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[Note]

Validation of Endoscopy for Determination of Maturity in Small Salmonids and Sex of Mature Individuals

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Abstract.—Fish maturity status, sex ratio, and age and size at first maturity are important parameters in population assessments and life history studies. In most empirical studies of these variables, fish are sacrificed and dissected to obtain data. However, maturity status and the sex of mature individuals can be determined by inserting an endoscope through a small incision in the body cavity wall and viewing the gonads. The objective of this study was to evaluate endoscopy in a field setting for gonadal assessment of stream-resident forms of salmonids that mature at small sizes. Ninety-one brook trout *Salvelinus fontinalis* (60–210 mm fork length [FL]) were obtained via electrofishing and anesthetized. Maturity status and sex of mature individuals were determined with an endoscope. After recovery, individuals were euthanized with anesthetic and were dissected to validate the endoscopic classification. Endoscopy correctly determined the maturity status and sex of mature individuals for 96% of the brook trout; the highest accuracy was observed for the smallest (60–70 mm FL) and largest (>140 mm FL) individuals examined. In the misclassifications, visceral fat hampered visibility and was mistaken for mature testes. Immediate postprocedure mortality was 3.3% and was limited to individuals smaller than 70 mm FL. Endoscopy is a useful technique that can be used in field settings to assess maturity status and sex of mature fish with a low rate of immediate mortality.

To effectively conserve fish populations, it is critical to understand mechanisms that contribute to population dynamics and resilience to disturbance, such as life history diversity. Important parameters used in population assessments and life history studies include sex ratio, maturity status, and age and size at first maturity (e.g., Meerburg 1986). We wished to identify a technique to accurately obtain these data from small resident salmonids (50–250 mm fork length [FL]) that would allow the fish to be collected, examined, and released in the field with a low risk of mortality. External characteristics that indicate maturity or sex (e.g., kype, gametes produced by pressing gonads) are not consistently apparent in small salmonids, particularly before the latter stages of gonadal development (e.g., Martin et al. 1995). Without such reliable external characteristics, fish have been typically sacrificed to obtain these data (Fleming 1998; Kennedy et al. 2003; Meyer et al. 2003).

We evaluate endoscopy as an alternative approach. Endoscopy is used in medical and veterinary settings to view internal organs via insertion of the instrument into the body cavity through a small incision (e.g., Wildhaber et al. 2005) or through the urogenital pore (e.g., Ortenburger et al. 1996). The endoscope consists of a thin tube (in our case, 2.7 mm in diameter) containing fiber optics to transmit light from an external source for illumination of internal organs that are viewed through an eyepiece or video camera attached to the tube. As with any surgical procedure, there are risks to subject organisms, but they are generally very low with endoscopy (e.g., Froehlich et al. 1999). Otoscopes (designed for viewing the human ear) and endoscopes have been successfully used as a generally nonlethal method of determining the sex or maturity of fish in laboratory settings, including European catfish *Silurus glanis* (Fijan 1975), largemouth bass *Micropterus salmoides* (Driscoll 1969), rainbow trout *Oncorhynchus mykiss* (Steucke and Atherton 1965; Moccia et al. 1984), Arctic char *Salvelinus alpinus* (Ortenburger et al. 1996), and shovelnose sturgeon *Scaphirhynchus*

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platorynchus (Wildhaber et al. 2005). Endoscopy has also been used in the field to assess maturity status and sex of small (100–250-mm) stream-resident forms of bull trout *Salvelinus confluentus*, a species listed as threatened under the U.S. Endangered Species Act (P.J.H., unpublished data), and small (54–217-mm) rainbow trout (E.A.S., unpublished data).

A variety of techniques besides endoscopy are available to nonlethally determine fish maturity status and sex; however, some approaches are costly or are limited to laboratory settings. Ultrasound has been employed for these purposes (e.g., Evans et al. 2004; Bryan et al. 2005), but the relatively high cost of the equipment may limit its use. An endoscope and related equipment cost approximately US$4,000, whereas a portable ultrasound unit costs between $13,000 and $18,000. Blood plasma indicators can also be useful, but this technique is highly invasive (Webb et al. 2002) and cannot be used nonlethally in fish less than 150 mm (Strange 1996).

