

## Notes

# Gonad Size Measured by Ultrasound to Assign Stage of Maturity in Burbot

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## Abstract

We measured gonad size (diameter and circumference) by ultrasound and used it as a metric to assign stage of maturity in Burbot *Lota lota* from Lake Roosevelt, Washington. We collected paired gonad tissue and ultrasound measurements monthly from November 2017 to March 2018 and processed gonad tissue for histological analysis to confirm stage of maturity. We measured gonad diameter and circumference by ultrasound. We also measured excised gonad diameter (i.e., true gonad diameter) by digital calipers and excised gonad circumference (i.e., true gonad circumference) by a measuring tape. All late vitellogenic (stage 6) ovaries measured by ultrasound had a diameter greater than 3.90 cm, suggesting a value of 3.90 cm or greater may be used to characterize females capable of spawning in the current reproductive cycle. One mid-spermatogenic (stage 3) and all ripe (stage 4) testes were too large to be measured and were assigned a diameter of 5.11 cm, the maximum value capable of being measured by our ultrasound transducer. A value of 5.11 cm or greater may be used to characterize males capable of spawning in the current reproductive cycle. Testis circumference measured by ultrasound is not reported because some testes were wider than the ultrasound transducer and could not be measured. Measurements of testis diameter did not differ between measurement methods (ultrasound versus true), but ultrasound measurements of ovary diameter and circumference were higher than true measurements. We attributed the difference between measurement methods to flattening of the ovary while applying the ultrasound transducer. Gonad diameter and circumference measured by ultrasound were highly correlated with gonadosomatic index and ovarian follicle diameter, indicating gonad size measured by ultrasound is an appropriate index of gonad development in Burbot.

Keywords: Burbot; reproductive biology; reproductive indices; ultrasound; stage of maturity

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## Introduction

Fisheries biologists determine the sex and reproductive condition of fishes to assess reproductive indices (e.g., sex ratio, reproductive structure, and spawning periodicity) of a population to characterize and monitor demographics, model growth, establish sustainable harvest regulations, monitor the effects of management actions, and monitor the effects of environmental stressors (Trippel 1993; Downs et al. 1997; Wildhaber et al. 2007; Power 2007; Morgan 2008; Schill et al. 2010). Despite the utility of reproductive indices to fisheries management and conservation, they are often difficult to assess because they require extensive effort and sacrificing fish (e.g., gonad histology). Sacrificing fish may not be possible or preferable; therefore, fisheries biologists have sought nonlethal tools to assess reproductive indices.

Ultrasound has been used to nonlethally assign sex, stage of maturity, and reproductive condition in fishes by detecting morphological and echogenic differences among gonads (Shields et al. 1993; Moghim et al. 2002; Colombo et al. 2004; Evans et al. 2004; Novelo and Tiersch 2016). In a previous study, we assessed the use of ultrasound to assign sex, stage of maturity, and reproductive condition in adult Burbot *Lota lota* (McGarvey et al. 2020). We used ultrasound to assign sex with 96% accuracy, but ultrasound could not be used to assign stage of maturity and reproductive condition by detecting morphological or echogenic differences among gonads. For example, the diameter of a ripe ovarian follicle was less than 1 mm and could not be delineated using our ultrasound transducer as has been done in previous studies of fishes with larger ovarian follicles (Shields et al. 1993; Evans et al. 2004; Novelo and Tiersch 2016).

Gonad size measured by ultrasound has previously been used to assign stage of maturity in fishes (Mattson 1991; Blythe et al. 1994; Evans et al. 2004; Newman et al. 2008; Whittamore et al. 2010; Du et al. 2017; Naeve et al. 2018). Gonad diameter measured by ultrasound was proposed as a metric to assign stage of maturity (ripe and nonripe) in adult Striped Bass *Morone saxatilis* (Blythe et al. 1994). Ultrasound was used to determine sex and measure gonad diameter at the perceived maximum cross-section (i.e., largest area determined by scanning the entire gonad with an ultrasound transducer). In female Striped Bass, an ovary diameter greater than 3.0 cm characterized ripe females, and in male Striped Bass, a testis diameter greater than 2.0 cm characterized ripe males. Gonad area measured by ultrasound has also been used as a metric to assign stage of maturity (prespawn and postspawn) in adult male steelhead *Oncorhynchus mykiss* (Evans et al. 2004). Ultrasound was used to measure gonad area at the perceived maximum cross-section. Gonad area was  $2.86 \pm 0.73 \text{ cm}^2$  (mean  $\pm$  SEM) in prespawn males and  $0.62 \pm 0.24 \text{ cm}^2$  (mean  $\pm$  SEM) in postspawn males. A gonad area of  $1.25 \text{ cm}^2$  or greater was used to characterize prespawn males. To our knowledge, gonad size measured by ultrasound has not been assessed as a metric to

