Use of portable ultrasonography to determine ovary size and fecundity non-lethally in northern pike (*Esox lucius*) and white sucker (*Catostomus commersoni*)

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**ABSTRACT**

The objective of this study was to investigate the feasibility of using field-based portable ultrasonography to accurately estimate ovary weight, gonadosomatic index (GSI) and fecundity in large-bodied fish species. These reproductive endpoints were estimated using ultrasound on prespawning female northern pike (*Esox lucius*) and white sucker (*Catostomus commersoni*) and compared with actual measured values determined post mortem. Using five cross-sectional ultrasound images in pike, estimated ovary weight and fecundity, but not GSI, were significantly correlated with measured values. All endpoints were overestimated by 21 to 23% using ultrasound in pike. In a subsequent experiment using 20 cross-sectional ovary images, estimated ovary weight, GSI and fecundity were significantly correlated with measured values in white sucker. Although underestimated by 5 to 12% using 20 cross-sectional images, there were no statistical differences among estimated and measured mean ovary weight, GSI and fecundity in white sucker using this approach. Based on the variances for GSI estimations in both species, power analysis indicated that the ultrasound technique could detect a 25% change in GSI using sample sizes of <20 fish. This study illustrates the utility of portable ultrasonography as a promising non-lethal technique for assessing reproductive endpoints in the field.

**Key words** | fecundity, fish, gonadosomatic index, non-lethal, ovary, ultrasound

**INTRODUCTION**

Reproductive physiology is one of the most sensitive processes impaired in fish following exposure to stressors such as environmental toxicants (*Donaldson 1990; Van Der Kraak et al. 1998*). Laboratory and field studies have demonstrated that exposure of female fish to polycyclic aromatic hydrocarbons is associated with perturbations in ovarian physiology such as reductions in ovary weight, egg size, fecundity, fertility, hatching success and circulating levels of sex steroid hormones (*Hose et al. 1981; Johnson et al. 1988*). Similar effects have been observed in female fish exposed to trace metals (*Thomas 1989; Brown et al. 1994*), halogenated aromatic hydrocarbons (*Von Westernhagen et al. 1981; Hose et al. 1989*) and complex industrial discharges such as pulp mill effluents (*Munkittrick et al. 1991; Van Der Kraak et al. 1992*). Epidemiological evaluations of the relationship between environmental toxicants and impaired reproductive performance in female fish have revealed strong evidence of causality (*Mac & Edsall 1991; McMaster et al. 1996*). Thus, environmental monitoring programs routinely utilize metrics of ovarian function, such as ovary weight, egg size and fecundity, as ecologically relevant measures of female reproductive status (*Environment Canada 2005*).

Environment Canada requires pulp mills and metal mines throughout Canada to undertake Environmental Effects Monitoring (EEM) under the Pulp and Paper Effluent Regulations and Metal Mining Effluent Regulations,
respectively, which are both under the authority of the Fisheries Act of Canada (Environment Canada 2005). Requirements include an assessment of potential effects on growth and reproduction of adult fish (two species) inhabiting aquatic ecosystems receiving liquid effluent from these operations. The basic EEM endpoints measured in adult female fish include condition factor, weight-at-age, ovary weight, egg size, fecundity (total number of eggs per female) and liver weight (Environment Canada 2005). The target sample size for adult fish surveys under EEM programs is 20 female fish per sampling site, and there may be several exposure and reference sites assessed in a given program. As determination of these endpoints requires destructive (lethal) sampling of fish, it may not be practical under certain circumstances. For example, collection of threatened fish species or sampling ecosystems with low productivity may not allow lethal sampling methods. Thus, there is a need to develop non-lethal techniques to accurately assess reproductive capacity in wild fish exposed to industrial discharges (Gray et al. 2002). To this end, recent advances in ultrasound technology have been used to assess reproductive status of female fish in aquaculture (e.g., Martin-Robichaud & Rommens 2001; Newman et al. 2008) and to a lesser extent in wild fish (Blythe et al. 1994; Will et al. 2002; Jennings et al. 2005; Whiteman et al. 2005; Bryan et al. 2007).

