A Comparison of Nonlethal Methods for Evaluating the Reproductive Status of Female Coastal Cutthroat Trout

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Abstract

Knowledge of the state of sexual development is important for management of coastal cutthroat trout *Oncorhynchus clarkii clarkii*, a fish species targeted for sport fishing throughout its range along the Pacific coast of North America. The purpose of this study was to compare the nonlethal methods of ultrasound imaging, body lipid content, and the measurement of plasma vitellogenin and estradiol levels for assessing the reproductive status of female coastal cutthroat trout. This was examined in a population living in Florence Lake, Alaska, during the spring–early autumn period of the annual reproductive cycle. All methods, except body lipid content, were effective at determining maturity status in either the spring (ultrasound imaging), or spring and autumn (plasma vitellogenin and estradiol). These approaches could be useful for conducting nonlethal assessments of length- or age-at-maturity on populations of coastal cutthroat trout that are small, have conservation concerns, or are heavily utilized by anglers.

Keywords: cutthroat trout; estradiol; lipids; sexual maturity; ultrasound; vitellogenin

Introduction

Knowledge of the state of sexual development relative to length or age is important for understanding the structure of fish populations in many fundamental or applied applications. Lethal sampling techniques to assess sexual maturity may be inappropriate for small populations or for those with conservation concerns. A variety of nonlethal techniques such as gonad ultrasound imaging (Martin et al. 1983), blood plasma indicators (Webb et al. 2002), and coelomic endoscopy (Swenson et al. 2007) have been used to infer sex or reproductive status in many species of fish. However none of these techniques have been developed or applied to coastal cutthroat trout *Oncorhynchus clarkii clarkii*.

Coastal cutthroat trout occur in streams and lakes along the northwestern coast of North America from northern California to the Kenai Peninsula, including the Alexander Archipelago in Southeast Alaska and Prince William Sound (Behnke 2002). Several life-history forms have been identified, including those that migrate from salt water to freshwater for winter refuge and/or spawning (typically referred to as sea-run or anadromous) as well as freshwater forms that do not enter salt water. The freshwater forms reside in either river systems (riverine), lake systems (lacustrine), or in headwater tributaries (Johnson et al. 1999).

There is extensive recreational angling of coastal cutthroat trout throughout their range and it is important for management purposes to know when fish attain sexual maturity. A key management objective in Washington (Leider 1997) and Alaska (Gresswell and Harding 1997) is to protect coastal cutthroat trout from
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harvest until they have had the opportunity to spawn at least once. Spawning in coastal cutthroat trout populations can occur anytime between the months of March through June, but most fish generally spawn in April or May (Trotter 1989) which generally coincides with the period when many Alaskan lakes in the range of coastal cutthroat trout begin to lose their winter ice coverage.

Significant development of gonads in female coastal cutthroat trout occurs in the late summer and autumn in the year prior to spawning (Foster 2003). This development period, termed vitellogenesis, is characterized by rapid growth of oocytes. Foster (2003) found that female coastal cutthroat trout gonads were sufficiently developed by October (i.e., diameter of oocytes ranged from 2.0 to 4.4 mm) of the previous year to infer maturity status (i.e., determine which females are preparing to spawn) 6–7 mo before spawning occurs.

Among populations, maturation rates may vary considerably in relation to size and age characteristics, but collecting this information can be difficult because salmonid fishes as a group are difficult to sex and determine maturity status from external visual observations. Therefore, it would be valuable to have nonlethal methods available to provide insight on the reproductive status of female coastal cutthroat trout. Additionally, some populations are small; nonlethal methods would be advantageous over lethal sampling to preserve the health of the population.

