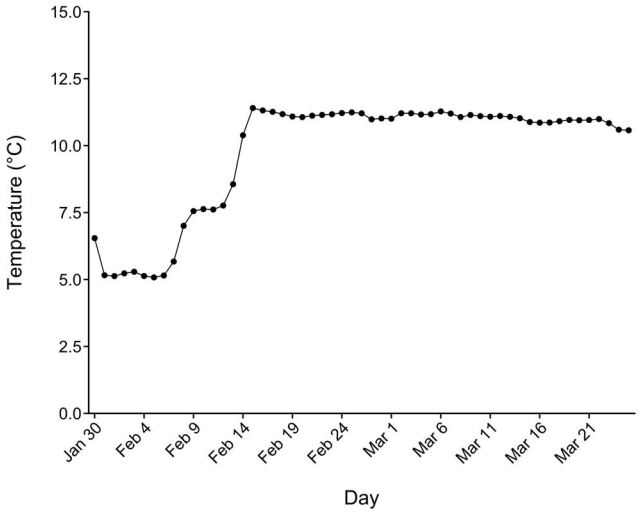

Figure 1. Thermal profile on which we maintained adult female Burbot *Lota lota* at the Bozeman Fish Technology Center, Bozeman, Montana. We increased water temperature from 5 to 11°C during a 2-wk period before the spawning season (from January 1, 2018, to February 14, 2018) to initiate mass ovarian follicular atresia.

Burbot fishery may be underused and able to support greater harvest (CTCR 2018).

Methods

Fish collection and maintenance

The Confederated Tribes of the Colville Reservation collected adult Burbot for laboratory research from Lake Roosevelt in October 2017. They set baited cod (genus *Gadus*) traps shallower than 10 m to prevent barotrauma in captured fish and retrieved traps the following day. They tagged each fish with a passive integrated transponder and transferred 45 fish to the Bozeman Fish Technology Center, Bozeman, Montana. For this study, we kept a subset of 10 fish in two 300-L circular tanks (91.4 × 45.7 cm) under a natural light cycle and constantly provided fish with live Rainbow Trout *Oncorhynchus mykiss* (150 mm) as feed. Burbot spawn at temperatures of 1–4°C in February or March (McPhail and Paragamian 2000); therefore, we increased water temperature from 5 to 11°C during a 2-wk period before the spawning season to induce mass ovarian follicular atresia (Figure 1). We attained maximum temperature by February 14, 2018.

Biological sampling

We repeatedly sampled 10 females in the late stages of gametogenesis each week from January 29, 2018, to March 25, 2018. We anesthetized fish by using 50 ppm tricaine methanesulfonate (MS-222) and continuously supplied fresh water to their gills during sampling. We collected an ovarian follicle sample, blood sample, and gonadal sonogram from each fish.

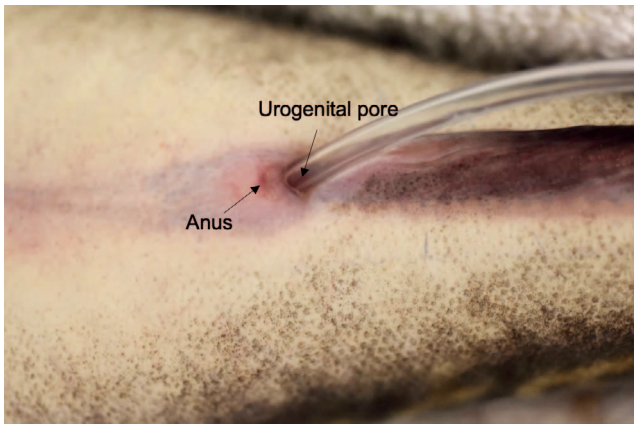

Figure 2. Collection of ovarian follicles by insertion of a flexible catheter through the urogenital pore into the ovary of an adult female Burbot *Lota lota* in February 2018.

Histology

We collected ovarian follicles by inserting a flexible catheter (Tygon tubing, 4-mm internal diameter, 6-mm external diameter) through the urogenital pore into the ovary and then applied suction by mouth (Rottmann et al. 1991; Figure 2). Given that Burbot have group synchronous gonadal development, we assumed that the sample was representative of the entire ovary. We preserved a subset of collected ovarian follicles ($n = 30$) in a 10% solution of phosphate-buffered formalin. We then embedded ovarian follicles in paraffin wax, sectioned them at 5 μm, and stained the sections with hematoxylin and eosin or periodic acid-Schiff for histological analysis (Luna 1968). We examined ovarian follicles under a DM compound scope (×250–450; Leica Biosystems Inc., Lincolnshire, IL) and assigned a stage of maturity by using our previous description of gametogenesis in female Burbot (McGarvey et al. 2020; Table 1). Because females assessed lethally with equal to or greater than 50% of vitellogenic oocytes undergoing atresia were not likely to spawn (Hunter and Macewicz 1985), we classified fish as atretic (stage 9) if histological signs of atresia (i.e., thickened and deformed follicular layers) were evident in more than 75% of ovarian follicles to be conservative with our classification. We assumed as per Rideout and Tomkiewicz (2011) that atresia continued, leading to the eventual resorption of all ovarian follicles.