The goal of this study was to evaluate the use of portable ultrasonography as a non-lethal technique to determine ovary weight and fecundity in two common bioindicator species inhabiting Canadian freshwaters, northern pike (Esox lucius) and white sucker (Catostomus commersoni). The focus was on using an approach that was amenable to rapid and accurate field-based evaluations of these endpoints as would be used in monitoring studies, and to evaluate the accuracy and reliability of the technique in comparison with actual measurements taken from the same individual fish.

**METHODS**

**Fish collection**

The study was conducted over two field seasons in northern Saskatchewan. In May 2005, prespawning female northern pike (body weight 1,125 ± 106 g, total length 59.0 ± 1.6 cm, n = 7) were collected using hoop nets from Davies Creek (57° 11’ N, 105° 34’ W). In June 2006, prespawning female white sucker (body weight 1,131 ± 124 g, total length 42.7 ± 1.6 cm, n = 7) were collected using hoop nets from Indigo Lake (58° 23’ N, 103° 48’ W). Both fish collection sites were reference sites used for our concurrent field work investigating selenium ecotoxicology downstream of uranium mine operations (Muscatello et al. 2006, 2008; Muscatello & Janz 2009a, b). Fish were held in net pens for 2–3 days before data collection, so that all fish could be processed on the same day. All procedures involving fish were conducted in accordance with an approved Animal Care and Use protocol at the University of Saskatchewan.

**Ultrasound imaging and data collection in the field**

Individual fish were anaesthetized with 0.2 g/L of MS-222 (3-aminobenzoic acid). Body weight and total length were recorded. Fish were immersed in a portable 0.2 × 0.2 × 1.0 m acrylic tank filled with site water. A Sonosite 180Plus portable ultrasound unit with a 10.5 MHz linear array transducer (Sonosite Canada, Markham, ON) was used to obtain cross-sectional images of the ovary along the ventral surface of each fish. Images were obtained from fish with the transducer at a water depth of 1–2 cm. To reduce handling time, ovaries were presumed to be bilaterally symmetrical and only the left lobe was assessed with ultrasonography. The transducer was adjusted to a penetration depth of 3.6 to 5.7 cm, depending on fish size, to obtain representative ovary images at each body location.

For the northern pike experiment conducted in 2005, cross-sectional images were recorded at five locations: immediately posterior to the pectoral fins, mid body, anterior to the pelvic girdle, posterior to the pelvic fins and anterior to the urogenital vent. To estimate ovary length, a straight-line measurement from pectoral fins to vent was recorded using a ruler. The total time required for ovarian imaging in pike was <5 min. For the white sucker experiment conducted in 2006, the anterior (pectoral fins) and posterior (urogenital vent) ends of the ovary were visualized using ultrasonography, and a straight-line measurement was recorded between these points to determine ovary length. Twenty equidistant cross-sectional
images were recorded along the entire length of the left ovary. The total time required for recording 20 ovarian images in white sucker was <8 min. After image collection, a 2–3 mL sample of eggs was manually expressed from pike and sucker, and egg density (g/mL) was determined by weighing 1.0 mL of eggs in a 5 mL graduated cylinder on a portable pan balance accurate to ±0.01 g. Egg samples were then stored on ice in a plastic vial containing physiological saline (0.9% NaCl) for subsequent fecundity estimations.

After imaging and oocyte collection, fish were euthanized with MS-222 (1.0 g/L) followed by cervical dislocation. Ovaries were dissected and total ovarian mass was recorded in order to compare this value with values estimated from ultrasonography. For white sucker, a 2–3 mL sample of oocytes was collected from cranial, mid and caudal regions of the left ovary and stored on ice in 0.9% NaCl to determine potential differences in egg size and fecundity among ovarian locations.

