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# Validity of an external sex determination method in Atlantic Sturgeon (*Acipenser oxyrinchus oxyrinchus*)

**Running Title**: Determining sex from urogenital morphology in sturgeon

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**Summary**

The validity of the shape of the urogenital opening was tested as a predicable means to determine the sex of Atlantic sturgeon captured from in the Saco River estuary, Maine. Evaluation of 121 individuals ranging in size from 107 to 182 cm fork length were compared to non-lethal radioimmunoassay determined sex data previously examined for these individuals within this estuary. The results suggested that using the shape of the urogenital opening is not a reliable means to determine sex as only 51% of Atlantic sturgeon were correctly identified female. Additionally, there was no significant difference in correctness relative to 10 cm fork length size classes.

## Introduction

An important basic dataset to obtain for any fish population is the sex of individuals, as it can allow for an evaluation of the sex ratio, indicating temporal and spatial sexual segregation or aggregation (e.g. Taubert, 1980b; Buckley & Kynard, 1985b; Collins & Smith, 1997). However, many fish do not exhibit external sexual dimorphism throughout their life stages, making baseline reproductive data difficult to obtain in some species. The sturgeon family, *Acipenseridae*, is a world-wide threatened and endangered group (IUCN, 2010) that lacks external sexual dimorphism (Vecsei, Litvak, Noakes, Rien, & Hochleithner, 2003). Since sturgeon populations are critically low, large datasets on sex ratios cannot viably be obtained via lethal methods (i.e. gross dissection).

With many fish populations declining in both marine and freshwater environments, research methods that use nonlethal assessment allow for data to be collected in large sample sizes without hindering the population (Chiotti, Boase, Hondorp, & Briggs, 2016). In response, non-lethal methodology development and implementation in fish research and management is a rapidly advancing area (e.g. Vecsei et al., 2003; Bryan et al., 2007; Chiotti et al., 2016). Moreover, non-lethal methods can be used in tandem with some other close research areas (i.e.

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feeding ecology and spatial usage) to determine essential fish habitat (Novak, Carlson, Wheeler, Wippelhauser, & Sulikowski, 2017).

Many non-lethal methods have been assessed in sturgeon including: endoscopy for inspection and collection of gonadal tissue (e.g. Wildhaber et al., 2005), sex steroid hormone levels (e.g. Webb et al., 2002), ultrasonography (e.g. Moghim, Vajhi, Veshkini, & Masoudifard, 2002), morphological features and measurements (e.g. Maltsev & Merkulov, 2006; Podushka, 2008), and a physical attempt to secrete eggs or milt from an adult fish (e.g. Heise, Bringolf, Patterson, Cope, & Ross, 2009). These techniques have varying levels of invasiveness, accuracy, and cost. Endoscopy for gonadal tissue collection an invasive technique requiring anesthetization, an incision in the abdominal cavity, and the insertion of an endoscope (Wildhaber et al., 2005). This method requires means to recover the fish for a period, as well as a high level of skill from the examiners to not only perform the surgery but histologically analyze the sampled tissue. Sex steroid hormone analysis to determine sex is a non-lethal method, using circulating levels of hormone ratios to determine sex (Webb, Feist, Foster, Schreck, & Fitzpatrick, 2002). This method is minimally invasive and requires a low amount of skill to collect a sample, but a high degree of time and expense to process samples in a laboratory setting. Ultrasonography is also non-invasive; however it requires proper equipment and training to not only correctly locate the gonad, but determine sex from imaging. While this method has varying degrees of success, when done properly, the mature gonads of each sex give off key signatures in an ultrasound images (Moghim et al., 2002).

Other more easily examined external characteristics have also been assessed to sex various species of sturgeon. Methods such as urogenital region morphometry (Billard, 2002) and craniological measurements (Maltsev & Merkulov, 2006) have been performed with some success, however Chebanov and Galich (2011) warned that these methods are not recommended given uncertainty despite ease. Although Atlantic sturgeon are not considered externally sexually dimorphic, it was suggested (Vecsei et al., 2003) that the urogenital opening shape may serve as an indicator of sex, thus, providing a fast and nonlethal method to differentiate males from females in Atlantic sturgeon. Results by Vecsei et al. (2003) indicated that a “Y” shaped urogenital opening indicated a male (Figure 1a), and while an “O” shaped urogenital opening

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indicated a female (Figure 1b). Although an 82% accuracy was observed, only 17 fish were evaluated.

In Wheeler, Novak, Wipplehauser, and Sulikowski (2016), Atlantic sturgeon in the Saco River estuary (SRE) in Maine were sexed using a combination of circulating blood hormones techniques. Results showed 93% of the 288 fish sampled were sexed, demonstrating the validity of this non-lethal method in this endangered species (Wheeler et al., 2016). The goal of this study herein was to use the previously determined Atlantic sturgeon sex data from Wheeler et al. (2016) to assess the validity of the external morphological feature described in Vecsei et al. (2003) using a more robust sample size.

## Materials and Methods

### *Sampling*

This research was part of a larger study assessing Atlantic sturgeon in the Saco River estuary. The details of capture and sampling methods can be found in Wheeler et al. (2016). After routine sampling, the urogenital opening was inspected for shape and photographed. After the sampling procedure, sturgeon were allowed to recover in a net-pen prior to release.