Plasma sex steroids

We collected blood from the caudal vasculature by using a heparinized 3-mL syringe with a 22-gauge needle (Webb et al. 2002). We separated plasma by centrifugation (1,228 × g for 5 min) and stored plasma at −80°C until analysis. We extracted testosterone (T) and estradiol-17β (E2) from the plasma following the methods of Fitzpatrick et al. (1987). In brief, we extracted steroids twice from 100 μL of plasma with 2 mL of diethyl ether. We vigorously vortexed tubes with ether and removed the aqueous phase by snap-

Table 1. Description of gonadal stages of maturity in female Burbot *Lota lota* assigned by histological analysis of ovarian follicles from McGarvey et al. (2020).

Sex	Stage of maturity	Description
Female	Oogonial proliferation (1)	Oogonia and potentially a few primary growth oocytes
	Primary growth (2)	Primary growth oocytes and oogonia
	Cortical alveolar (3)	Cortical alveoli on periphery of ovarian follicles
	Early vitellogenic (4)	Yolk granules accumulating in periphery of ovarian follicles, cortical alveoli on periphery of ovarian follicles, one layer of zona radiata
	Midvitellogenic (5)	Yolk globules with a mean diameter of 7.93 μm (5.97–9.89 μm; 95% CI) accumulating toward center or may be present throughout ovarian follicles, few cortical alveoli on periphery of ovarian follicles, one layer of the zona radiata
	Late vitellogenic (6)	Yolk fusing into larger globules with a mean diameter of 19.62 μm (14.55–24.69 μm; 95% CI) throughout ovarian follicle, one layer of zona radiata, central germinal vesicle (i.e., nucleus)
	Ripe (7)	Yolk fused but not completely coalesced in all ovarian follicles, offset germinal vesicle
	Postovulatory (8)	Postovulatory follicles present with primary growth oocytes
	Atretic (9)	Greater than 75% atretic ovarian follicles, no postovulatory follicles present with primary growth oocytes

freezing in liquid nitrogen. We resuspended extracted steroids in 1 mL of phosphate-buffered saline with gelatin. We determined extraction recovery efficiencies for T and E2 by adding tritiated steroids to tubes containing pooled plasma ($n = 4$) and then we extracted as described above. Extraction recovery efficiencies were 87–91% for T and 80–87% for E2. We corrected all steroid concentrations for the extraction recovery efficiency.

We quantified plasma T and E2 concentrations by radioimmunoassay as described by Fitzpatrick et al. (1986) and modified by Feist et al. (1990). We assayed samples in duplicate. For quantified plasma sex steroid concentrations below the minimum quantifiable concentration of the radioimmunoassay, we assigned the minimum quantifiable concentration ($n = 1$ for T; $n = 5$ for E2; see *Supplemental Material*, Data S1). For plasma sex steroid concentrations that were not quantifiable (below the assay detection limit), we assigned half of the minimum quantifiable concentration for statistical purposes ($n = 0$ for T; $n = 15$ for E2; Data S1; Croghan and Egeghy 2003). The intra- and interassay coefficient of variation for all assays was less than 5 and 10%, respectively.

Ultrasonography

We used an Edge ultrasound machine (SonoSite Inc., Bothell, WA) with a linear transducer (6–15 MHz) to measure gonad size in anesthetized fish. We used the Small Parts exam type with the optimization set to general. We set the ultrasound scanning depth between 2.2 and 2.8 cm. We oriented fish ventral side up and placed the ultrasound transducer on the abdomen to locate the right gonad. We always measured the right gonad for standardization. We scanned the gonad until the perceived maximum cross section was in view (Blythe et al. 1994; Evans et al. 2004). The gonad was elliptical, with major and minor axes. We defined gonad diameter as the length of the major axis and measured gonad diameter (± 0.01 cm) by using the ultrasound caliper function (McGarvey et al. 2021). We assigned a value of 5.11 cm (the width of the ultrasound transducer) if the gonad was wider than the ultrasound transducer and unmeasurable (McGarvey et al. 2021). We measured gonad circumference in the same location by using the ultrasound caliper function on manual trace (McGarvey et al. 2021). We did not measure circumference if the gonad was wider than the ultrasound transducer (> 5.11 cm).

Ovarian follicle diameter

We collected ovarian follicles (described above) by inserting a flexible catheter through the urogenital pore into the ovary and then applying suction by mouth. We preserved a subset of collected ovarian follicles ($n = 20$) in Ringer's solution (Dettlaff et al. 1993). We measured 15 ovarian follicles (± 1.0 μm; Johnson 1971) from each female by image analysis by using SPOT Advanced software (SPOT Imaging, Sterling Heights, MI).