**Image analysis and calculations**

**Ovary weight and gonadosomatic index**

Ovary weight was estimated based on left ovarian lobe volume determined using ultrasound images collected in the field. The area of each ovarian cross-section was determined using image analysis software (Image-Pro Discovery version 4.5., Media Cybernetics, Bethesda, MD). The image analysis software was calibrated with a standard 4.0 cm length measurement obtained by the ultrasound unit at the initiation of image collection in the field. Twenty points were manually assigned around the circumference of the ovary for each cross-sectional image. A best-fit circle was created in association with these points by the imaging software and used to calculate the area of each cross-section. Ovaries were assumed to be cylindrical and the volume of the left ovary was calculated as \( \pi r^2 h \), where \( \pi r^2 \) = mean ovarian cross-sectional area and \( h \) = ovary length. For northern pike, mean ovarian cross-sectional area was obtained from five cross-sectional images. For white sucker, mean ovarian cross-sectional area was obtained from 20 equidistant cross-sectional images collected along the entire length of the left ovary and compared with the mean cross-sectional area obtained from five equidistant images (i.e. images 1, 5, 10, 15 and 20).

Total ovarian volume was estimated by doubling the value estimated from the left ovary. Estimated total ovarian volume and egg density determined in the field were used to calculate ovarian mass as total ovarian volume \( (\text{cm}^3) \times \) egg density \( (\text{g/cm}^3) \). Gonadosomatic index (GSI) was calculated as total ovarian mass/total body mass \( \times 100\% \). Estimates of ovary mass, GSI and fecundity estimated from ultrasound images were then compared with actual values obtained from post mortem dissection of ovaries.

**Fecundity and egg size**

Fecundity was calculated as the number of eggs/g (from manually expressed eggs) \( \times \) total ovarian mass (estimated using ultrasound and measured post mortem). For white sucker, fecundity was also compared among anterior, mid and posterior regions of the ovary and calculated as number of eggs/g \( \times \) actual ovarian mass. In addition, egg diameter was determined in eggs collected from anterior, mid and posterior regions of the ovary. Egg diameter was determined in 20 randomly chosen eggs from each ovary location using an Olympus SZ61 dissecting microscope and Image-Pro software. Egg diameters were measured to the nearest 0.01 mm after calibrating microscope images with a stage micrometer.

**Statistical analyses**

All data were initially tested for normality and homogeneity of variance, and passed these tests without requiring data transformation. Relationships between ultrasound-estimated and directly measured values for ovary mass, GSI and fecundity were determined using linear regression. For northern pike, differences between mean estimated and measured ovarian mass, GSI and fecundity were detected using unpaired Student’s \( t \)-tests. For white sucker, differences among mean estimated (five images), mean estimated (20 images) and directly measured ovarian mass, GSI and fecundity were detected using one-way ANOVA. Differences among fecundity and egg diameter from anterior, mid and posterior ovary regions were also detected using one-way ANOVA. Statistical
significance was set at $\alpha = 0.05$. Based on the variance observed for GSI estimates using ultrasound, a posteriori power analyses ($1 - \beta = 0.95$) were performed (Dupont & Plummer 1990) to determine the sample sizes required to detect 25% differences in mean GSI values for each fish species (Munkittrick et al. 2009).

RESULTS

Following euthanasia in northern pike, measured (actual) ovary weight ranged from 75.1 to 171.8 g, GSI ranged from 7.5 to 11.0% and fecundity ranged from 10,376 to 21,397 total eggs per female. Following euthanasia in white sucker, measured ovary weight ranged from 105.4 to 266.5 g, GSI ranged from 11.0 to 16.6% and fecundity ranged from 18,509 to 46,089 total eggs per female.