Several nonlethal approaches to gauge female reproductive development were evaluated in this study: ultrasound imaging, body lipid content, and the measurement of vitellogenin (VTG) and estradiol-17β (E2) levels in blood plasma. Ultrasound and body lipid content measurements are the least invasive and most rapid to conduct. Ultrasound uses cyclic sound pressure waves (>20 kHz) to visualize internal organs within the body cavity through the skin (Martin et al. 1983). Body lipid content can be measured using handheld microwave devices that determine water content of a sample, following the concept that there is an inverse relationship between water and lipid contents (Kent 1993). Quantification of blood-borne factors (e.g., VTG, hormones) requires more handling of the fish because anesthesia is required to obtain a blood sample for laboratory analysis. Vitellogenin and E2 are well-studied indicators of sexual development in female fishes (Lambert et al. 1978, Whitehead et al. 1978, Scott and Sumpter 1983, Bon et al. 1997). Vitellogenin is a glycoporpholipoprotein secreted by the liver, sequenced from the bloodstream by the ovary, and used to form the yolk within the oocytes (So et al. 1985, Copeland et al. 1986). In salmonid fishes it is the primary component of the yolk and increases substantially in females undergoing ovarian development during the reproductive cycle (Tyler and Sumpter 1996). Similarly, plasma E2 is an excellent indicator of female reproductive development because it increases substantially in the blood and stimulates the liver to synthesize VTG in maturing females (Whitehead et al. 1978, Campbell and Idler 1980, van Bohemen et al. 1982, Scott and Sumpter 1983). The goal of this study was to compare these nonlethal methods to evaluate the reproductive status of female coastal cutthroat trout during the spring–summer–early autumn period of the annual reproductive cycle.

Study Site

The site for this study was Florence Lake (United States, 134°4’W; 58°3’N), which is located approximately 50 km southwest of Juneau, Alaska, on the west side of Admiralty Island. The 431-ha lake is narrow (~1 km wide) and about 7.2 km long, and has a maximum depth of approximately 27 m. The lake outlet flows about 1 km before entering Chatham Strait and passes over a barrier falls about 400 m upstream of tidewater, blocking the lake to upstream fish passage.

Methods

The sampling dates for the ultrasound evaluation were 13–15 April 2004. For the remaining techniques, we conducted five sampling trips to Florence Lake in 2006 (20–24 April, 14–16 June, 25–28 July, 23–25 August, 3–4 October). The timing of these trips was intended to occur periodically throughout the annual reproductive cycle of coastal cutthroat trout and in conjunction with the portion of the year in which the lake is not covered by ice.

We captured fish by setting hoop traps that were baited with salmon eggs that were disinfected in a povidone–iodine solution. The hoop traps were 1.4 m long and consisted of four 0.6-m-diameter hoops with 9-cm-diameter throats attached to the first and third hoops, and a mesh size of 1 cm. We set the traps across the lake in a uniform distribution without regard to depth. Traps were set for 2–14 h in 2004 and <3 h in 2006.

We anesthetized fish with Aqui-S® (Aqui-S New Zealand Ltd, Lower Hutt, New Zealand) following the manufacturer’s instructions to facilitate sampling procedures listed below. Afterward, we euthanized all fish by a blow to the head. After weighing the fish, we dissected them to determine sex and examine the gonads. We weighed ovaries to allow for calculation of a gonadosomatic index or GSI (i.e., ovary weight / fish weight × 100).

Foster (2003) found that female cutthroat trout in Florence Lake could be classified, based on a GSI, into two distinct groupings in October; fish with larger ovaries (GSI ≥ 1.8) had relatively large oocytes (diameters ranged from 2.0 to 4.4 mm) and were, presumably, preparing to spawn the following spring. In contrast, fish with small ovaries (GSI < 1.8) and much smaller oocytes were deemed unlikely to spawn the following spring. Foster (2003) concluded that fish could be easily classified into these two groupings based on visual inspection of the ovaries alone (Figure 1). However, Foster (2003) determined that cutthroat trout could not be classified into distinct groupings on the basis of GSI or macroscopic inspection of ovaries in the summer. Therefore, in the absence of data for histological classification, we restricted our categorization of fish into groupings of small versus large ovaries to April and
October; the reproductive status of fish in the summer months was simply considered unknown. Sample sizes in 2006 were April = 17 fish with large ovaries, 15 fish with small ovaries; June = 43 fish of unknown status; July = 43 fish of unknown status; August = 43 fish of unknown status; and October = 13 fish with large ovaries, 16 fish with small ovaries (data available in Table S1).