Data analyses

We used linear mixed effects models for mean comparisons (plasma sex steroid concentrations, gonad diameter, and ovarian follicle diameter) among stages of maturity and weeks (ovarian follicle diameter only) while accounting for repeated measures among individuals (e.g., plasma sex steroid concentration ~ stage of maturity + (1|fish ID)). We determined gonad diameter and ovarian follicle diameter data to be normally distributed by examining the fitted model residuals. We determined steroid (T and E2) data to be nonnormally distributed and normalized steroid data by using the $\log_e$ transformation for analyses. We followed the mixed effect model indicating differences in ovarian follicle diameter among weeks by a pairwise mean comparison using a Bonferroni correction. We completed all statistical analyses using R software (version 3.3.2; https://cran.r-project.org). Data are presented as mean ± SD.

Table 2. Number of female Burbot *Lota lota* by week and stage of maturity determined by gonadal histology (n = 10 at initiation of study). We maintained Burbot at the Bozeman Fish Technology Center, Bozeman, Montana, and exposed them to increasing water temperatures before the spawning season to initiate mass ovarian follicular atresia. We collected data weekly from January 28, 2018, to March 25, 2018, and attained maximum temperature (11°C) by February 14.

Week	Late vitellogenic (stage 6)	Atretic (stage 9)	Total by week
January 28	10		10
February 5	10		10
February 12	10		10
February 19	8		8[a]
February 26	6	3	9[b]
March 5	1	7	8[c]
March 12	1	6	7[d]
March 19		5	5[e]
March 25		6	6

[a] We could not histologically analyze ovarian follicles from two females.
[b] We could not collect blood from one female.
[c] Two females died before sampling.
[d] We could not analyze serum from one female.
[e] Two females died before sampling on this date, and we could not histologically analyze ovarian follicles from one female.

Results

Histology

All females were late vitellogenic (stage 6) on January 28, the beginning of the study (Table 2; Data S1). Females initiated atresia at varying times of the study. All females were atretic (stage 9) by March 19 (Table 2).

Plasma sex steroids

Plasma T concentrations varied from 0.35 to 23.47 ng/mL. Concentrations differed between stages of maturity ($F = 94.57$; $df = 1$; $P < 0.001$). Plasma T concentration decreased from 10.30 ± 5.06 ng/mL (mean ± SD) during late vitellogenesis (stage 6) to 2.41 ± 1.58 ng/mL during atresia (stage 9; Table 3; Data S1). Plasma E2 concentrations varied from nondetectable to 12.61 ng/mL. Concentrations differed between stages of maturity ($F = 60.49$; $df = 1$; $P < 0.001$). Plasma E2 concentration decreased from 3.34 ± 2.91 ng/mL (mean ± SD) during late vitellogenesis (stage 6) to 0.29 ± 0.31 ng/mL during atresia (stage 9; Table 4; Data S1).

Ultrasonography

Gonad diameter measured by ultrasonography varied from 2.55 to 5.11 cm (the maximum value capable of being measured by the ultrasound transducer) and differed between stages of maturity ($F = 7.58$; $df = 1$; $P = 0.008$). We assigned one individual ($n = 1$) a value of 5.11 cm for gonad diameter and no value for gonad circumference because the gonad was too wide to be measured. Gonad diameter measured by ultrasonography decreased from 4.05 ± 0.60 cm (mean ± SD) during late vitellogenesis (stage 6) to 3.65 ± 0.50 cm during atresia (stage 9; Table 5; Data S1). Gonad circumference measured by ultrasonography varied from 6.60 to 11.10 cm and did not differ between stages of maturity ($F = 1.10$; $df = 1$; $P = 0.298$). Gonad circumference measured by ultrasonography was 9.53 ± 1.23 cm (mean ± SD) during late vitellogenesis (stage 6) and 9.04 ± 1.24 cm during atresia (stage 9; Data S1).

Ovarian follicle diameter

Ovarian follicle diameter varied from 501 to 825 μm and did not differ between stages of maturity ($F = 1.73$; $df = 1$; $P = 0.19$). Ovarian follicle diameter was 677 ± 43 μm (mean ± SD) during late vitellogenesis (stage 6) and 655 ± 99 μm during atresia (stage 9; Table 6; Data S1). However, mean ovarian follicle diameter did differ during the last week of the study (i.e., comparing mean diameter among weeks instead of stages of maturity: $F = 12.66$; $df = 8$; $P < 0.001$).