The primary purpose of this study was to validate the use of endoscopy to determine the maturity status of small salmonids and to determine the sex of mature individuals. We applied the technique to eastern brook trout *Salvelinus fontinalis* (approximately 60–200 mm FL). Brook trout are nonnative to the study area (Boise River system, Idaho) and consequently could be sacrificed to verify endoscopic classifications without raising conservation concerns. In this region, brook trout appear to spawn at small sizes, similar to that observed for other small, resident salmonids in the area (e.g., rainbow trout; A.E.R., unpublished data). Our intent was not to identify stages or gradations of maturity (e.g., Wildhaber et al. 2005) but instead to determine whether fish were ripening during late summer before the spawning season in the fall. Specific objectives were to (1) use endoscopy in the field to classify brook trout as immature or mature, (2) distinguish the sex of mature fish by endoscopy, (3) validate endoscopic classifications by dissection of the same fish, and (4) determine the extent of immediate (but not long-term) mortality associated with the procedure.

Methods

Brook trout were collected from Beaver Creek, a tributary to the Crooked River in the Boise River drainage of southwestern Idaho. Sampling with a backpack electrofisher (Smith-Root, Inc., Vancouver, Washington; Model LR-24 or 12B) with pulsed DC took place on August 5, 2005, before the autumn reproductive season. Voltage, pulse, and frequency were adjusted to maximize capture probability with minimal fish injury (settings range: voltage = 400–700 V, frequency = 30–50 Hz, pulse width = 2–8 ms). Once captured, fish were held in live wells containing ambient stream water outside the influence of the electrofishing unit.

The endoscope operator had prior experience using endoscopy on wild rainbow trout (A.E.R., unpublished data). However, 10 brook trout were initially used to reacquaint the operator with the procedure. After endoscopy, these individuals were humanely euthanized and dissected to reorient the operator with the location and appearance of the peritoneal organs, particularly the gonads, which vary in color and seasonal development among trout and char species. After this initial training, 91 individual brook trout were examined for the study.

Individual fish were removed from live wells and placed in a tub filled with stream water containing the minimum amount of tricaine methanesulfonate (MS-222) needed for a fish to lose equilibrium within 5 min. Each fish was then moved to a small, portable platform, placed on its side, and measured for FL to the nearest millimeter. During the endoscopy procedure, the operator practiced standard aseptic techniques while an assistant irrigated gills every 30–40 s by injecting water with anesthetic into the mouth by use of a 10-mL syringe. A small (3–5 mm) incision was made directly above the base of the pelvic fin on the left side of the fish for insertion of the endoscope into the body cavity. To improve visibility, 10% saline solution was injected into the peritoneum until excess solution began to seep out of the incision. The fish was then gently held by the operator while the gonads were viewed. The endoscope was a rigid Richard Wolf 25° Panoview Plus (Richard Wolf Medical Instruments Corp., Vernon Hills, Illinois) equipped with a fiber optic light source (Welch Allyn, Model Solarc LB-21) powered by a 7-Ah, 12-V battery.

Gonads were categorized as mature or immature; if mature, the sex of the individual was determined using categories similar to those described by Bonar et al. (1989) in a study validating the use of ultrasound for identifying sex and maturity of Pacific herring *Clupea pallasii*. Previous use of this technique and dissection indicated that identification of the sex of immature gonads in other small salmonids is difficult; therefore, we did not document the gender of immature individuals.

After the endoscope was removed from the fish, the fish was returned to the portable platform. The incision was closed with Nexaband surgical glue (usually a drop). If the incision appeared large or likely to open, the operator also used a nylon suture to close the wound. On average, the endoscopy procedure lasted 4 min. Fish identified as mature were then marked with

TABLE 1.—Maturity status of brook trout and sex of mature individuals captured from Beaver Creek, Idaho. The number of immature fish, mature females, and mature males in each size-class was determined endoscopically (number determined by dissection is given in parentheses).

Fork length (mm)	Total	Mortalities	Female	Male	Immature
60–70	43	3	0 (0)	0 (0)	43 (43)
71–120	12	0	0 (0)	4 (2)	8 (10)
121–140	19	0	1 (1)	12 (10)	6 (8)
141–209	17	0	7 (8)	8 (7)	2 (2)
All size-classes	91	3	8 (9)	24 (19)	59 (63)

TABLE 2.—Contingency table summarizing the accuracy of endoscopy (relative to dissection) for determining maturity status of brook trout and sex of mature individuals captured from Beaver Creek, Idaho.

	Endoscope		
Dissection	Immature	Mature females	Mature males
Immature	59	0	4
Mature females	0	8	1
Mature males	0	0	19

an upper or lower caudal fin clip to indicate sex and placed in a recovery bucket filled with water at ambient stream temperatures. After sufficient time had passed to assess initial recovery of individuals (fish swimming unaffectedly in holding bucket), they were euthanized with MS-222 and dissected to validate the endoscopic classifications through direct examination of the gonads (Crim and Glebe 1990).