assign stage of maturity in Burbot. The objective of this study was to assess gonad size (diameter and circumference) measured by ultrasound as a metric to assign stage of maturity in Burbot from Lake Roosevelt. Using ultrasound to assign sex (McGarvey et al. 2020) and stage of maturity will enable fisheries biologists to nonlethally and noninvasively assess indices of reproductive potential (e.g., sex ratio, reproductive structure, and spawning periodicity) for the Burbot population in Lake Roosevelt.

## Study Area

Lake Roosevelt is located in northeast Washington. The reservoir was formed after the construction of Grand Coulee Dam on the Columbia River. The reservoir is 1–3 km wide with a maximum depth of 122 m extending 241 km upstream from the Grand Coulee Dam to the Canadian border (Polacek et al. 2006). The reservoir supports many recreational fisheries, including a Burbot fishery. The Burbot fishery is relatively small with approximately 485 angler trips (2,942 angler hours) and 1,325 harvests per year, as estimated from a reservoir-wide creel survey (Spokane Tribe of Indians unpublished data). However, these estimates are considered low because the creel survey design does not adequately capture the Burbot fishery, which often occurs at nonsurvey shoreline locations and at night. Currently, the Burbot fishery may be underused and able to support greater harvest (CCT 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 traps shallower than 10 m to prevent barotrauma in captured fish and retrieved the traps the following day. They tagged each fish with a passive integrated transponder and transferred the fish to the Bozeman Fish Technology Center, Bozeman, Montana. We kept fish in four 485 L tanks ( $244 \times 56 \times 36 \text{ cm}$ ) at a maximum stocking density of 51.3 g/L. We maintained fish under a natural light cycle and a thermal profile ranging from 3.8–16.7°C to match Lake Roosevelt (USBR 2020). We constantly provided fish with live rainbow trout (150 mm) as feed.

### Biological sampling

We euthanized six fish by an overdose of MS-222 (500 ppm) monthly from November 2017 to March 2018 (seven fish were euthanized in January 2018) for biological sampling. We selected equal numbers of females and males using ultrasound (McGarvey et al. 2020). We collected the following biological data from individual fish: total length ( $\pm 0.1 \text{ cm}$ ), body weight ( $\pm 0.01 \text{ g}$ ), gonad weight ( $\pm 0.01 \text{ g}$ ), ultrasound measurements of gonad diameter and circumference ( $\pm 0.01 \text{ cm}$ ), measurements of excised gonad diameter and circumference ( $\pm 0.01 \text{ cm}$ ; i.e., true gonad diameter and



**Table 1.** Reproductive condition and stages of maturity identified from histological analysis of gonad tissue from Burbot *Lota lota* held at the Bozeman Fish Technology Center from November 2016 to March 2018 (McGarvey et al. 2020). Stage numbers are in parentheses.

Sex and reproductive condition	Stage of maturity	Description
<b>Females</b>		
Nonreproductive	Oogonial proliferation (1)	Oogonia and potentially a few primary growth oocytes
Nonreproductive	Primary growth (2)	Primary growth oocytes and oogonia
Reproductive	Cortical alveolar (3)	Cortical alveoli on periphery of ovarian follicles
Reproductive	Early vitellogenic (4)	Yolk granules accumulating in periphery of ovarian follicles, cortical alveoli on periphery of ovarian follicles, one layer of zona radiata
Reproductive	Mid-vitellogenic (5)	Yolk globules with a mean diameter of 7.93 $\mu\text{m}$ (5.97–9.89 $\mu\text{m}$ , 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
Reproductive	Late vitellogenic (6)	Yolk fusing into larger globules with a mean diameter of 19.62 $\mu\text{m}$ (14.55–24.69 $\mu\text{m}$ , 95% CI) throughout ovarian follicle, one layer of zona radiata, central germinal vesicle (i.e., nucleus)
Reproductive	Ripe (7)	Yolk fused but not completely coalesced in all ovarian follicles, offset germinal vesicle
Nonreproductive	Postovulatory (8)	Postovulatory follicles present with primary growth oocytes
Nonreproductive	Atretic (9)	>75% atretic ovarian follicles, no postovulatory follicles present with primary growth oocytes
<b>Males</b>		
Nonreproductive	Spermatogonial proliferation (1)	Cysts contain only spermatogonia
Reproductive	Early spermatogenic (2)	Cysts contain spermatogonia and spermatocytes
Reproductive	Mid-spermatogenic (3)	Few cysts contain spermatogonia, >50% of the cysts contain spermatocytes and spermatids
Reproductive	Ripe and initiating spermiation (4)	Cysts filled with spermatozoa, approximately 20% of cysts emptying of spermatozoa
Reproductive	Mid-spermiation (5)	Cysts with reduced or residual spermatozoa, some cysts may be empty
Nonreproductive	Post-spermiation (6)	Cysts with residual spermatozoa and cysts empty of spermatozoa

circumference), and gonad tissue (Data S1, *Supplemental Material*).