Table 3. Plasma testosterone (T) concentrations (ng/mL) in female Burbot *Lota lota* exposed to increasing water temperatures before the spawning season to initiate mass ovarian follicular atresia at the Bozeman Fish Technology Center, Bozeman, Montana. We collected data weekly from January 28, 2018, to March 25, 2018, and attained maximum temperature (11°C) by February 14. We observed females in the late vitellogenic (stage 6) and atretic (stage 9) stages of maturity. Data are sample size (n) and mean ± SD (minimum–maximum). Dashes indicate no data available. Different letters (A, B) indicate differences in mean plasma T concentrations between stages of maturity.

	Late vitellogenic (stage 6)		Atretic (stage 9)	
Week	*n*	Concentration	*n*	Concentration
January 28	10	10.46 ± 3.08 (6.37–17.46)	—	—
February 5	10	13.91 ± 5.56 (4.83–21.78)	—	—
February 12	10	11.48 ± 5.26 (7.30–23.47)	—	—
February 19	8	7.93 ± 5.02 (0.96–17.58)	—	—
February 26	6	6.46 ± 3.47 (2.09–10.58)	3	2.84 ± 1.89 (1.14–4.87)
March 5	1	6.39	7	2.87 ± 1.13 (1.35–4.55)
March 12	1	6.99	6	2.40 ± 2.36 (0.35–6.27)
March 19	—	—	5	2.44 ± 1.47 (0.68–4.62)
March 25	—	—	6	1.65 ± 1.28 (0.37–3.56)
Mean by stage	46	10.30 ± 5.06 A	27	2.41 ± 1.58 B

Table 4. Plasma estradiol (E2) concentrations (ng/mL) in female Burbot *Lota lota* exposed to increasing water temperatures prior to spawning season to initiate mass ovarian follicular atresia at the Bozeman Fish Technology Center, Bozeman, Montana. We collected data weekly from January 28, 2018, to March 25, 2018, and attained maximum temperature (11°C) by February 14. We observed females in the late vitellogenic (stage 6) and atretic (stage 9) stages of maturity. Data are sample size (*n*) and mean ± SD (minimum–maximum). We assigned a value of 0.07 ng/mL for nondetectable concentrations. Dashes indicate no data available. Different letters (A, B) indicate differences in mean plasma E2 concentration between stages of maturity.

	Late vitellogenic (stage 6)		Atretic (stage 9)	
Week	***n***	**Concentration**	***n***	**Concentration**
January 28	10	5.62 ± 1.62 (2.96–8.21)	—	—
February 5	10	5.84 ± 3.35 (1.19–12.61)	—	—
February 12	10	2.52 ± 1.82 (0.47–6.40)	—	—
February 19	8	0.71 ± 0.58 (0.07–1.76)	—	—
February 26	6	0.81 ± 0.74 (0.07–163)	3	0.43 ± 0.63 (0.07–1.16)
March 5	1	2.06	7	0.43 ± 0.29 (0.17–0.81)
March 12	1	1.48	6	0.17 ± 0.18 (0.07–0.52)
March 19	—	—	5	0.29 ± 0.35 (0.07–0.90)
March 25	—	—	6	0.20 ± 0.21 (0.07–0.21)
Mean by stage	46	3.34 ± 2.91 A	27	0.29 ± 0.31 B

Table 5. Gonad diameter measured by ultrasonography (cm) in female Burbot *Lota lota* exposed to increasing water temperatures before spawning season to initiate mass ovarian follicular atresia at the Bozeman Fish Technology Center, Bozeman, Montana. We collected data weekly from January 28, 2018, to March 25, 2018, and attained maximum temperature (11°C) by February 14. We observed females in the late vitellogenic (stage 6) and atretic (stage 9) stages of maturity. Data are sample size (*n*) and mean ± SD (minimum–maximum). Dashes indicate no data available. Different letters (A, B) indicate differences in mean gonad diameter measured by ultrasonography between stages of maturity.

	Late vitellogenic (stage 6)		Atretic (stage 9)	
Week	***n***	**Diameter**	***n***	**Diameter**
January 28	10	4.05 ± 0.56 (3.19–5.02)	—	—
February 5	10	3.97 ± 0.70 (2.55–4.92)	—	—
February 12	10	3.92 ± 0.71 (2.74–4.87)	—	—
February 19	8	4.06 ± 0.50 (3.34–4.78)	—	—
February 26	6	4.12 ± 0.46 (3.64–4.71)	3	3.89 ± 0.59 (3.22–4.32)
March 5	1	4.74	7	3.90 ± 0.42 (3.25–4.40)
March 12	1	5.11[a]	6	3.56 ± 0.35 (3.11–4.10)
March 19	—	—	5	3.56 ± 0.64 (2.87–4.53)
March 25	—	—	6	3.43 ± 0.53 (3.01–4.47)
Mean by stage	46	4.05 ± 0.60 A	27	3.65 ± 0.50 B

[a] Maximum value capable of being measured by ultrasound transducer.