We conducted ultrasonography only once (April 2004) on a sample of 63 coastal cutthroat trout; we used a Sonosite model 180 using the small parts mode with a L38 linear probe (10-5 MHz). The ultrasound sampling duties were divided between two persons, the sonographer and the fish handler. The sonographer was responsible for interpreting the ultrasound images and classifying each fish as either a female with large discernable ovaries in the body cavity (n = 21) or unknown (i.e., no discernable ovaries; a male or nonspawning female; n = 42). To prevent possible bias introduced by observing fish coloration, size, or other characteristics, the station was set up such that the sonographer was unable to see the fish. The fish handler gently held the fish upside down in a small bin of water (approx. 20 L) and guided the transducer (also held underwater) perpendicularly along the ventral abdominal surface of the fish from the vent to the operculum. The examinations typically lasted ~20 s.

We measured body lipid content with a Distell model FM 992 fatmeter (Distell, West Lothian, Scotland) following the manufacturer's technical manual. It employs a microstrip transmission sensor (2 mW microwave power) to measure water content. The "Trout-2" factory calibration setting was selected and accuracy was verified before each sampling session by ensuring that test readings on the corresponding Distell reference pads were within specified tolerances. For each fish, we took duplicate readings on each side by positioning the sensor along the lateral line, posterior to the operculum. The median value was used for data analysis.

We used a heparinized syringe to draw approximately 1–1.5 mL of blood from the caudal vasculature. The blood samples were immediately centrifuged at 7,200 gravities for 5 min. The plasma was removed and then divided into two or three vials with 5–10 trypsin inhibitor units of aprotinin (Sigma Chemical, St. Louis, MO) added per mL of plasma to stabilize the VTG protein. We initially stored the samples in a portable freezer (–20°C) and then transferred them to a –80°C freezer within 10 d.

We measured plasma levels of VTG using a rainbow trout *O. mykiss* Vitellogenin Enzyme-Linked Immunosorbent Assay (ELISA; Cat. No. V01004402-480, Cayman Chemical Company, Ann Arbor, MI). The rainbow trout VTG ELISA was used because an ELISA specific for cutthroat trout *O. clarkii* was not available. However, the rainbow trout is a congener with cutthroat trout and serially diluted female coastal cutthroat trout plasma samples were parallel to the rainbow trout VTG standard.

Figure 1. Comparison of oocytes from female coastal cutthroat trout *Oncorhynchus clarkii clarkii* sampled in October 1997 in Florence Lake, Alaska. The large oocytes on the left are representative of females with larger, more developed ovaries; whereas, the smaller ovary on the right is typical of a female that is unlikely to spawn the following spring. Adapted from “Maturity, fecundity, growth, and sustained yield of coastal cutthroat trout at Florence Lake, Southeast Alaska,” by M. B. Foster, 2003, Master’s thesis, University of Alaska - Fairbanks. Adapted with permission.
Coastal cutthroat trout plasma samples were serially diluted 1:1000, 1:10,000, or 1:100,000, and then triplicate technical replicates were assayed in the ELISA according to the manufacturer’s instruction manual provided with the kit.

We measured plasma levels of E2 by first performing a double solvent extraction with diethyl ether. This was followed by serial dilutions at 1:10 or 1:100, and then triplicate technical replicates were assayed in an E2 enzyme immunoassay (EIA; Cat No. 582251.1, Cayman Chemical Company) following the instruction manual provided with the kit.

We used a two-sample permutation test (Good 2000) to compare E2, VTG, and lipid levels between groups. We used an $\alpha$ level of 0.05 to establish statistical significance.

### Results

Ultrasound imaging during April 2004 correctly identified 100% of the female coastal cutthroat trout with large ovaries ($n = 21$). The remaining fish ($n = 42$) consisted of 15 females with small ovaries (presumably nonspawning fish) and 27 males. The ultrasound machine was not available for the other sampling trips.