### Histology

We preserved gonad tissue samples in 10% phosphate-buffered formalin. We then embedded tissue samples in paraffin wax, sectioned them at 5  $\mu\text{m}$ , and stained sections with periodic acid-Schiff for histological analysis (Webb and Erickson 2007). We examined slides under a Leica DM compound microscope (250–450 $\times$ ; Leica Biosystems Inc., Lincolnshire, IL) and assigned a stage of maturity to germ cells in gonad tissue using our description of gametogenesis in Burbot (McGarvey et al. 2020; Table 1).

### Ultrasound

We used a SonoSite Edge ultrasound (SonoSite Inc., Bothell, WA) with a linear transducer (6–15 MHz) to measure gonad size in euthanized fish. We used the Small Parts exam type with the optimization set to General and the 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 with the ultrasound transducer until the perceived maximum cross-section was in view (Blythe et al. 1994; Evans et al. 2004). The gonad was elliptical in shape with major and minor axes. We defined gonad diameter as the length of

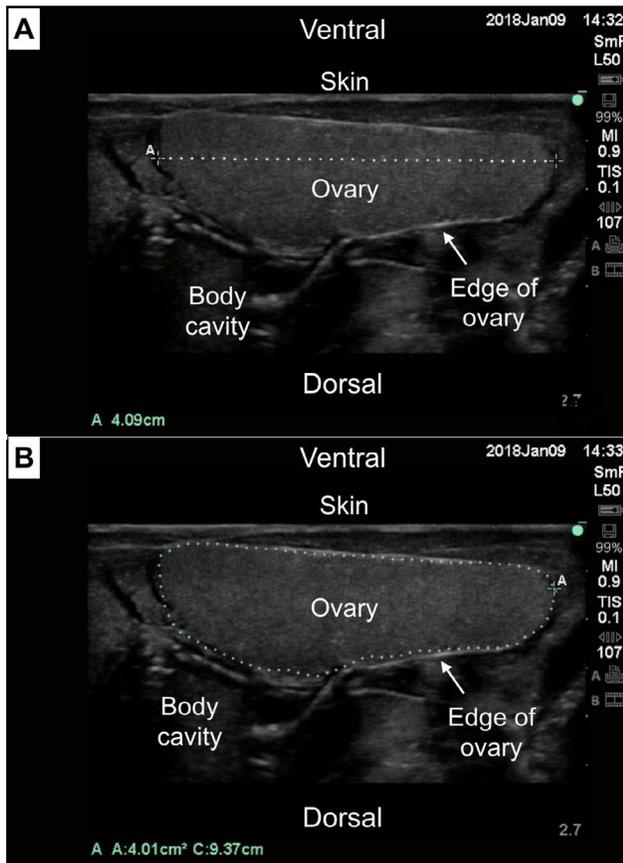
the major axis. We measured gonad diameter using the ultrasound caliper function (Figure 1). We assigned a value of 5.11 cm (the width of the ultrasound transducer) if the gonad was wider than the ultrasound transducer and unable to be measured (Figure 2). We measured gonad circumference in the same location using the ultrasound caliper function on manual trace (Figure 1). We could not measure circumference if the gonad was wider than the ultrasound transducer (5.11 cm). We repeated measurements three times for each individual, and mean measurements were used in analyses.

### True measure of gonad diameter and circumference

We excised gonads to measure true diameter using Mitutoyo digital calipers (150 mm range  $\pm$  0.01 mm; Mitutoyo American Corp., Aurora, IL) and true circumference using a measuring tape. We repeated measurements three times for each individual and used mean measurements in analyses. We compared gonad measurements by ultrasound to excised gonad measurements to assess the accuracy of ultrasound measurements.