Results and Discussion

We found endoscopy to be a highly accurate method for determining maturity status and sex of mature brook trout. Of the 91 fish examined using endoscopy and dissected for validation, 96% ($n = 87$) were correctly classified as immature or mature (Tables 1, 2). Similarly, the sex of 96% (27 of 28) of the mature adults was correctly classified. Mature females had pale yellow eggs (approximately 3–5 mm in diameter) in ovaries running the entire length of the body cavity. In mature males, long, highly vascularized, opaque, smooth, milky white testes were visible along the length of the body cavity. Immature individuals had small, thin, transparent or translucent gonads. Occasionally, small, grain-sized (~1 mm) developing oocytes were observed in immature individuals. However, we were able to distinguish these small oocytes from larger eggs of mature females. Through the endoscope, individual or multiple developing oocytes were easily viewed in their entirety and were semitransparent, whereas an egg was typically similar to or larger than the field of view and was more opaque.

Overall accuracy for both maturity status and sex classifications was highest for the smallest and largest size-classes (Table 1). Misidentification of fat stores as mature testes was the cause of endoscope error. Misclassification of immature fish as mature males accounted for error in maturity classifications, and one mature female was incorrectly classified as male (Table 2). This may be a common source of error for this procedure; the ability of the endoscope to distinguish between fat and testes was limited in endoscopic studies of shovelnose sturgeon (Wildhaber et al. 2005). Endoscopy could introduce bias in comparative maturity data between locations where fish differ in condition. In addition to fat stores, full stomachs occasionally impeded visibility of gonads; evacuation of stomach contents before the endoscopy could eliminate this source of error. Regardless, the accuracy evident in our results support other studies that have found endoscopy to be a useful technique for determining maturity status and sex of mature fish (Moccia et al. 1984; Ortenburger et al. 1996; Wildhaber et al. 2005); our results further indicate that use of endoscopy can be expanded to individuals that mature at relatively small sizes (80–100 mm).

In addition, risk of immediate mortality from the procedure appears to be low. Three of the 91 fish (3.3%) that underwent endoscopy died before euthanization for dissection (Table 1). These fish were 60–70 mm FL, the smallest size-class examined. In smaller individuals, it was more difficult to view the internal organs, and the gonads were much less apparent in immature fish. As a result, the procedure may have taken longer. In addition, in smaller fish, the incision was larger relative to body size. Although the specific cause of mortality was uncertain and could include factors not directly related to the endoscopy (e.g., electrofishing, reaction to anesthesia), smaller individuals may be at greater risk from this procedure than larger fish. The relationship between fish size and risk of mortality from the procedure may be an important consideration when using this technique on rare fishes. If the technique is primarily used to determine the sex of mature fish rather than timing of maturity, endoscopy can be limited to larger individuals, which are most likely to be mature. To avoid bias, this would require prior data collection regarding minimum size at maturity for males and females.

Although immediate mortality may be low, this study does not rule out the possibility of delayed complications from the procedure, such as reopening of the incision, infection, and injury to internal organs.

Incidental loss of epidermal mucus, increases in body temperature, drying of the skin, or a combination of these factors could contribute to eventual mortality in fish that undergo the procedure. Previous study indicates that this is unlikely. Moccia et al. (1984) noted that necropsy of fish maintained under controlled laboratory conditions revealed no evidence of internal damage from endoscopy, such as internal bruising or infection; without antibiotics, entry incisions healed 1 week after application of tissue adhesive. However, fish released into wild settings after endoscopy may be more susceptible to these and other sources of related mortality, such as subsequent predation. Further study is needed to evaluate the long-term lethal and sublethal effects of endoscopy in natural settings. However, studies of radio-tagging, a procedure that is more invasive than endoscopy, suggest that these problems are minimal. Radio tags in largemouth bass and dummy acoustic transmitters in juvenile Atlantic salmon *Salmo salar* had few long-term effects on fish in the wild (Cooke et al. 2003; Lacroix et al. 2004).

The endoscope was a useful tool for identifying maturity of small brook trout and the sex of mature fish with a low rate of immediate mortality. The equipment is compact, portable, and—with exception of the light source—water resistant. These characteristics make it practical for field applications, even in remote settings. However, endoscopy requires knowledge of fish anatomy and experience in observing gonads at various stages of development. This could potentially lead to operator-to-operator differences in error. Gonadal and gametic differences among species, such as size, color, and seasonal development, should also be considered. Before using this technique for scientific study, training and validation may minimize operator error as well as incidental injury or mortality to the fish. In addition, endoscopy can be useful for purposes other than observing sexual organs. The spleen, liver, visceral fat, and intestinal tract were also observed through the endoscope (see also Moccia et al. 1984).

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