The first experiment in northern pike used five cross-sectional images of the left ovary to estimate total ovary weight, GSI and fecundity. Using this approach, ovary weight and fecundity estimates were significantly ($p < 0.001$) correlated with post mortem measurements ($r^2 = 0.94$ and $r^2 = 0.91$, respectively; Figures 1(a) and (c)). Estimated GSI was not significantly correlated with measured GSI ($p = 0.056$, $r^2 = 0.55$; Figure 1(b)). Mean ovary weight, GSI and fecundity in pike were overestimated by 21% ($p = 0.28$), 23% ($p = 0.014$) and 21% ($p = 0.16$), respectively, compared with measured values (Figures 2(a)–(c)). The statistical power

![Figure 1](https://iwaponline.com/wqrj/article-pdf/46/1/43/379715/43.pdf)

**Figure 1** | Linear regression analyses of relationships between estimated and measured values for (a) ovary mass ($y = 0.95x + 27.3; r^2 = 0.94; p < 0.001$), (b) gonadosomatic index (GSI; $y = 0.87x + 3.1; r^2 = 0.55; p = 0.056$) and (c) fecundity ($y = 0.89x + 4720; r^2 = 0.91; p < 0.001$) in $n = 7$ female northern pike. Estimated values were obtained using five cross-sectional ultrasound images of the left ovary and manual expression of a small egg sample. Measured values were obtained post mortem.

![Figure 2](https://iwaponline.com/wqrj/article-pdf/46/1/43/379715/43.pdf)

**Figure 2** | Mean (±SEM) estimated and measured values for (a) ovary mass, (b) gonadosomatic index (GSI) and (c) fecundity in $n = 7$ female northern pike. Estimated values were obtained using five cross-sectional ultrasound images of the left ovary and manual expression of a small egg sample. Measured values were obtained post mortem. *: Significantly different from measured values using Student’s $t$-test ($p = 0.014$).
using \( n = 7 \) pike to estimate a 25% change in GSI from ultrasound-estimated ovary weight and actual body weight was 92%.

The second experiment, conducted the following year in white sucker, was designed to modify the ultrasonography approaches used for northern pike in order to increase accuracy. Twenty cross-sectional images were collected along the length of the ovary and compared with five images (i.e. every fifth image collected) and to measured values post mortem. In this experiment, ovary weight \((p < 0.001, r^2 = 0.97)\), GSI \((p = 0.003, r^2 = 0.85)\) and fecundity \((p < 0.001, r^2 = 0.99)\) estimates using 20 cross-sections were significantly correlated with post mortem measurements (Figures 3(a)–(c)). There were no significant differences among mean ovary weight \((p = 0.79)\), GSI \((p = 0.25)\) or fecundity \((p = 0.48)\) when comparing the data collected from 20 images, five images or measured post mortem values (Figures 4(a)–(c)). Ovary weight, GSI and fecundity were underestimated by 5, 6 and 13%, respectively, when comparing measured values with those estimated from 20 cross-sectional images (Figure 4). Ovary weight, GSI and fecundity were underestimated by 14, 14 and 23%, respectively, when comparing measured values with those estimated from five cross-sectional images (Figure 4). The statistical power using \( n = 7 \) white sucker to estimate a 25% difference in GSI from ultrasound-estimated ovary weight and actual body weight was 68 and 58% when using 20 and five cross-sectional images, respectively. Mean fecundity estimates

![Figure 3](https://iwaponline.com/wqrj/article-pdf/46/1/43/379715/43.pdf)  
Linear regression analyses of relationships between estimated and measured values for (a) ovary mass \((y = 1.05x - 15.5; r^2 = 0.97; p < 0.001)\), (b) gonadosomatic index (GSI; \(y = 1.04x - 0.014; r^2 = 0.85; p = 0.003\)) and (c) fecundity \((y = 0.95x-2025; r^2 = 0.99; p < 0.001)\) in \( n = 7 \) female white sucker. Estimated values were obtained using 20 cross-sectional ultrasound images of the left ovary and manual expression of a small egg sample. Measured values were obtained post mortem.

![Figure 4](https://iwaponline.com/wqrj/article-pdf/46/1/43/379715/43.pdf)  
Mean (± SEM) estimated and measured values for (a) ovary mass, (b) gonadosomatic index (GSI) and (c) fecundity in \( n = 7 \) female white sucker. Estimated values were obtained using either 20 (20× Est) or five (5× Est) cross-sectional ultrasound images of the left ovary and manual expression of a small egg sample. Measured values were obtained post mortem.
determined using eggs dissected from anterior (27,331), mid (26,425) and posterior (23,234) regions of the ovary were not significantly different (p = 0.14; data not shown). Mean egg diameters among anterior, mid and posterior regions of the ovary (range 2.12 to 2.44 mm) were not significantly different (p = 0.92; data not shown). For calculation of ovary weight (ovary volume × egg density), egg density values were 1.04 ± 0.006 g/mL for northern pike and 1.05 ± 0.011 g/mL for white sucker.