### Gonadosomatic index and ovarian follicle diameter

We excised and weighed gonads ( $\pm$ 0.01 g) for calculation of gonadosomatic index (GSI = [gonad weight/total body weight]  $\times$  100). We collected ovarian follicles from excised ovaries and preserved them in Ringers solution (Dettlaff et al. 1993). We measured 15

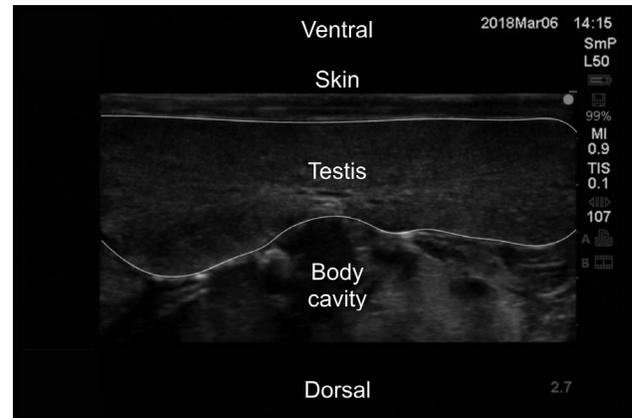


**Figure 1.** Sonogram showing the diameter (A) and circumference (B) at the perceived maximum cross-section of a late vitellogenic (stage 6) ovary from an adult female Burbot *Lota lota* in January 2018. The white, dashed line represents the gonad diameter measured by ultrasound (A), and the green, dotted line represents gonad circumference measured by ultrasound (B). We held Burbot at the Bozeman Fish Technology Center from November 2017 to March 2018 and exposed them to a natural photoperiod and thermal profile similar to Lake Roosevelt, Washington.

ovarian follicles (Johnson 1971;  $\pm 1.0 \mu\text{m}$ ) from each female by image analysis with SPOT Advanced software, version 5.2 (SPOT Imaging, Sterling Heights, MI).

### Data analyses

We used one-way analyses of variance (ANOVAs) for mean comparisons of gonad size measured by ultrasound among stages of maturity and paired *t*-tests for mean comparisons of gonad size between measurement methods (ultrasound versus true). We determined data to be normally distributed by examining the fitted model residuals. The ANOVAs indicating differences were followed by pairwise comparisons with a Bonferroni correction. We calculated correlation coefficients (*r*) to assess the linear relationship between gonad size measured by ultrasound and indices of gonad development. We completed statistical analyses using R software, version 3.3.2.



**Figure 2.** Sonogram taken at the perceived maximum cross-section of a ripe (stage 4) testis from an adult male Burbot *Lota lota* in March 2018. The white line delineates testicular tissue. The testis was wider than the ultrasound transducer; therefore, a value of 5.11 cm (the width of the ultrasound transducer) was assigned for gonad diameter measured by ultrasound. We held Burbot at the Bozeman Fish Technology Center from November 2017 to March 2018 and exposed them to a natural photoperiod and thermal profile similar to Lake Roosevelt, Washington.

## Results

### Biological sampling

Female total length did not vary among stages of maturity ( $F = 0.21$ ;  $df = 2,12$ ;  $P = 0.810$ ). Mean female total length was 51.0 cm (47.9–52.3 cm, 95% CI). Male total length also did not vary among stages of maturity ( $F = 1.23$ ;  $df = 3,12$ ;  $P = 0.343$ ). Mean male total length was 51.4 cm (48.1–54.7 cm, 95% CI).

### Histology

We observed females in the early vitellogenic (stage 4;  $n = 3$ ), mid-vitellogenic (stage 5;  $n = 6$ ), and late vitellogenic (stage 6;  $n = 6$ ) stages of maturity (see Table 1 for reproductive condition) and no females in the oogonial proliferation (stage 1), primary growth (stage 2), cortical alveolar (stage 3), ripe (stage 7), postovulatory (stage 8), or atretic (stage 9) stages of maturity. We observed males in spermatogonial proliferation (stage 1;  $n = 2$ ), early spermatogenic (stage 2;  $n = 7$ ), mid-spermatogenic (stage 3;  $n = 4$ ), and ripe (stage 4;  $n = 3$ ) stages of maturity (see Table 1 for reproductive condition) and no males in the mid-spermiation (stage 5) or postspermiation (stage 6) stages of maturity.