### *Sex determination*

The sex of Atlantic sturgeon captured in the SRE was determined in vivo via a comparison of two calculations using circulating levels of sex steroid hormones. The full details of the analysis can be found in Wheeler et al. (2016). Briefly, testosterone (T) and 17β-estradiol ($E_2$) was quantified via radioimmunoassay (RIA) methods modified from Tsang and Callard (1987) and Sulikowski, Tsang, and Howell (2004). The ratios of these hormones in circulation were compared to mean hormone concentrations from Van Eenennaam et al. (1996) in addition to application in discriminant function analysis from Webb et al. (2002). Sex output from these two methods correlated in 93% of Atlantic sturgeon, showing the validity of this method as one non-lethal sex determining method (Wheeler et al., 2016). Therefore, we were able to directly compare morphological shape of the urogenital region herein to steroid hormone sex determination from previous work. Images obtained during sampling from urogenital photography were read by two readers in conjunction without prior knowledge of the specimen's

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sex as determined via RIA. Each reader assigned sex individually which was then discussed if determinations conflicted and a final sex status was given to each specimen. If an individual did not strictly adhere to either shape it was not included in the statistical data analysis. Here forward we use the term relative correctness to describe the relationship between urogenital morphology sex and Wheeler et al. (2016) RIA sex, as we do not have dissection or histological validation of the sex of any individuals in the SRE.

**Statistic*al Analysis***

A logistic regression with forward selection was performed to assess if relative correctness was influenced by 10 cm fork length size classes (ranging from 100-190 cm) as well as months sampled (May-November 2013 & 2014) (*SYSTAT (Systat Software, San Jose, CA)).*

**Results**

Atlantic sturgeon used within this study ranged from 106-182 cm fork length (n=140), with 121 individuals adhering to a Y or O shape. Overall, sex determination was correct to Wheeler *et al.*, (2016) sex data in 51% of fish, ranging from 0.0-76.9% correctness when assessed by 10 cm size classes (Figure 2). The resulting logistic regression was not significant (AIC=167.130; df=6; p=0.107), therefore we could not conclude any influence of 10 cm size classes or months on relative correctness (Figure 2 & 3).

**Discussion**

Our findings suggest assessment of urogenital shape in SRE sturgeon was highly variable. Some individuals did have a defined “Y” or “O” shape (Figure 1) and other lacked adherence to one shape or the other (Figure 4). However, this is in contrast to Vecsei et al. (2003), which found this method to be 82% accurate in live wild Atlantic sturgeon, when validated with gross dissection.

Other work has found urogenital shape as a good indicator of sex. For example, this technique correctly sexed 91% of males and 94% of females in mature Northern pike (*Esox lucius*) (Casselman 1974), with 3-4 month old young of the year being sexed correctly 72% of the time. Similarly, using a modified method applied to muskellunge (*Esox masquinongy*), sex

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was determined correctly in 92-98% of juveniles, and 100% in adults (Lebeau & Pageau, 1989). Finally, high accuracies of this technique applied in yellow perch (*Perca flavescens*) have also been reported ranging from 82.7-97.4% depending on total length (Malison, Held, & Kaatz, 2011). In some cases, discrepancies were reported due to reader skill levels (Guerrero, 1982).

Despite the aforementioned findings and based on the difficulties observed in the current study, the use of urogenital shape as an indicator of sex in Atlantic sturgeon appears complex. However, it is important to note the current study's limitations, where sex was not confirmed via dissection or histology, and maturity stages of our individuals were not known. In the future, this method needs further validation across maturity stages and seasons for the *Acipenseridae* family before application in the field.

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**Figure Legends**

Figure 1: Urogenital opening morphology of a male "Y" shape **(A)** and female "O" shape **(B)** in Atlantic sturgeon caught in the Saco River estuary. The posterior ends are represented by the arrows - as we have two specimens and two arrows.

Figure 2. The percent relative correctness of urogenital morphology sex to RIA sex data from Wheeler et al. (2016) over 10 cm fork length size classes. There was no statistical significance between any of the groups at $\alpha \leq 0.05$. N values are represented by numbers above each bar.

Figure 3. The percent relative correctness of urogenital morphology sex to RIA sex data from Wheeler et al. (2016) over months of sampling in 2013 and 2014 combined. There was no statistical significance between any of the groups at $\alpha \leq 0.05$. N values are represented by numbers above each bar.

Figure 4: Urogenital opening morphology of an Atlantic sturgeon with unidentifiable sex externally. Note the lack of adherence to a "Y" or "O" shape. The posterior end is represented by the arrow.

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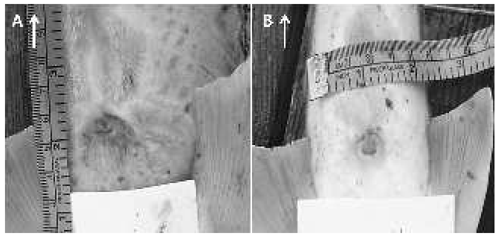


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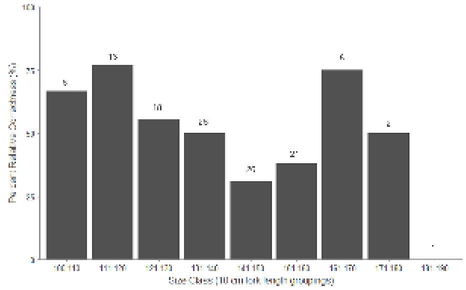


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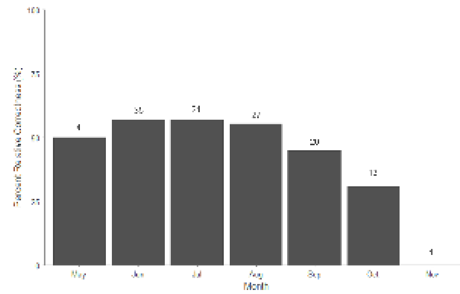


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