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A noninvasive technique for determining sex of live adult North American sturgeons

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Synopsis

We have developed a noninvasive technique to determine the sex of adult North American sturgeon by examination of the external morphology of live individuals. We analyzed four sturgeon species taken by commercial and scientific harvest: white *Acipenser transmontanus*, green *A. medirostris*, Atlantic *A. oxyrinchus* and shortnose *A. brevirostrum*. Males have a urogenital opening in the shape of a letter **Y**, while the opening of females is in the shape of the letter **O**. We accurately sexed 26 of 36 sturgeon using this criterion. Accuracy was highest in live fish (82%), and significantly lower in dead fish (29%). Dead sturgeon usually have the rectum prolapsed so that the urogenital opening is protruded, and thus making the sexes indistinguishable.

Introduction

A knowledge of the sex of individual fish is useful for application to aquaculture or conservation management. However, the determination of the sex of individuals with minimal handling or disruption of their ongoing activities can be a challenge. Sexual dimorphism of adult fishes with specialized reproductive behavior is usually obvious, it includes differences in body shape, coloration, fin size or nuptial tubercles (e.g., Pitcher 1993, Godin 1997, Helfman et al. 1998). In some other species sexual dimorphism is less obvious and in yet other species there appears to be no visible external morphological differences between adult males and females. The latter species are, of course, the most challenging to assess.

Sturgeons spawn over rock and gravel substrate (Breder & Rosen 1966, Balon 1975). No complex courtship displays or aggression has been reported in sturgeons and researchers have concluded that there is no external sexual dimorphism in sturgeon morphology (Berg 1962, Scott & Crossman 1973, Doroshov et al. 1983). Vladykov (1931) mentioned that the paired fins of female sterlet, *Acipenser ruthenus* are slightly longer than on males. However, such a criterion requires the measurement and comparison of fin lengths of a large number of fish of each sex, and it would not be particularly useful to assess the sex of a given individual. Thus most authorities have concluded that there is no reliable method for determining the sex of adult sturgeons from external morphology.

Suggestions that concentrations of steroid hormones or other compounds circulating in the blood of sturgeons could be used to determine sex of individuals have not yet proved to be feasible, as they have in Atlantic salmon, *Salmo salar* (Idler et al. 1981). The procedure requires restraint and invasive blood sampling of fish, and then a sequence of sophisticated analytical procedures to measure specific chemicals (Doroshov et al. 1997). Attempts to use these procedures have been inconclusive and seem unlikely to provide unambiguous sex determination of individual fish (G. J. Van Der Kraak pers. comm.). However one of us, (M H) believed that the shape of the urogenital opening was a sexually dimorphic character in sturgeon, as it is for some other fishes (Guerrero 1982). Thus we undertook a detailed analysis of selected North American species to test this hypothesis.

Methods

We conducted our study of sexual dimorphism in sturgeon on watersheds where there are significant harvests of adults of four different species. We had to be able to confirm the sex of the individual sturgeon by internal examination of gonads and that is possible only if fish are killed and dissected after external examination or if they are held to produce mature ova and sperm. We chose the St John River, New Brunswick, Canada and the Columbia River, Oregon, U.S.A. as our study sites. The former site has the only commercial harvest for Atlantic sturgeon, *A. oxyrinchus* and sampling of shortnose sturgeon. There is a commercial harvest and scientific sampling for white, *A. transmontanus*, and green, *A. medirostris*, sturgeons on the Columbia River. All field observations and photography were carried out by one of us (PV).

The St John River is subject to an intense fishery for Atlantic sturgeon in July and August during the spawning migration from the Bay of Fundy to the mid-upper reaches of the river. Commercial fisherman on this river are in close collaboration with a local aquaculture company (Canadian Caviar) and sell mature adults to that organization as brood stock. Since all fish are potential candidates, they are kept alive in the water after capture by 33 cm mesh gill nets. This gave us the opportunity to examine several adult specimens. This would have been impossible anywhere else for this species, since stocks have collapsed and there is no commercial harvest or any scientific sampling. For example, the next most productive system for Atlantic sturgeon, the Hudson River produced only one adult female Atlantic sturgeon during an intense biological sampling survey during the 1998 spawning season (D. Peterson pers. comm.).

The Oregon Department of Fish and Wildlife conducts an annual stock assessment of white sturgeon on the Columbia River. While over 340 white sturgeon were captured, tagged and released in August 1999, the vast majority was sexually immature juveniles. We examined few adults that were captured in this assessment. In addition, a 12 h commercial harvest was allocated to zone 6 in the estuary of the Columbia River. These fish were all brought into the processing plants in Astoria and were measured and sexed by opening the body cavity.