### Ultrasound

Ovary diameter measured by ultrasound differed among stages of maturity ( $F = 19.99$ ;  $df = 2,12$ ;  $P \leq 0.001$ ). Mean ovary diameter measured by ultrasound increased from 2.46 cm during early vitellogenesis (stage 4) to 4.42 cm during late vitellogenesis (stage 6; Table 2). Ovary circumference measured by ultrasound also differed among stages of maturity ( $F = 17.73$ ;  $df = 2,12$ ;

**Table 2.** Gonad diameter (cm) and circumference (cm) measured by ultrasound in adult female Burbot *Lota lota* by stage of maturity. Data are means, 95% confidence interval (CI), and sample sizes (*n*). Different letters among stages of maturity indicate differences within a measurement. We held Burbot at the Bozeman Fish Technology Center from November 2017 to March 2018 and exposed them to a natural photoperiod and thermal profile similar to Lake Roosevelt, Washington. We did not observe females in the primary growth (stage 1), cortical alveolar (stage 2), ripe (stage 7), postovulatory (stage 8), or atretic (stage 9) stages of maturity.

Stage of maturity	<i>n</i>	Diameter (cm)	Circumference (cm)
Early vitellogenic (stage 4)	3	2.46 (1.87–3.04) <sup>z</sup>	5.81 (5.23–6.40) <sup>z</sup>
Mid-vitellogenic (stage 5)	6	2.99 (2.33–3.66) <sup>z</sup>	7.10 (5.64–8.55) <sup>z</sup>
Late vitellogenic (stage 6)	6	4.42 (3.99–4.84) <sup>y</sup>	10.12 (8.98–11.25) <sup>y</sup>

$P \leq 0.001$ ). Mean ovary circumference measured by ultrasound increased from 5.81 cm during early vitellogenesis (stage 4) to 10.12 cm during late vitellogenesis (stage 6; Table 2).

Testis diameter measured by ultrasound differed among stages of maturity ( $F = 20.10$ ;  $df = 3,12$ ;  $P \leq 0.001$ ). Mean testis diameter measured by ultrasound increased from 0.99 cm during spermatogonial proliferation (stage 1) to 5.11 cm when testes were ripe (stage 4; Table 3). All ripe (stage 4) testes were too large to be measured by ultrasound and assigned a diameter of 5.11 cm. Testis circumference measured by ultrasound was not reported because the circumference of testes wider than the ultrasound transducer could not be measured.

### True measurement of gonad diameter and circumference

We compared all ultrasound measurements of gonad diameter and circumference, regardless of stage of maturity, to all true measurements of excised gonad diameter and circumference (i.e., measured by calipers and a measuring tape, respectively). Measurements of ovary diameter differed between the two methods ( $t = -8.24$ ;  $df = 14$ ;  $P \leq 0.001$ ). Mean ovary diameter measured by ultrasound was 3.45 cm (2.93–3.98 cm, 95% CI), and mean ovary diameter measured by calipers was 2.74 cm (2.34–3.14 cm, 95% CI). There was a 21% difference between the two measurement methods. Measurements of ovary circumference also differed between the two methods ( $t = -3.31$ ;  $df = 14$ ;  $P = 0.005$ ). Mean ovary circumference measured by ultrasound was 8.05 cm (6.88–9.21 cm, 95% CI), and mean ovary circumference measured by a measuring tape was 7.49 cm (6.45–8.53 cm, 95% CI). There was a 7% difference between the two measurement methods.

Measurements of testis diameter did not differ between the two methods ( $t = -0.23$ ;  $df = 14$ ;  $P = 0.823$ ). Mean testis diameter measured by ultrasound was 3.68 cm (2.87–4.48 cm, 95% CI), and mean male gonad diameter measured by calipers was 3.56 cm (2.61–4.50, 95% CI). There was a 3% difference between the two measurement methods.

**Table 3.** Gonad diameter (cm) measured by ultrasound in adult male Burbot *Lota lota* by stage of maturity. Gonad diameters wider than the ultrasound transducer could not be measured and were assigned a value of 5.11 cm (the width of the ultrasound transducer). All ripe (stage 4) gonad diameters were assigned a value of 5.11 cm. Data are means, 95% confidence interval (CI), and sample sizes (*n*). Different letters among stages of maturity indicate differences. We held Burbot at the Bozeman Fish Technology Center from November 2017 to March 2018 and exposed them to a natural photoperiod and thermal profile similar to Lake Roosevelt, Washington. We did not observe males in the mid-spermatiation (stage 5) or post-spermatiation (stage 6) stage of maturity.

Stage of maturity	<i>n</i>	Diameter (cm)
Spermatogonial proliferation (stage 1)	2	0.99 (0–2.39) <sup>z</sup>
Early spermatogenic (stage 2)	7	3.14 (2.28–4.00) <sup>y</sup>
Mid-spermatogenic (stage 3)	4	4.88 (4.28–5.47) <sup>x</sup>
Ripe (stage 4)	3	5.11 (N/A) <sup>x</sup>

Abbreviations: N/A = not applicable.

