Comparison of Gravimetric and Volumetric Methods to Estimate Brook Trout (Salvelinus Fontinalis) Fecundity

Research Note

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Abstract

Estimating fish fecundity is important for developing accurate population models and informed management decisions. Fecundity can be determined by tedious, complete oocyte counts. Researchers save time by counting a subsample of oocytes, measuring the subsample and total ova volume and weight, and extrapolating to produce fecundity estimates using volumetric and gravimetric methods. Volumetrically- and gravimetrically-generated fecundity estimates from 70 brook trout (*Salvelinus fontinalis*, range 143–356 mm) captured from Long Creek, Oregon, were compared to total oocyte counts to evaluate the accuracy and precision of each method. The average total oocyte count was 775 (SD ± 354.8). The mean difference between total oocyte count and extrapolated count based on the gravimetric method was 111.2 (SD ± 154.0) and 165.9 (SD ± 279.0) for the volumetric method. Gravimetric and volumetric fecundity estimates were closely correlated with total oocyte counts, although both were positively biased. Volumetric estimates were on average 1.100 times true fecundity (95% CI = 1.05, 1.15) and gravimetric estimates were 1.086 times true fecundity (95% CI = 1.05, 1.12).

Keywords: Brook trout, fecundity, extrapolation methods

Introduction

Fecundity is often used as an indicator of reproductive potential in fish populations (Marshall et al. 2003, Murua et al. 2003, Rawat et al. 2017) and is important for developing accurate population models and informed management decisions (Jakobsen et al. 2016). Information on reproductive potential may be used in conjunction with survival data to evaluate species reproductive success (Lambert 2008). Population viability analyses that rely on accurate fecundity estimates are used to identify vulnerable life stages. Such information can be leveraged to suppress non-native species or promote conservation of native species (Peterson et al. 2008, Benjamin et al. 2017). Indeed, fecundity data are often imperative for informing alternative management strategies and assessing population dynamics (Lambert 2008, Jakobsen et al. 2016).

Fecundity can be determined by counting all oocytes from individual female fish, which constitutes fecundity (Crim and Glebe 1990). Although total oocyte counts confer accuracy, this method may be time consuming, particularly for highly fecund species. For instance, absolute fecundity of blue sucker (*Cycleptus elongatus*) has been reported to average 150,704 oocytes per female (Daugherty et al. 2008). As a result, researchers often choose to save time and resources by counting a subsample of oocytes and making inferences on those subsample counts (Kelso et al. 2012). Thus, implementation of rapid, accurate methods for estimation of fecundity is of interest to managers.

Commonly, fecundity estimates are determined by measuring the volume of water displaced by a subsample of ova, counting ova in the subsample, and extrapolating the count by the volume displaced by all ova (volumetric method). Likewise, fecundity may be determined by measuring the weight of the subsample of ova, counting ova in the subsample, and extrapolating the count by the total ova weight (gravimetric method; Crim and Glebe 1990). Although both volumetric and gravimetric methods have been used to estimate fecundity (Crim and Glebe 1990, Murua et al. 2003),

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rarely are the estimates compared to total counts to examine the accuracy and precision of these methods (Phillips 1969). Given the apparent paucity of literature validating fecundity extrapolation, the objective of this study was to compare the accuracy and precision of fecundity estimates based on volumetric and gravimetric methods.

Methods

Prior to fall spawning, non-native brook trout (*Salvelinus fontinalis*) were captured by electrofishing from 16 July to 16 August 2018, within Long Creek (latitude 42°49′45.9984″N, longitude 121°10′42.9996″E) in the Klamath River basin, Oregon, during a population suppression effort. Brook trout are not native to Long Creek and have been the subject of occasional removal to prevent hybridization and competition with native species. Multiple electrofishing passes were completed to maximize capture of female brook trout from varying size classes. After sampling, captured brook trout were euthanized by cranial concussion and dissected to determine sex. Fork length (to nearest millimeter), weight (to nearest 0.1 gram), and sex were recorded. Harvested ovaries were preserved separately in 10% formalin solution (MWI Veterinary Supply Co., Boise, Idaho) and transported to a laboratory.