In 2006, our categorization of females based on macroscopic examination of the ovaries was consistent with GSI data (Figure 2). Females categorized as having large ovaries (April: 1.95–15.34; October: 1.32–3.45). With the exception of one fish, GSI levels in females with large ovaries were much higher in April than in October (Figure 2).

Body lipid data were collected on four (April, July, August, and October) of the five sampling trips in 2006 because the fatmeter was not available for the June trip. The data show a gradual increase in fatmeter readings, from 1% to 4% during April up to 5–6% for July through October (Figure 3). Only during April was there a statistically significant difference ($t = 82$, two-sided $P < 0.001$) in lipid content between fish with large ovaries compared with fish with small ovaries. An overlap in fatmeter readings was observed in April, with lower levels in fish with large ovaries compared with fish with small ovaries.

The plasma sample for two females with large ovaries that were sampled in April could not be analyzed because of a laboratory error. In addition, we were unable to measure E2 levels in six females (April: two females with large ovaries, one female with small ovaries; June: three females of unknown status) because the amount of plasma collected for these fish was insufficient.

Plasma VTG levels ranged from 0 to 24,254 $\mu$g/mL in females sampled during the April to October, 2006 period (Figure 4). During April and October, VTG levels were distinctly bimodal; females with small ovaries had a range of low levels—1–655 $\mu$g/mL (April) and 0–35 $\mu$g/mL (October), and females with large ovaries were much higher.

![Figure 2](image-url)
higher—6,917–24,255 μg/mL (April) and 7,274–19,344 μg/mL (October). For both April and October, VTG levels of the two groups were significantly different from one another (April: \(t = 307\), one-sided \(P < 0.001\), October: \(t = 260\), one-sided \(P < 0.001\)). During these 2 mo, there was no overlap in VTG levels between females with large ovaries compared with females with small ovaries. Sampling from June to August showed a broad distribution (0.5–12,500 μg/mL) of generally lower VTG levels with no distinct bimodality.

Plasma E2 levels ranged from 0 to 35,998 pg/mL in females sampled during April to October, 2006 (Figure 5). There was distinct bimodality in E2 levels for three (April, August, and October) of the five sampling trips. In April, females with small ovaries had low levels of E2 (28–252 pg/mL; Figure 5) that were significantly lower (\(t = 307\), one-sided \(P < 0.001\)) than females with large ovaries (107–35,998 pg/mL). In August, there were two distinct groups; one group had low levels of E2 (0–32 pg/mL; Figure 5) that were significantly lower (\(t = 146\), one-sided \(P < 0.001\)) than the second group (205–2,313 pg/mL). In October, females with small ovaries had low levels of E2 (19–87 pg/mL; Figure 5) that were significantly lower (\(t = 307\), one-sided \(P < 0.001\)) than females with large ovaries (1,579–13,552 pg/mL). Sampling from June to August showed a broad distribution (14–1,168 pg/mL) of fairly low E2 levels with no distinct bimodality.

**Discussion**

In Alaska, sexual maturity rates of female coastal cutthroat trout, relative to fish length, is one of the principal biological considerations used to develop and evaluate sport fishing regulations (Gresswell and Harding 1997). Nonlethal methods to identify female coastal cutthroat trout that are preparing to spawn would be beneficial for sampling small populations or those with other conservation or management concerns.

Of the four methods we tested, body lipid content was the only one that was not useful for providing insight regarding the reproductive status of female coastal cutthroat trout. Although there was a significant difference in body lipid content between the two groups of female coastal cutthroat trout in April (but not at any other sampling times; Figure 3) there was some overlap in the ranges of the two groups (i.e., some females with small ovaries had lower fatmeter readings than females with large ovaries). Thus, it would be problematic to draw inference on the relative size of ovaries in female cutthroat trout, with 100% certainty, based on body lipid content in the months sampled. Body lipid levels were generally lower in April than in other months, which is to be expected because trout have not had the opportunity to rebuild energy stores that were depleted over winter. In April, females with larger ovaries tended to have even lower lipid levels than females with small ovaries (Figure 3), perhaps due to the high energetic burden imposed on females that are preparing to spawn.