Table 6. Ovarian follicle diameter (μm) in female Burbot *Lota lota* exposed to increasing water temperatures before spawning season to initiate mass ovarian follicular atresia at the Bozeman Fish Technology Center, Bozeman, Montana. We collected data weekly from January 28, 2018, to March 25, 2018, and attained maximum temperature (11°C) by February 14. We observed females in the late vitellogenic (stage 6) and atretic (stage 9) stages of maturity. Data are sample size (*n*) and mean ± SD (minimum–maximum). Dashes indicate no data available. Identical letter (A) indicates no difference in ovarian follicle diameter between stages of maturity.

	Late vitellogenic (stage 6)		Atretic (stage 9)	
Week	***n***	**Diameter**	***n***	**Diameter**
January 28	10	649 ± 30 (588–688)	—	—
February 5	10	675 ± 57 (593–794)	—	—
February 12	10	669 ± 30 (603–722)	—	—
February 19	8	689 ± 29 (650–732)	—	—
February 26	6	703 ± 36 (637–735)	3	744 ± 59 (697–811)
March 5	1	748	7	750 ± 48 (669–825)
March 12	1	754	6	643 ± 70 (522–729)
March 19	—	—	5	607 ± 83 (539–750)
March 25	—	—	6	552 ± 59 (501–656)
Mean by stage	46	677 ± 43 A	27	655 ± 99 A

Discussion

Fisheries scientists have recognized the need to incorporate the frequency of skipped spawning into population models (Marshall et al. 2003; Rideout et al. 2005; Jørgensen et al. 2006; Rideout and Tomkiewicz 2011). Failing to account for mass ovarian follicular atresia among females in a population is a possible source of error when estimating reproductive potential and may lead to ineffective management actions (Jørgensen et al. 2006; Corriero et al. 2021). High rates of mass ovarian follicular atresia may also be an indicator of a population experiencing poor nutrition, altered temperatures, and/or environmental pollution (Corriero et al. 2021). Nonlethal tools to assess the frequency of mass ovarian follicular atresia provide fisheries biologists with alternative methods to gonadal histology and make the assessment of mass ovarian follicular atresia more accessible.

Several studies examined the relationship between plasma sex steroids and ovarian follicular atresia in commercially raised sturgeon species (e.g., Webb et al. 2001; Linares-Casenave et al. 2002; Talbott et al. 2011) because yield and quality of caviar are negatively affected by ovarian follicular atresia (Talbott et al. 2011). In White Sturgeon, Talbott et al. (2011) used plasma T concentrations to identify mass ovarian follicular atresia. They did not recommend use of plasma E2 concentrations to identify mass ovarian follicular atresia because plasma E2 concentrations were nondetectable in both maturing and atretic females. We also observed very low plasma E2 concentrations in both late vitellogenic (stage 6) and atretic (stage 9) female Burbot. Plasma E2 concentrations rapidly decrease once vitellogenesis is complete in female teleosts (Scott et al. 1980; Fitzpatrick et al. 1986; Kime 1993; Kagawa 2013); therefore, plasma E2 concentrations may decrease with the completion of vitellogenesis or the initiation of atresia. We recommend the use of plasma T concentrations to identify mass ovarian follicular atresia in female Burbot because plasma T concentrations remain elevated after vitellogenesis is complete (McGarvey et al. 2020), but decrease during mass ovarian follicular atresia.

Time is an important consideration when using plasma T concentrations to identify atretic (stage 9) females. Decreased plasma T concentrations can be used to identify atretic (stage 9) females, but postovulatory (stage 8) females also show decreased plasma T concentrations (McGarvey et al. 2020). Collecting plasma before spawning prevents the misidentification of postovulatory (stage 8) females as atretic (stage 9) females.

Plasma sex steroid concentrations (T and E2) in late vitellogenic (stage 6) females were lower than plasma sex steroid concentrations previously observed in late vitellogenic (stage 6) female Burbot (McGarvey et al. 2020). In our previous description of gametogenesis in Burbot, we observed late vitellogenic (stage 6) females with a mean plasma T concentration of 24.36 ng/mL and a mean plasma E2 concentration of 7.56 ng/mL (McGarvey et al. 2020). In the current study, we observed late vitellogenic (stage 6) females with a mean plasma T concentration of 10.30 ng/mL and a mean plasma E2 concentration of 3.34 ng/mL. Plasma sex steroid concentrations observed in the current study were likely lower than plasma sex steroid concentrations previously observed because females had initiated atresia (i.e., approximately 20% of ovarian follicles exhibited signs of atresia), but did not meet the criteria for classification as atretic (i.e., $>$ 75% of ovarian follicle exhibiting signs of atresia). In White Sturgeon, several studies document that plasma sex steroid concentrations decrease when early signs of atresia, such as deterioration of the vitelline envelope and zona radiata, become histologically evident (Webb et al. 2001; Linares-Casenave et al. 2002; Talbott et al. 2011). We classified females initiating atresia, and therefore decreasing plasma sex steroid production, as late vitellogenic (stage 6), accounting for the lower plasma sex steroid concentrations.