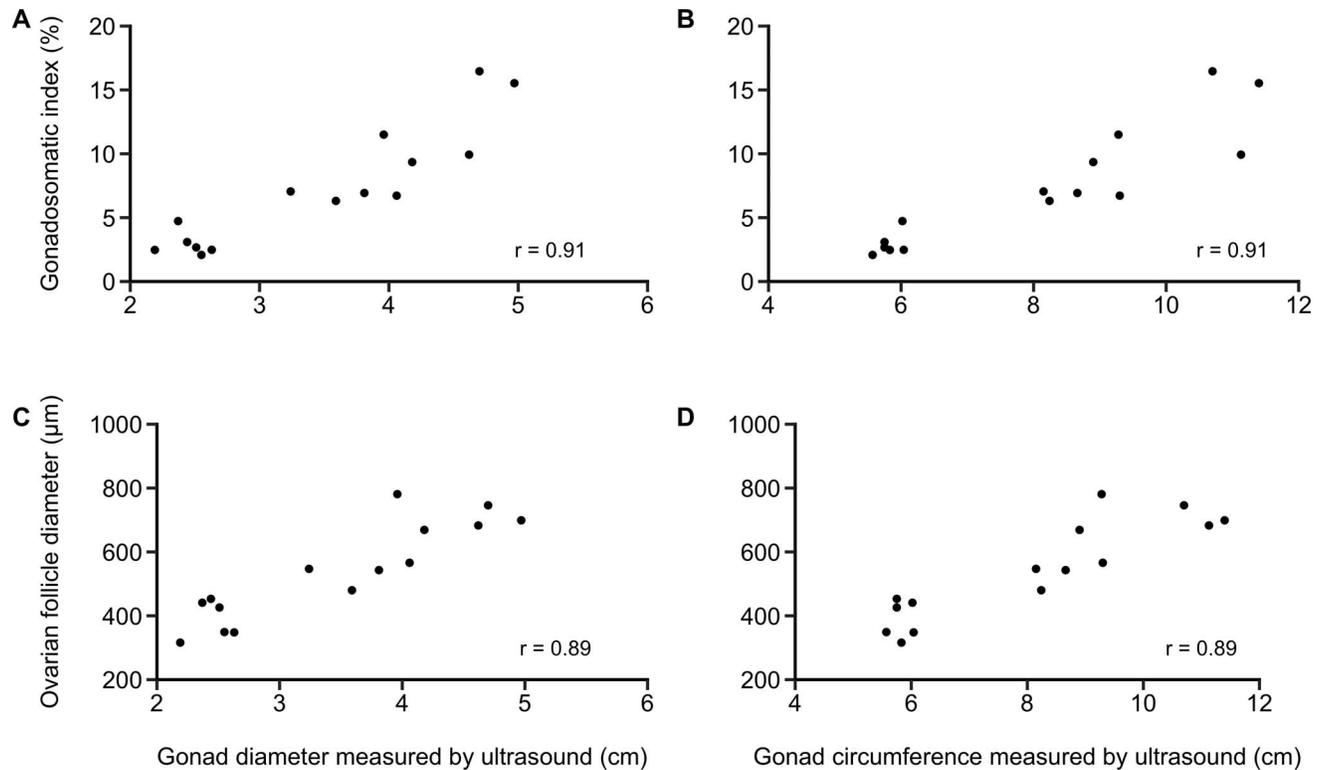
### Gonadosomatic index and ovarian follicle diameter

Female GSI increased from 2.34 (1.78–2.9, 95% CI) during early vitellogenesis (stage 4) to 11.58 (7.63–15.53, 95% CI) during late vitellogenesis (stage 6). Ovarian follicle diameter increased from 338  $\mu\text{m}$  (291–384  $\mu\text{m}$ , 95% CI) during early vitellogenesis (stage 4) to 691  $\mu\text{m}$  (613–768  $\mu\text{m}$ , 95% CI) during late vitellogenesis (stage 6). Female gonad diameter and circumference measured by ultrasound were strongly correlated with GSI ( $r = 0.91$ ) and ovarian follicle diameter ( $r = 0.89$ ; Figure 3).

