

Identification and Location of Testes in the Invasive *Channa argus* Cantor (Northern Snakehead)

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Abstract - *Channa argus* (Northern Snakehead) has been established in the Potomac River since at least 2004. Although ovaries of females have previously been discovered, to date, no testes had been confirmed in males from the Potomac River or any other North American waterbody. Dissection of individual Northern Snakeheads and subsequent histology confirmed discovery of testes in males taken from Quantico Creek, VA. Our discussion includes implications of this finding and methods for properly dissecting males to find testes.

Introduction. *Channa argus* Cantor (Northern Snakehead) was first discovered in the Potomac River watershed in 2004 (Odenkirk and Owens 2005). Since its discovery, Northern Snakehead has expanded throughout much of the watershed, covering over 193 river-km (Fuller et al. 2014). Moreover, the species has invaded several major waterbodies along the eastern US coast including tributaries of the Chesapeake Bay and Delaware Bay watersheds (Fuller et al. 2014). Due to the ability of Northern Snakehead to rapidly colonize new areas and to potentially impact ecosystems where it is found, researchers have been studying this species in its new environment. Some research has focused on habitat preferences (Lapointe et al. 2010, 2013), while other work has focused on interactions between Northern Snakehead and its potential prey and/or competitors (Love and Newhard 2012, Saylor et al. 2012). However, little research has been completed on the reproductive nature of Northern Snakehead, especially in regards to sexual differentiation (see Gascho Landis et al. 2011). In fact, one of the research priorities of the national control and management plan for members of the snakehead family is to determine methods for sexing snakeheads, including histology of testes (ANSTF 2014). Currently, it is known that the reproductive potential of Northern Snakehead is relatively high, with a single female generally producing about 40,000 eggs but capable of producing up to 100,000 eggs (USFWS 2014). Limited information is available about the ova of Northern Snakehead, and no testes had been described from Northern Snakehead in North American waters to date.

Observations. We captured 8 Northern Snakeheads via boat electrofishing from Quantico Creek, VA (Potomac River) on 5 August 2014. Following acceptable standard operating procedures, we placed all fish on ice upon capture for transport to the lab for stomach-content analysis and dissection. Historically, sex of mature fish was determined based on the presence or absence of egg-laden ovaries (J.J. Newhard, pers. observ.). If no ovaries were found, a fish was classified as a male even though no testes were positively identified. Upon examining the body cavity of a suspected male fish, we observed a structure that appeared to be testes (Fig. 1). The suspected testes were connected to the urogenital pore of the fish and were located toward the posterior portion of the body cavity (Fig. 1). There was no obvious milt expressed from the tissue, nor was the tissue white as testes are in many other fish species. We had to remove a significant amount of fatty material in order to properly observe the suspected testes. The accumulation of this fatty tissue may be one reason for previous confusion about testes location. We examined other Northern Snakeheads

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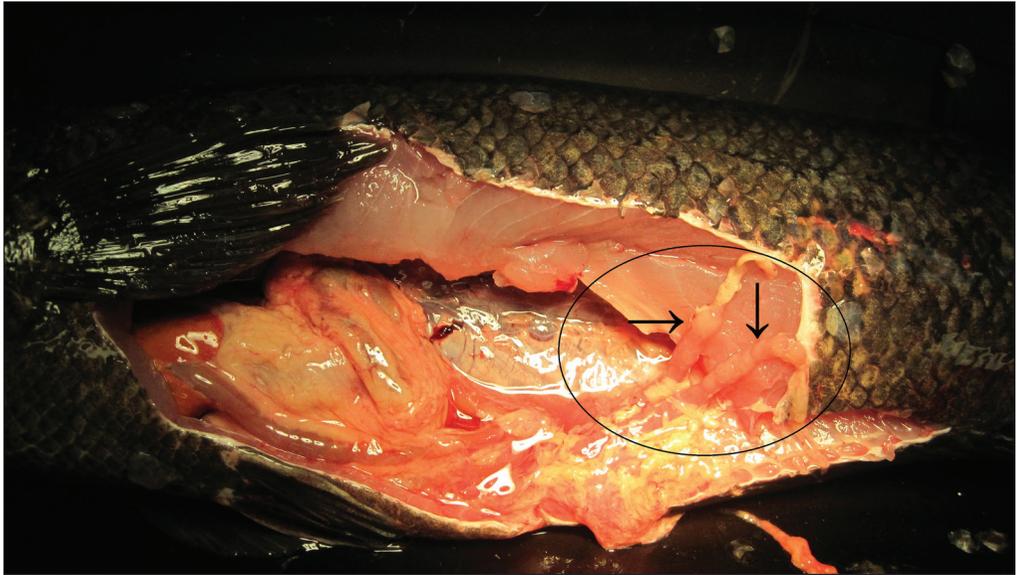


Figure 1. Dissected view of a male Northern Snakehead collected from Quantico Creek, VA. Note the location of the structure positively identified as testes in the black circle (indicated by arrows). Photograph by Joshua Newhard, US Fish and Wildlife Service.