A sample of 50 green sturgeon caught during the 12 h commercial sturgeon fishery on August 3, 1999 was purchased by the Oregon Department of Fish and Wildlife from the fish processing plant in Astoria. We examined these fish both externally and internally. Photography and direct measurements of large sturgeon under field or processing conditions are demanding. It is not easy to obtain good quality photographs of entire fish, or even parts of external morphology, with the uniform illumination and depth of focus we would prefer. These observations are more difficult when live fish are the subjects, since the welfare of both the fish and people handling them are of concern. Fortunately, we have had a good deal of prior experience with similar observations of many sturgeon species (Vecsei 1997, 1999), and we used that experience to design our observation procedures.

Fine grained film (Fujichrome 50 and 100 ASA) was used for photographs. All photos were taken with a Nikkor 24–120 aspherical zoom lens set at 120-mm focal length to minimize parallax distortion. The lighting of the subjects varied greatly and sometimes affected the clarity of detail. A Vivitar 280 variable output flash was used for some photographs, but it tended to bleach out detail so we used natural light whenever possible.

We projected slides of the pelvic regions of the fish onto a Linetech illustration board, then traced details using a Koh-I-Noor technical pen (Rapidograph 0.25 mm point) (Figure 1).

Total lengths of sturgeon were measured to the nearest cm (Scott & Crossman 1973). Sex of fish was judged by examination of the pelvic region. Our prediction was that the female urogenital opening would be larger and more circular, and the male opening would be smaller and more narrow. In most cases this external examination was carried out while the fish were still alive, but this was not possible in all cases. In particular, all the green sturgeon available to us were dead by the time we could examine them. Sex was confirmed by examination of gonads following dissection of fish

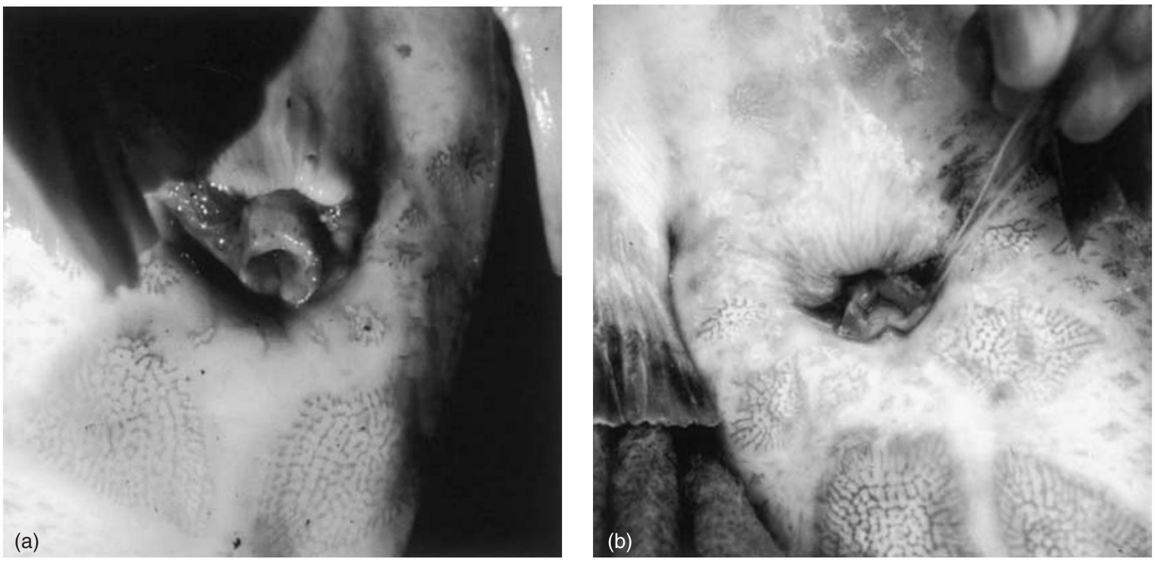

Figure 1. Photograph of (a) female and (b) male Atlantic sturgeon, *A. oxyrinchus*, urogenital region. Anterior is to the left of both photographs.

after they were killed by the commercial fisherman or in the case of shortnose sturgeon, after production of gametes. We analyzed data with a combination of chi-square and binomial tests, the former for sample sizes greater than 15, the latter for smaller sample sizes. We tested whether we were more likely to sex females or males correctly using external morphology, for sturgeon either alive or dead at the time of external examination. We performed these analyses for the four species, as well as for all species combined.