Based on the variances observed for GSI estimated using ultrasound images and measured post mortem, power analyses were performed to determine sample sizes required to detect a 25% change in mean GSI values. Setting α = β (power = 95%), detecting a 25% change in GSI for northern pike would require n = 7 fish for measured values and n = 9 pike for estimated (ultrasound) values. For white sucker, a sample size of n = 10 fish would be required to detect this difference using measured values, compared with n = 14 and n = 17 for GSI values estimated using 20 and 5 cross-sectional ultrasound images, respectively.

DISCUSSION

The present study compared ovary weight, GSI and fecundity values measured using traditional (lethal) sampling methods versus values estimated using non-lethal ultrasonographic imaging of ovaries and a small, manually expressed sample of eggs. Adult northern pike and white sucker collected for the present study were captured immediately prior to spawning, which occurs in mid-May and early June, respectively, at this latitude (Muscatello et al. 2006; Muscatello & Janz 2009a). Modification of the technique by increasing the number of cross-sectional ovary ultrasound images from five to 20 resulted in more accurate estimates of ovary weight, GSI and fecundity compared with actual values determined post mortem in white sucker. The procedure took less than 8 min to complete in anaesthetized fish held under water, which would allow for the recovery and release of female fish in aquatic ecotoxicological studies such as those routinely conducted as part of EEM programs. Thus, a non-lethal field sampling program that uses portable ultrasound to estimate ovary weight and fecundity would allow for determination of body length, body weight, GSI, fecundity and age (if structures such as scales or fin rays can be used), but not hepatosomatic index (liver to body weight ratio). Such an approach should be considered for EEM programs, particularly when fish species at risk are being assessed or when low productivity aquatic systems, such as in northern Canada, are being monitored. In addition, this approach would be conducive to monitoring individuals over time in catch and release studies, which may be useful in assessing ecosystem recovery.

Ultrasound technology has most commonly been used in aquaculture to determine sex ratios and maturation status of brood stock (Martin-Robichaud & Rommens 2001) and a recent summary of published aquaculture studies employing ultrasound is available (Newman et al. 2008). However, there are few studies that have used ultrasound to assess female reproductive status in wild fish. The most extensive studies have been conducted in striped bass (Morone saxatilis) from the Savannah River, GA (Blythe et al. 1994; Will et al. 2002; Jennings et al. 2005). In these studies, ovary volume estimated using ultrasound was used to develop fecundity models for different age classes. This approach was also used in a related study of red hind (Epinephelus guttatus) at the US Virgin Islands (Whiteman et al. 2005). Ultrasound in combination with endoscopic imaging of ovarian follicles has recently been used to assess ovary volume, fecundity and maturation status of shovel nose sturgeon (Scaphirhynchus platynychus) in the Missouri River, USA (Bryan et al. 2007). Collectively these studies have indicated that the use of ultrasound to estimate ovary size and fecundity shows great promise as a non-lethal monitoring approach.

The present study identified several sources of variation in estimating ovary size and fecundity, which may be reduced in future studies. Accurate estimation of mean ovary width is a key component affecting the calculation of ovarian volume and thus mass and GSI. The gradual tapering of northern pike ovaries at the posterior end (from pelvic girdle to vent) was likely an important cause of the overestimated ovary volume upon which estimates of GSI and fecundity were based. In contrast, the white sucker ovary has a more consistent diameter along its length. Incorporation of the additional cross-sectional images reduced the differences between estimated and
measured values, and this approach is recommended in future studies. Depending on the species, the use of ten cross-sectional ultrasound images is recommended as a trade-off between accuracy and time required (and thus physiological stress to fish) to acquire images. In addition, assuming a circular cross-section for each image may have contributed to error in estimating ovary volume. Future studies may consider using mathematical formulas for oval or polygonal cross-sections to reduce this variation. Differences in ovarian morphology may mean that species-specific models are necessary to successfully apply this approach to long-term monitoring studies.