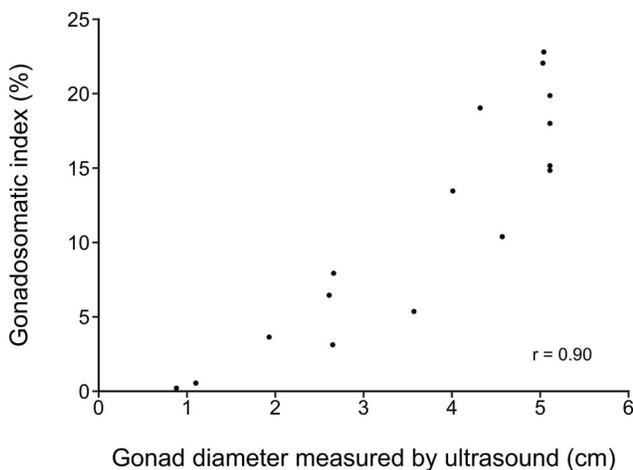
Male GSI increased from 0.38 (0.01–2.54, 95% CI) during spermatogonial proliferation (stage 1) to 19.76 (14.24–25.29, 95% CI) during mid-spermatogenesis (stage 3). Male gonad diameter was strongly correlated with GSI ( $r = 0.90$ ; Figure 4). Male gonad circumference could not be correlated with GSI because the circumference of all testes could not be measured by ultrasound.

## Discussion

We have demonstrated that ultrasound is a promising nonlethal tool to assign stage of maturity in Burbot. Ultrasound has been used to assign stage of maturity by measuring reproductive structures in other fishes, such as Atlantic Salmon *Salmo salar* (Mattson 1991; Naeve et al. 2018), Striped Bass (Blythe et al. 1994), steelhead (Evans et al. 2004), Murray Cod *Maccullochella peelii* (Newman et al. 2008), Small-spotted Catshark *Scyliorhinus canicula* (Whittamore et al. 2010), Thornback Ray *Raja clavata* (Whittamore et al. 2010), and Chinese Sturgeon *Acipenser sinensis* (Du et al. 2017). Our results are similar to other studies that found gonad size measured by ultrasound was highly correlated with GSI and ovarian follicle diameter, two commonly used indices of gonad development in fishes (e.g., Blythe et al. 1994; Newman et al. 2008; Naeve et al. 2018). The strong correlation between gonad size measured by ultrasound and indices of gonad development (GSI and ovarian follicle diameter) suggests that gonad size



**Figure 3.** Correlations of ovary size measured by ultrasound and indices of gonad development in adult female Burbot *Lota lota*. **(A)** Correlation ( $r = 0.91$ ) between ovary diameter measured by ultrasound (cm) and gonadosomatic index (GSI) of adult female Burbot. **(B)** Correlation ( $r = 0.91$ ) between ovary circumference measured by ultrasound (cm) and gonadosomatic index (GSI) of adult female Burbot. **(C)** Correlation ( $r = 0.89$ ) between ovary diameter measured by ultrasound (cm) and ovarian follicle diameter ( $\mu\text{m}$ ) of adult female Burbot. **(D)** Correlation ( $r = 0.89$ ) between ovary circumference measured by ultrasound (cm) and ovarian follicle diameter ( $\mu\text{m}$ ) of adult female Burbot. We held Burbot at the Bozeman Fish Technology Center from November 2017 to March 2018 and exposed them to a natural photoperiod and thermal profile similar to Lake Roosevelt, Washington.



**Figure 4.** Correlation ( $r = 0.90$ ) between testis diameter measured by ultrasound (cm) and gonadosomatic index (GSI) in adult male Burbot *Lota lota*. The maximum gonad diameter capable of being measured by ultrasound was 5.11 cm. We held Burbot at the Bozeman Fish Technology Center from November 2017 to March 2018 and exposed them to a natural photoperiod and thermal profile similar to Lake Roosevelt, Washington.

measured by ultrasound is a nonlethal and noninvasive index of gonad development in fishes.

Only a few studies have documented how gonad size measured by ultrasound changes with stage of maturity determined by gonad histology (e.g., Newman et al. 2008; Du et al. 2017). In Murray Cod, gonad diameter measured by ultrasound differed among stages of maturity but with a high degree of overlap, indicating the assignment of stage of maturity based solely on an ultrasound measurement of gonad diameter was prone to error (Newman et al. 2008). We also observed overlap in the distributions of gonad diameter measured by ultrasound among stages of maturity. However, we did not observe overlap in the distributions of testis diameter measured by ultrasound between nonreproductive stages of maturity and reproductive stages of maturity in males, suggesting that testis diameter measured by ultrasound may be used to assign reproductive condition. Females were only observed in the reproductive stages of maturity; therefore, we could not compare gonad diameter measured by ultrasound between nonreproductive and reproductive stages of maturity. Gonad diameter measured by ultrasound may be used to assign reproductive condition in Burbot, but a study including all stages of maturity during the annual

reproductive cycle and larger sample sizes would be useful to validate our initial results.