Results

As expected, all the fish we examined were adults and sexually mature. For white, green and Atlantic sturgeon, testes of all the males were enlarged, clearly visible upon dissection and uniformly pink or white in color. All females of those species had oocytes clearly developing in their ovaries. The state of maturity of the gonads and ovaries varied among individuals, but all were clearly identifiable. In all but green sturgeon, we were able to discriminate two classes of sturgeon, based upon external appearance of their urogenital openings (Figure 2). The urogenital openings of females were **O**-shaped, those of males were **Y**-shaped. The green sturgeon were exceptional in this regard. After failing to identify the sex of the first four individuals, we

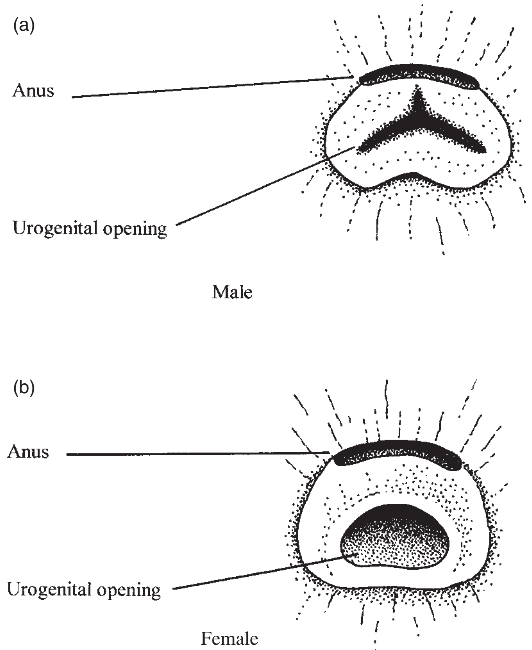

Figure 2. Schematic diagram of external urogenital region of sturgeon used for field identification of females and males by external examination. Anterior end is indicated by direction of arrow. (a) Urogenital region of female Atlantic sturgeon, *A. oxyrinchus*. (b) Urogenital region of male Atlantic sturgeon, *A. oxyrinchus*. Anterior is to the bottom.

Table 1. Sex of adult North American sturgeon as determined by external morphology and internal gonadal examination.

Species (length cm)	Status	N	Sex gonadal	External			Accuracy (%)
				M	F	U	
Shortnose (80–160)	Live	4	M	3	1	0	75
		3	F	1	2	0	67
		7	Combined				71
Atlantic (138–225)	Live	8	M	6	0	2	75
		9	F	0	8	1	89
		17	Combined				82
	Dead	2	M	0	2	0	0
		0	F	—	—	—	—
		2					63
	Combined						74
White (144–165)	Live	2	M	2	0	0	100
		2	F	0	2	0	100
		4	Combined				100
	Dead	2	M	1	1	0	50
		3	F	2	1	0	33
		5	Combined				40
	Combined						67
All species	Live	28	Combined				82
	Dead	7	Combined				29
		35	Combined				71

concluded that it was not possible to sex green sturgeon. All the first four individuals were males, but they did not have the characteristic **Y**-shaped urogenital opening. Examination of the external morphology of the remaining species confirmed our inability to distinguish the sexes in those fish. Both male and females had a very small **O**-shaped urogenital opening.

We have summarized our observations on sexual dimorphism of adults of the four sturgeon species in Table 1. Our success in determining the sex of fish from external morphology was 71% for all species combined, 71% for shortnose, 74% for Atlantic, 67% for white, and 0% for green sturgeon. If we categorize our results according to whether the fish were alive or dead at the time we examined external morphology, a clear pattern emerges. Our success rate for determining sex based on external morphology was 82% for fish examined alive but only 29% for fish examined after death (based on a species breakdown). This difference is highly significant ($p < 0.01$, binomial test). There was no significant difference in the sexing of male versus female sturgeons ($\chi^2_{(1)} = 0.11$, N.S.) for all species combined, or ($\chi^2_{(1)} = 0.01$, N.S.) for *A. oxyrinchus*. There was a significant difference in sexing live versus dead fish of all species combined ($\chi^2_{(1)} = 6.18$, $p < 0.05$). Results were significant for live white sturgeon ($p < 0.05$) but not significant for white sturgeon when both live and dead fish were incorporated into the formula, or when dead white sturgeon were looked at independently ($p > 0.05$, binomial test). The binomial test was insignificant for shortnose sturgeon ($p > 0.05$).

Recently one of us has examined approximately 20 live adult lake sturgeon, *A. fulvescens*, to test this technique (Paul Vecsei, unpubl. observ.). The gonads of each fish were viewed with a fiber optic device through a small incision in the body wall. The sex and maturity of each fish could be determined from direct examination of the gonads, but it was not possible to reliably identify sex from examination of the external urogenital openings.