In the laboratory, ovaries from each individual fish were removed from the solution, blotted with a paper towel to remove excess liquid, and ovarian tissue removed. Whole ova weight was recorded (to nearest 0.1 g) using a calibrated scale (A&D Co., model HL-3000WP, San Jose, California). Volume (milliliter) of water displaced by ova was measured using a graduated cylinder. A 0.2–0.9 g subsample (3–13% of total average weight) was taken, and the respective weight and displacement volume was recorded. The number of oocytes was enumerated using a handheld counter to prevent counting errors. Individual fecundity was estimated by dividing the number of ova in the subsample by the subsample displacement volume and multiplying the value by the entire displacement volume. Fecundity estimates produced by the gravimetric method were similarly estimated using weight instead of volume. Manual counts of all oocytes in the ova were conducted to assess the accuracy of volumetric and gravimetric methods.

Bivariate plots were made to visualize the relationship between total oocyte count and volumetric and gravimetric fecundity estimates. Since a true oocyte count of zero would lead to an estimated count of zero, a linear model with the intercept forced through the origin was used to evaluate fit of the data. When fecundity estimates precisely match total oocyte counts, all observations fall on the same plot line (i.e., a one-to-one relationship). Thus, an identity (1:1) line was added to the plots for comparison. The absolute average difference between total count and estimated counts was examined for accuracy. To examine the magnitude and direction of bias, the relationship between estimated and true fecundity was modeled by calculating the geometric mean and standard error of the log ratios (estimated:true) and back-transforming to generate estimates and 95% confidence intervals. To determine if the model fit the data, the log residuals were plotted against true fecundity. RStudio version 1.4.1106 was used for analyses (RStudio Team 2021).

Results

Two hundred and fifteen female, 149 male, and 16 brook trout of unknown sex were captured during sampling. Seventy mature female brook trout were retained for analyses; insufficient resources precluded examination of additional fish. Average length was 231.4 mm (range 143–356 mm; Figure 1), average weight was 155.5 g (range 36.0–603.4 g), and average total oocyte count was 775.2 (range 340–1,914).

The linear model of total oocytes and volumetric estimates revealed these estimates were positively biased (i.e., estimated counts fell above the 1:1 line; Figure 2). Mean difference between total oocyte count and estimated count based on the volumetric method was 165.9 ± 279.0 (mean ± SD). Fecundity estimated using the volumetric method was on average 1.100 times true fecundity (95% CI = 1.05, 1.15); the plot of log residuals against true fecundity exhibited no pattern, indicating the model was appropriate for the data.

The linear model of total oocytes and gravimetric estimates revealed these estimates were positively biased (i.e., estimated counts fell above the 1:1 line; Figure 3). Mean difference between total oocyte count and extrapolated count based on the gravimetric method was 111.2 ± 154.0 (mean ± SD). Fecundity estimated using the gravimetric method was on average 1.086 times true fecundity (95% CI = 1.05, 1.12); the plot of log residuals against true fecundity exhibited no pattern, indicating the model was appropriate for the data.

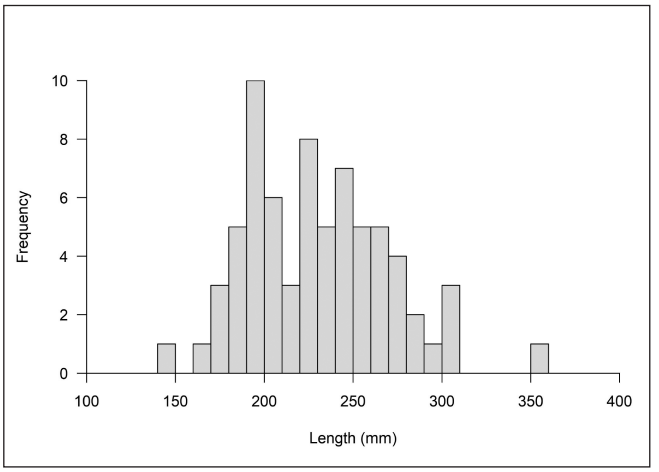

Figure 1. Length-frequency histogram of brook trout collected from Long Creek, Oregon in 2018. Bin width is 10 mm.