There is typically an association of ovarian follicular atresia with decreased fish condition (Rideout et al. 2000; Kraus et al. 2008; Skjaeraasen et al. 2009); however, few studies describe how gonad size and ovarian follicle diameter change during mass ovarian follicular atresia. Gonadosomatic index is directly related to gonad size and was previously used by researchers with ovarian follicle diameter as metrics to assess mass ovarian follicular atresia in Piau *Leporinus reinhardti* (Miranda et al. 1999), a total spawning teleost like Burbot. Both gonadosomatic index and ovarian follicle diameter decreased during mass ovarian follicular atresia, but the complete absorption of ovarian follicles occurred over a 4-mo period. The current study occurred over a 9-wk period until we observed the initial signs of mass ovarian follicular atresia in all fish. Gonad diameter measured by ultrasonography differed between late vitellogenic (stage 6) and atretic (stage 9) females; however, we only observed a 0.40-cm decrease in mean gonad diameter. We observed a 22-μm decrease in mean ovarian follicle diameter between late vitellogenic (stage 6) and atretic (stage 9) females because we included all stages of ovarian follicular atresia (i.e., initial to advanced) in our study. A more marked decrease in gonad size and ovarian follicle diameter would likely occur in females undergoing advanced ovarian follicular atresia, when yolk is phagocytized and resorbed (Miranda et al. 1999). The distributions of gonad diameter measured by ultrasonography and ovarian follicle diameter overlapped between late vitellogenic (stage 6) and atretic (stage 9) females, indicating both tools may misidentify atretic (stage 9) females. However, ovarian follicle diameter differed during the final week of the study, suggesting that ovarian follicle diameter may be used to identify advanced ovarian follicular atresia in female Burbot.

We demonstrated plasma T concentration can be used as a nonlethal tool to identify mass ovarian follicular atresia in female Burbot. Gonad diameter measured by ultrasonography and ovarian follicle diameter may also be used to identify advanced mass ovarian follicular atresia. It would be valuable for fisheries biologists interested in using these nonlethal tools to identify mass

ovarian follicular atresia to perform laboratory studies to determine plasma T, gonad diameter measured by ultrasonography, and ovarian follicle diameter thresholds indicative of mass ovarian follicular atresia in their species of interest, which may vary among populations or species. The nonlethal tools assessed in this study enable fisheries biologist to assess the frequency of skipped spawning among female Burbot. Incorporation of this information into population growth models may aid fisheries biologists in more accurately estimating population growth and establishing effective management actions.

Supplemental Material

Please note: The *Journal of Fish and Wildlife Management* is not responsible for the content of functionality of any supplemental material. Queries should be directed to the corresponding author.

Data S1. Data collected to detect mass ovarian follicular atresia by plasma sex steroid concentrations, gonad diameter measured by ultrasonography, and ovarian follicle diameter in Burbot *Lota*. We reference column headings in metadata tab of datafile. We held Burbot at the Bozeman Fish Technology Center, Bozeman, Montana, and exposed fish to increasing water temperatures before the spawning season to initiate mass ovarian follicular atresia. We collected data weekly from January 28, 2018, to March 25, 2018, and attained maximum temperature (11°C) by February 14.

Available: https://doi.org/10.3996/JFWM-22-018.S1 (17 KB XLSX)

Acknowledgments

We thank Dr. Christine Verhille for comments to improve an early version of this manuscript; Matt Toner for help to maintain Burbot; and Charlee Capaul, Matt Howell, and all the technicians who helped catch Burbot. We also thank the journal reviewers and Associate Editor for suggestions to improve this manuscript. The Confederated Tribes of the Colville Reservation and the Bonneville Power Administration funded this study. The Montana Cooperative Fishery Research Unit is jointly sponsored by the U.S. Geological Survey, Montana Fish, Wildlife and Parks, Montana State University, and the U.S. Fish and Wildlife Service. We performed this study under the auspices of Montana State University institutional animal care and use protoc ol 2016-34.

Any use of trade, product, website, or firm names in this publication is for descriptive purposes only and does not imply endorsement by the U.S. Government.

References

Blythe B, Helfrich LA, Beal WE, Bosworth B, Libey GS. 1994. Determination of sex and maturational status of Striped Bass (*Morone-saxatilis*) using ultrasonic-imaging. Aquaculture 125:175–184.

[CTCR] Confederated Tribes of the Colville Reservation. 2018. Lake Roosevelt Burbot project. Colville Tribes Fish and Wildlife News 29:2. Available: https://static1.squarespace.com/static/56f45574d51cd42551248613/t/5ac7ddb58a922da5caa60be2/1523047885098/ColvilleTribesF%26WD_March2018Newsletter.pdf (August 2022)

Corriero A, Zupa R, Mylonas CC, Passantino L. 2021. Atresia of ovarian follicles in fishes, and implications and uses in aquaculture and fisheries. Journal of Fish Diseases 44:1271–1291.