Discussion

We have shown that it is possible to determine the sex of live adult sturgeon from external morphology. This is in contrast to earlier reports stating sturgeon are sexually monomorphic. Our success rate on live fish was about the same (80%) as that reported for skilled technicians assessing the sex of live tilapias from examination of the external genital papilla (Guerrero 1982).

Currently, biologists on the Columbia River, Oregon, are testing a surgical biopsy technique on white sturgeon to determine sex and maturity of the fish. To ensure fish health and survival, the incision used is less than 2-cm long. However, this procedure requires that fish be immobilized by anesthesia, and that a tube be inserted through the incision to remove a sample of gonadal tissue. Gonadal biopsies are intrusive, and potentially stressful to the fish and human operators. Even with anesthesia fish are subjected to considerable physical manipulation and exposure to air (Tufts et al. 1991). Also the gonads of nonreproductively active fish can be very small and difficult to examine *in situ.*

To date, there is no noninvasive field procedure that will allow sex determination of white sturgeon. Unpublished laboratory analyses of white sturgeon blood plasma have demonstrated that sex determination can be highly accurate depending on the source population and the individual's state of sexual maturity (G. Feist pers. comm.). However, laboratory analyses are relatively expensive and time consuming. Such analysis would typically be intended to describe gonad development stage, which can be used to describe the maturation cycle (Doroshov et al. 1997). Studies of white sturgeon mucus and urine chemistry also hold promise for a noninvasive laboratory (or even field) technique to determine sex and maturity (M. Webb pers. comm.), similar to procedures developed for Atlantic salmon (Idler et al. 1981). However, these procedures are still in development and all will require specialized equipment for analysis. Noninvasive sexing, such as examination by direct observation or photography of the urogenital region of live sturgeon is less disruptive, more immediate and much less expensive. This technique was proven 100% reliable in Gobies (Balon & Bruton 1994).

The confirmation of our noninvasive procedure has far-reaching potential for the management of the study species, and for sturgeon in general. The harvest of Atlantic sturgeon in the Saintt John River is primarily for their flesh. Most females caught are not in final stages of ovarian maturation. Thus they do not yield any quality caviar and so, are of no additional commercial value, compared to males. Males outnumber females in the spawning runs, as in most other sturgeon species, so there is no need or reason to harvest any females in this situation. There is typically a preponderance of males among spawning adults of sturgeons (Binkowski & Doroshov 1985, Holcik 1989, Birstein et al. 1997). This almost certainly is because males have an earlier age of initial sexual maturation than females, and males become sexually mature more frequently than females after their initial maturation (Beamish et al. 1996). The present quota for commercial harvest of sturgeon in the St John River is an annual total weight limit. In this situation the annual quota could be satisfied by harvesting more males. Females could be identified externally while still alive, and released to complete their spawning migration. Even more importantly, size quotas could be implemented on females, such as imposed on the Columbia River. This would ensure the release of small, first-time returning females, and perhaps larger females as well. The imposition of slot size restrictions on harvest of white sturgeon below the Bonneville Dam has lead to a dramatic recovery of their populations (Rieman & Beamesderfer 1990).

External identification of the sex of any sturgeons on the Columbia River would be important for both management and basic scientific studies. External identification of the sex of any sturgeon would be a significant advantage, whether for conservation, stocking, or other management procedures. Commercial aquaculture production of sturgeon depends significantly on whether fish are to be produced for caviar only, flesh only, or some combination of these two products (Logan et al. 1995). Similarly, the production of hatchery brood stock would be greatly enhanced if the sex of individual fish could be verified. The stocking of fish for restoration or rehabilitation would also be enhanced if the sex of individual fish were known.

Postmortem prolapsis of the rectum, with subsequent distortion of the adjacent urogenital openings, is common in large elasmobranchs (Clark & Castro 1995), and the coelacanth, *Latimeria chalumnae* (Balon 1991). This seems to occur in sturgeons, and thus limits the application of our external examination to living individuals. All green sturgeon were dead when we examined them externally, and we were not able to distinguish any external differences in their urogenital openings. This appears to be a result of a combination of factors: the fish were dead at the time of examination, their urogenital openings are small, and both females and males appear to have prolapsis of the rectum after death. Since all green sturgeon were dead before we examined them, this seems the most likely explanation for our lack of success in determining sex from external morphology in this species. The alternative explanation, that sex of green sturgeon can not be determined from external examination of live adult fish, is a possibility but seems less likely. The validation of this technique in other sturgeon species, and the application of the results to management and conservation of sturgeons should take the highest priority.

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