Discussion

In this study, gravimetric methods produced brook trout fecundity estimates that closely approximated total counts. Other studies have similarly demonstrated a low bias of gravimetric estimates. Johnston and McKenna (1977) reported a 0.4% difference between gravimetric estimates and total counts for brook trout, although the direction of bias was not described. Similarly, an average 7.5% positive bias of gravimetric estimates has been shown for brook trout (Halfyard et al. 2008). Kucera and Kennedy (1977) demonstrated a 5.7% positive bias of the gravimetric fecundity estimation method relative to total counts from cutthroat trout (*Oncorhynchus clarkii*). Given the results reported in this study

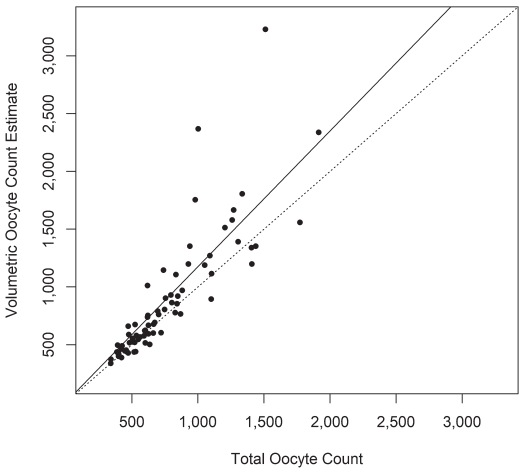

Figure 2. Comparison of total oocyte count to volumetric count estimates from brook trout collected in Long Creek, Oregon in 2018. The linear model (through the origin) of total count and estimated count is y = 1.17(x) (r^2 = 0.92). The dashed line represents the 1:1 line.

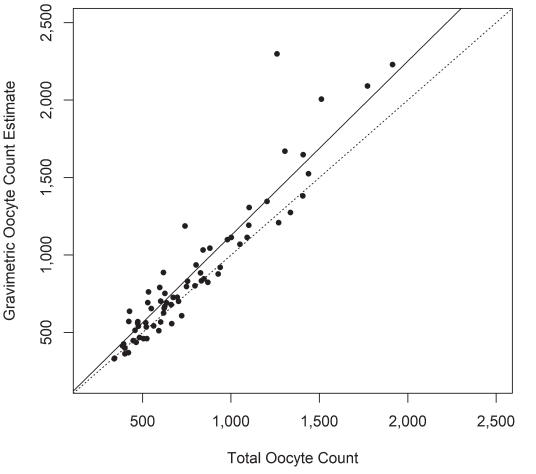

Figure 3. Comparison of total oocyte count to gravimetric count estimates from brook trout collected in Long Creek, Oregon in 2018. The linear model (through the origin) of total count and estimated count is y = 1.13(x) (r^2 = 0.97). The dashed line represents the 1:1 line.

and elsewhere, these observations reveal the gravimetric method provides an accurate method to estimate brook trout fecundity.

Based on the results of this study, it was not possible to determine whether the volumetric or gravimetric performed better because of the overlapping confidence intervals. However, the estimated gravimetric coefficient was slightly less than the volumetric coefficient, which is consistent with results reported by others. Phillips (1969) found the gravimetric method to produce a lower error rate than the volumetric method for estimating Southern redbelly dace (*Chrosomus erythrogaster*) fecundity. Similarly, in a comparative evaluation of methods to estimate oocyte counts, Witthames and Walker (1987) determined the volumetric method exhibited greater bias than estimates based on the gravimetric method. Although the results presented here do not confirm this pattern, they are consistent with the findings of these authors, suggesting the gravimetric method may be preferable.

The specific causes of bias differ between methods and may be explained in several ways. First, the gravimetric method requires the use of a calibrated scale to precisely weigh ovaries. A benefit of using a scale is that recalibration is completed between each use, which permits accurate weight measurements. Fecundity estimates may be positively or negatively biased, however, if readings from an uncalibrated scale are higher or lower than true weight. Second, observer error may occur when reading the quantity of water displaced by ovaries through variability in readings at the meniscus in the graduated cylinder. Third, incomplete drying of a graduated cylinder between each use may leave water clinging to the surface, which could lead to inaccurate readings on subsequent occasions. In both of these cases, excess water may lead to positively biased fecundity estimates.

Subsampling can save time when estimating fecundity. However, potential bias should be considered and minimized, as it may lead to errors when modeling non-native fish suppression or eradication (Cox et al. 2013, Klein et al. 2016, Benjamin et al. 2017) and reproductive dynamics (Ganias 2013, Jakobsen et al. 2016). The results presented here demonstrate that the use of volumetric and gravimetric estimation methods can be accurately used to estimate brook trout fecundity.

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