Croghan C, Egeghy PP. 2003. Methods of dealing with values below the limit of detection using SAS. Southeastern SAS User Group, St. Petersburg, Florida.

Dettlaff AJ, Ginsburg AS, Schmalhausen OI. 1993. Sturgeon fishes: developmental biology and aquaculture. Berlin: Springer Verlag.

Evans AF, Fitzpatrick MS, Siddens LK. 2004. Use of ultrasound imaging and steroid concentrations to identify maturational status in adult steelhead. North American Journal of Fisheries Management 24:967–978.

Evenson MJ. 2000. Reproductive traits of Burbot in the Tanana River, Alaska. Pages 61–70 in Paragamian VL, Willis DW, editors. Burbot: biology, ecology, and management. Spokane, Washington: American Fisheries Society.

Feist G, Schreck CB, Fitzpatrick MS, Redding JM. 1990. Sex steroid profiles of Coho Salmon (*Oncorhynchus-kisutch*) during early development and sexual-differentiation. General and Comparative Endocrinology 80:299–313.

Fitzpatrick MS, Redding JM, Ratti FD, Schreck CB. 1987. Plasma testosterone concentration predicts the ovulatory response of Coho Salmon (*Oncorhynchus-kisutch*) to gonadotropin-releasing-hormone analog. Canadian Journal of Fisheries and Aquatic Sciences 44:1351–1357.

Fitzpatrick MS, Van der Kraak G, Schreck CB. 1986. Profiles of plasma sex steroids and gonadotropin in Coho Salmon, *Oncorhynchus-kisutch*, during final maturation. General and Comparative Endocrinology 62:437–451.

Grier H, Uribe-Aranzábal M, Patiño R. 2009. The ovary, folliculogenesis, and oogenesis in teleosts. Pages 25–84 in Jamieson B, editor. Reproductive biology and phylogeny of fishes (Agnathans and Neotelestomi). Enfield, New Hampshire: Science Publishers.

Hunter JR, Macewicz BJ. 1985. Rates of atresia in the ovary of captive and wild Northern anchovy, *Engraulis mordax*. Fishery Bulletin 83:119–136. Available: https://swfsc-publications.fisheries.noaa.gov/publications/CR/1984/8442.PDF (August 2022)

Johnson JE 1971. Maturity and fecundity of Threadfin Shad, *Dorosoma-petenense* (Gunther), in central Arizo-