With regard to methods that were effective (i.e., ultrasonography, plasma VTG, and plasma E2), there are several considerations relative to their application for determining reproductive status. Ultrasonography was tested only in the spring (April) because this was the only month the machine was available. However, subsequent to this study, one author (Bangs) tested the equipment in...
a preliminary fashion on fish sampled in October and found that females with large developing oocytes were identifiable. Therefore, it is likely that ultrasonography could also be useful in autumn (and possibly other months) to assess ovary size in female coastal cutthroat trout, but this would need to be confirmed by further testing. Continual technological improvements in ultrasound machines (notably in the resolution and overall quality of images) may increase the utility of the devices in maturity studies. Specialized training to ensure proper use and interpretation of images by field technicians may yield similar benefits.

Plasma VTG levels are high in spring because mature female coastal cutthroat trout, like other prespawning salmonids, incorporate large amounts of VTG into the oocytes that will be cleaved to form yolk (Scott and Sumpter 1983, Copeland et al. 1986). Dramatic increases in E2 levels precede corresponding increases in VTG levels, which can be explained by the fact that E2 stimulates the liver to synthesize VTG in salmonids (Campbell and Idler 1980, van Bohemen et al. 1982). The measurement of plasma VTG and E2 showed an ability to discriminate between female coastal cutthroat trout with large and small ovaries, respectively, at two different times of the year, early spring (April) and autumn (October). The data clearly show two groups of females in both months; females with large ovaries had high levels of VTG and E2, whereas females with small ovaries had low levels. However, one of the females with large ovaries that was sampled in April had a much lower level of E2 (107 pg/mL) and GSI (1.95) compared with the rest of the females with large ovaries. This fish exhibited spawning coloration but, upon dissection, we found very few ova (about 15), which is much lower than expected for a spawning coastal cutthroat trout (Foster 2003). Therefore, we suspect this female spawned shortly before capture and the remaining ova were residual. Despite the low level of E2, this fish had a relatively high level of VTG (8,308 μg/mL), which is not surprising because Nagler et al. (2012) found that female rainbow trout have high levels of VTG for several weeks after spawning.

The appearance of two cohorts in the autumn, based on plasma VTG and E2 levels, is apparent because females that are preparing to spawn the following spring have begun the process of vitellogenesis, whereas nonspawning females have not. This fits well with what is known about the development of ovaries in these two cohorts in autumn (see above; Foster 2003). Indeed, the E2 data from this study show two cohorts of females were observable as early as midsummer (August; Figure 5). The period between July and August may correspond with the time when some female coastal cutthroat trout make the physiological “decision” to halt
ovarian development and forgo spawning the following spring. For females that spawned previously, they would be considered “skip spawners,” which is a known life-history characteristic of cutthroat trout (Giger 1972, Tomasson 1978, Trotter 1989, Foster 2003). This would explain the large number of females with low levels in E2 in August (Figure 5). However, because there are no discernable differences in ovaries based on macroscopic examination or GSI (Foster 2003) in August, histological studies would be necessary to better understand ovarian development before drawing inferences on the reproductive status of females based on plasma E2 levels in August. Although plasma VTG or E2 measurements are capable of discriminating between females with small ovaries versus those with large ovaries in spring and autumn, these methods are the most technically challenging because they require blood sampling and subsequent analysis in a suitably equipped laboratory by trained personnel. Therefore several considerations, including technical expertise and laboratory capabilities, must be made before adopting any of these methods for evaluating the reproductive status of female coastal cutthroat trout.

**Supplemental Material**

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**Table S1.** Data from: Length, weight, sex, plasma vitellogenin, plasma estradiol-17β (E2), body lipids, fish weight, ovary weight, and gonadosomatic index of coastal cutthroat trout (*Oncorhynchus clarkii clarkii*) sampled at Florence Lake, Alaska, in 2006.

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