Ovarian maturation status and the pattern of oogenesis and are also important factors to be considered when using ultrasound to estimate ovary size and especially fecundity. Both species in the present study are synchronous spawners, making fecundity estimates simpler than species that have an asynchronous (batch) pattern of oogenesis such as bass (Blythe et al. 1994; Will et al. 2002). Even within synchronous spawning species, however, potential differences in egg size within the ovary may introduce error into fecundity estimates. Although we did not detect statistically significant differences among egg densities or egg diameters in anterior, mid or posterior regions of the white sucker ovaries, expressed eggs from the posterior (caudal) region used for fecundity estimates had lower mean densities (150 eggs/g) compared with mid (175 eggs/g) and anterior (181 eggs/g) regions. Future studies should examine the homogeneity of egg densities within the ovary to evaluate this source of variation in fecundity estimates. In the present study, fish were collected just prior to spawning, allowing manual expression of a small sample of eggs. Collection of fish at other stages of ovarian maturation may not allow for fecundity estimates, although catheterization (Will et al. 2002) and endoscopy (Bryan et al. 2007; Divers et al. 2009) have been used in these situations previously.

In addition to ensuring accuracy when using ultrasound analysis to estimate reproductive endpoints in fish, statistical power is also an important consideration. This is particularly important in aquatic ecotoxicological studies, where Type II errors (accepting the null hypothesis of ‘no effect’ when it should be rejected) can influence management decisions and regulations influencing sustainability of native fish populations. Although the sample size of $n = 7$ fish was low in the present study, the variances observed for GSI were used to determine sample sizes required to detect a 25% difference in GSI, which has been suggested as a critical response in fish gonadal assessments (Munkittrick et al. 2009). Based on the variances observed for GSI in pike and white sucker, and $\alpha = \beta$ (i.e. power = 95%), the required sample sizes to detect a 25% difference in GSI ranged from 8 to 17 fish. As these sample sizes are smaller than the recommended $n = 20$ female fish required for EEM programs (Environment Canada 2005), this further demonstrates the potential for ultrasonography to be used as an alternative, non-lethal approach to assess female reproductive status. However, the variance estimates used in the present pilot study were obtained from the same (reference) fish populations, and greater heterogeneity in GSI may occur in wild fish populations collected from different study sites and in fish at different stages of their reproductive cycle.

Fish body size may limit the use of ultrasound to estimate ovary weight and fecundity. The linear array ultrasound transducer (probe) used in the present study had a width of 5 cm, making it ideal for large-bodied fish such as pike and white sucker, and would also be appropriate for salmonids and larger species of centrarchids and percids. An increasing variety of transducer configurations is available, making application of this technique to small-bodied fish species feasible. Jennings et al. (2005) compared ultrasound probes of different configurations and frequencies and recommended that fish anatomy and image acquisition software capabilities should be considered when initiating work of this nature. Recent advancements in ultrasound technology, particularly with respect to cost, portability and flexibility, should allow applications in many areas of fish biology.

CONCLUSION

The present study demonstrated the feasibility of using portable ultrasonography to accurately determine ovary weight, GSI and fecundity in white sucker. Using 20 cross-sectional ultrasound images in white sucker, estimated values were highly correlated and not significantly different from actual values determined post mortem in the same fish. Although
the ovary weight, GSI and fecundity were overestimated by 21 to 23% in northern pike, use of additional cross-sectional images in this species may increase accuracy in future studies. Power analysis indicated that the ultrasound approach could detect biologically significant differences in GSI using sample sizes of fewer than 20 female northern pike and white sucker. Thus, the use of portable ultrasonography for assessing female reproductive status in fish should be considered in certain aquatic monitoring scenarios such as EEM programs.

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