na reservoirs. Transactions of the American Fisheries Society 100:74–85.
Jørgensen C, Ernande B, Fiksen O, Dieckmann U. 2006. The logic of skipped spawning in fish. Canadian Journal of Fisheries and Aquatic Sciences 63:200–211.
Kagawa H. 2013. Oogenesis in teleost fish. AquaBioScience Monographs 6:99–127.
Kime DE. 1993. Classical and nonclassical reproductive steroids in fish. Reviews in Fish Biology and Fisheries 3:160–180.
Kraus G, Tomkiewicz J, Diekmann R, Koster FW. 2008. Seasonal prevalence and intensity of follicular atresia in Baltic Cod *Gadus morhua callarias* L. Journal of Fish Biology 72:831–847.
Linares-Casenave J, Van Eenennaam JP, Doroshov SI. 2002. Ultrastructural and histological observations on temperature-induced follicular ovarian atresia in the White Sturgeon. Journal of Applied Ichthyology 18:382–390.
Lowerre-Barbieri SK, Brown-Peterson NJ, Murua H, Tomkiewicz J, Wyanski DM, Saborido-Rey F. 2011. Emerging issues and methodological advances in fisheries reproductive biology. Marine and Coastal Fisheries 3:32–51.
Lubzens E, Young G, Bobe J, Cerda J. 2010. Oogenesis in teleosts: how fish eggs are formed. General and Comparative Endocrinology 165:367–389.
Luna LG. 1968. Manual of histological staining methods of the Armed Forces Institute of Pathology. New York: McGraw-Hill Book Company.
Marshall CT, O'Brien L, Tomkiewicz J, Köster FW, Kraus G, Marteinsdottir G, Morgan MJ, Saborido-Rey F, Blanchard JL, Secor DH, Wright PJ, Mukhina NV, Björnsson H. 2003. Developing alternative indices of reproductive potential for use in fisheries management: case studies for stocks spanning an information gradient. Journal of Northwest Atlantic Fishery Science 33:161–190.
Maskill PAC, Crossman JA, Webb MAH, Marrello MM, Guy CS. 2022. Accuracy of histology, endoscopy, ultrasonography, and plasma sex steroids in describing the population reproductive structure of hatchery-origin and wild white sturgeon. Journal of Applied Ichthyology 38:3–16.
McGarvey LM, Halvorson LJ, Ilgen JE, Guy CS, McLellan JG, Webb MAH. 2020. Gametogenesis and assessment on nonlethal tools to assign sex and reproductive condition in Burbot *Lota*. Transactions of the American Fisheries Society 149:225–240.
McGarvey LM, Ilgen JE, Guy CS, McLellan JG, Webb MAH. 2021. Gonad size measured by ultrasound to assign stage of maturity in Burbot. Journal of Fish and Wildlife Management 12:241–249.
McPhail JD, Paragamian VL. 2000. Burbot biology and life history. Pages 11–23 in Paragamian VL, Willis DW, editors. Burbot: biology, ecology, and management. Spokane, Washington: American Fisheries Society.
Medina A, Magro A, Paullada-Salmeron JA, Varela JL. 2021. An autofluorescence-based survey of late follicular atresia in ovaries of a teleost fish (*Thunnus thynnus*). Journal of Fish Biology 99:765–772.
Miranda ACL, Bazzoli N, Rizzo E, Sato Y. 1999. Ovarian follicular atresia in two teleost species: a histological and ultrastructural study. Tissue & Cell 31:480–488.
Morgan MJ. 2008. Integrating reproductive biology into scientific advice for fisheries management. Journal Northwest Atlantic Fishery Science 41:37–51.
Morgan MJ, Perez-Rodriguez A, Saborido-Rey F. 2011. Does increased information about reproductive potential result in better prediction of recruitment? Canadian Journal of Fisheries and Aquatic Sciences 68:1361–1368.
Polacek MC, Baldwin CM, Knuttgen K. 2006. Status, distribution, diet, and growth of Burbot in Lake Roosevelt, Washington. Northwest Science 80:153–164.
Pulliainen E, Korhonen K. 1990. Seasonal-changes in condition indexes in adult mature and nonmaturing Burbot, *Lota-lota* (L), in the north-eastern Bothnian Bay, Northern Finland. Journal of Fish Biology 36:251–259.
Rideout RM, Burton MPM, Rose GA. 2000. Observations on mass atresia and skipped spawning in Northern Atlantic Cod, from Smith Sound, Newfoundland. Journal of Fish Biology 57:1429–1440.
Rideout RM, Rose GA, Burton MPM. 2005. Skipped spawning in female iteroparous fishes. Fish and Fisheries 6:50–72.
Rideout RM, Tomkiewicz J. 2011. Skipped spawning in fishes: more common than you might think. Marine and Coastal Fisheries 3:176–189.
Rottmann RW, Shireman JV, Chapman FA. 1991. Determining sexual maturity of broodstock for induced spawning of fish. Stoneville, Mississippi: Southern Regional Aquaculture Center.
Scott AP, Bye VJ, Baynes SM. 1980. Seasonal-variations in sex steroids of female Rainbow-Trout (*Salmo-gairdneri richardson*). Journal of Fish Biology 17:587–592.
Skjaeraasen JE, Kennedy J, Thorsen A, Fonn M, Strand BN, Mayer I, Kjesbu OS. 2009. Mechanisms regulating oocyte recruitment and skipped spawning in Northeast Arctic Cod (*Gadus morhua*). Canadian Journal of Fisheries and Aquatic Sciences 66:1582–1596.
Talbott MJ, Van Eenennaam JP, Linares-Casenave J, Doroshov SI, Guy CS, Struffenegger P, Webb MAH. 2011. Investigating the use of plasma testosterone and estradiol-17 beta to detect ovarian follicular atresia in farmed White Sturgeon, *Acipenser transmontanus*. Aquaculture 315:283–289.
Trippel EA. 1999. Estimation of stock reproductive potential: history and challenges for Canadian Atlantic gadoid stock assessments. Journal of Northwest Atlantic Fishery Science 25:61–81.

Tyler CR, Sumpter JP. 1996. Oocyte growth and development in teleosts. Reviews in Fish Biology and Fisheries 6:287–318.

Webb MAH, Feist GW, Foster EP, Schreck CB, Fitzpatrick MS. 2002. Potential classification of sex and stage of gonadal maturity of wild White Sturgeon using blood plasma indicators. Transactions of the American Fisheries Society 131:132–142.

Webb MAH, Van Eenennaam JP, Feist GW, Linares-Casenave J, Fitzpatrick MS, Schreck CB, Doroshov SI. 2001. Effects of thermal regime on ovarian maturation and plasma sex steroids in farmed White Sturgeon, *Acipenser transmontanus*. Aquaculture 201:137–151.

Wootton RJ, Smith C, editors. 2014. Gametogenesis. Pages 46–80 in Reproductive biology of teleost fishes. Hoboken, New Jersey: John Wiley & Sons.