Late vitellogenic (stage 6) ovary diameter measured by ultrasound differed from early vitellogenic (stage 4) and mid-vitellogenic (stage 5) ovary diameter measured by ultrasound. However, there was only a 0.15-cm difference between the distribution of late vitellogenic (stage 6) ovary diameter measured by ultrasound and the distributions of early vitellogenic (stage 4) and mid-vitellogenic (stage 5) ovary diameter measured by ultrasound. Characterizing late vitellogenic (stage 6) females would be of interest because these females are capable of spawning (Brown-Peterson et al. 2011). Values of ovary diameter were proposed in other fishes to characterize females capable of spawning (Blythe et al. 1994; Whittamore et al. 2010; Du et al. 2017). All late vitellogenic (stage 6) ovaries had a diameter measured by ultrasound greater than 3.90 cm; therefore, we suggest an ovary diameter of 3.90 cm may be used to characterize late vitellogenic (stage 6) females.

We assigned the diameter of one mid-spermatogenic (stage 3) and all ripe (stage 4) testes a value of 5.11 cm (i.e., the width of the ultrasound transducer) because the testes were wider than our ultrasound transducer and unable to be measured. Our results suggest a testis diameter measured by ultrasound of 5.11 cm could be used to characterize mid-spermatogenic (stage 3) and ripe (stage 4) males, both of which would be expected to spawn during the current reproductive cycle (Brown-Peterson et al. 2011). The shape and frequency of the ultrasound transducer should be considered if further investigating the use of gonad size measured by ultrasound to assign stage of maturity and reproductive condition in male Burbot. In large female Striped Bass, the entire cross-section of the ovary could not be delineated using a linear transducer (6–8 MHz) but could be delineated using a curved transducer (3–5 MHz; Will et al. 2002). A curved transducer may be used to measure the entire cross-section in male Burbot by providing a wider field of view at a lower resolution (Will et al. 2002; Jennings et al. 2005).

Testis diameters were similar between ultrasound and true measurements. Other studies have also shown a high correlation between ultrasound and true measurements of gonad size (e.g., Mattson 1991; Jennings et al. 2005; Bryan et al. 2007; Whittamore et al. 2010; Naeve et al. 2018). Ovary diameter and circumference differed between ultrasound and true measurements. We attributed the difference between measurement methods to flattening of the ovary when applying the ultrasound transducer. Therefore, it is critical to be aware of the pressure exerted on the ovary by the ultrasound transducer. Several other studies have observed a difference in ultrasound and true measurements of reproductive structures (Bryan et al. 2007; Whittamore et al. 2010). For example, ultrasound measurements were 52% smaller than true measurements of ovarian follicle diameter in Shovelnose Sturgeon *Scaphirhynchus platorhynchus* (Bryan et al. 2007). The discrepancy between measurement methods was attributed to the ellipsoid shape of ovarian follicles in sturgeon, making it difficult

to measure the major axis (Bryan et al. 2007). An adjustment for the ultrasound error (i.e., the average difference between the two methods) was applied to all ultrasound measurements of ovarian follicle diameter (Bryan et al. 2007). A similar adjustment could be added to measurements of ovary diameter in Burbot; however, the high correlation observed between ovary diameter measured by ultrasound and indices of gonad development (GSI and ovarian follicle diameter) indicates it is an appropriate index of gonad development.

Ultrasound can be used to assign sex (McGarvey et al. 2020) and is a promising tool to assign stage of maturity in Burbot. Ultrasound is nonlethal and noninvasive, therefore eliminating the need to sacrifice fish and reducing stress experienced by fish compared with other indices of gonad development (e.g., gonad histology, GSI, ovarian follicle diameter, and plasma sex steroids; Blythe et al. 1994). Ultrasound offers immediate results unlike other indices of gonad development that require time to analyze samples in a laboratory. However, an ultrasound unit is expensive (\$6,000 to \$30,000), and using ultrasound requires adequate training to become familiar with the visceral anatomy of the species of interest (32–40 h; Webb et al. 2017). Despite the associated cost and training, we believe ultrasound is a preferable tool when sacrificing fish is unacceptable (e.g., threatened and endangered species), undesirable, or results are required immediately.

## Supplemental Material

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**Data S1.** Data Collected to Assess Gonad Diameter as a Metric to Assign Stage of Maturity in Burbot *Lota lota*. Column headings are referenced in the metadata tab of the datafile. We held Burbot at the Bozeman Fish Technology Center and exposed them to a natural photoperiod and thermal profile similar to that in Lake Roosevelt, Washington. We collected data monthly from December 2017 to March 2018.

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