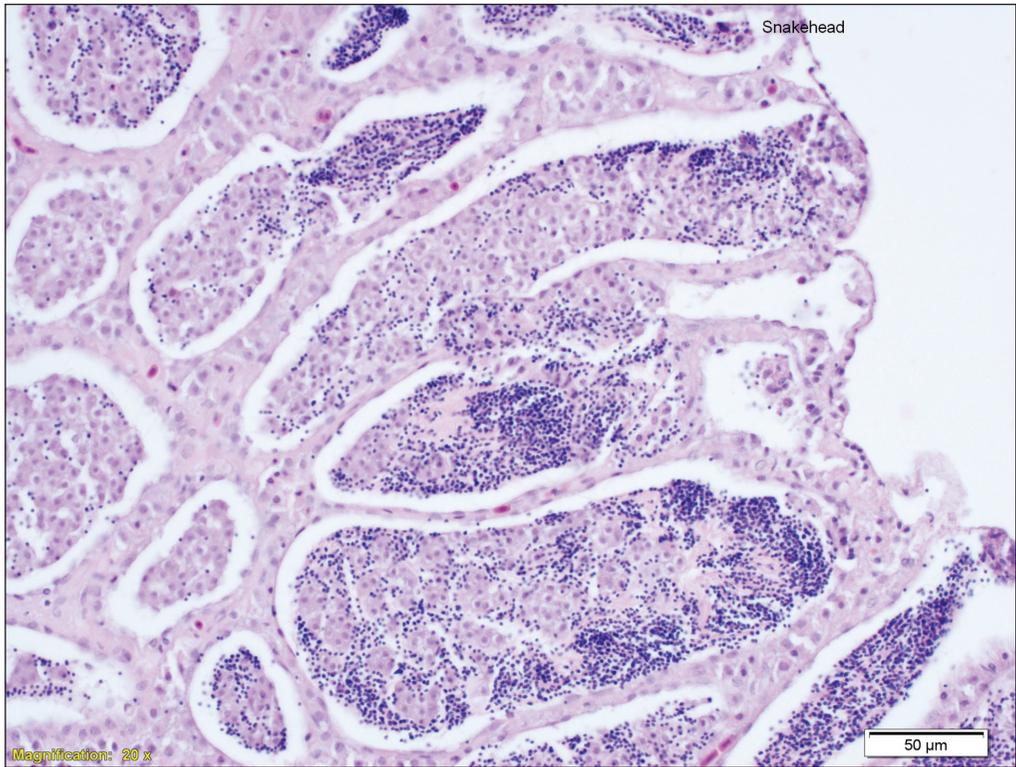


Figure 2. Stained gonadal tissue from testes of a male Northern Snakehead collected from Quantico Creek, VA. Darker dots are spermatozoa. Photograph by Joe Marcino, Maryland Department of Natural Resources.

collected at Quantico Creek for the presence of testes. In all, we found structures tentatively identified as testes in 5 fish ranging in size from 502 to 871 mm total length (TL). The remaining 3 fish were females or sexually immature (<150 mm TL).

In order to confirm that the observed structure was testes, we took tissue samples and preserved them in 10% formalin. We sent tissue samples from an individual of 591 mm TL to the Maryland Department of Natural Resources (MDDNR) Cooperative Oxford Laboratory (Oxford, MD) for histological analysis whereby subsamples of tissue were removed, mounted, stained, and viewed under a microscope. Upon examination, spermatozoa were observed in the gonad (Fig. 2), thus confirming the structure as testes.

Discussion. Our report is the first known positive identification of testes in Northern Snakehead from North America. While it previously was accepted that males and females existed in the Potomac River population, only the absence of ovaries was considered as identification of a male fish. Researchers can now positively identify male Northern Snakeheads. This information can aid in determining sex ratios of a population, improve population assessments, and facilitate investigations into potential sexually dimorphic behaviors or features. For example, body size may differ between males and females. To date, the largest Northern Snakehead collected by the US Fish and Wildlife Service (892 mm TL) was previously identified as a male (based on absence of ovaries), whereas the largest female collected was 782 mm TL (J.J. Newhard, unpubl. data). In addition, 1 fish from this study was positively identified as male (testes were observed) at 871 mm TL, suggesting that males may grow to larger sizes than females. Now that males can be confirmed, study of sexual differences of Northern Snakeheads can be properly assessed, thus meeting a priority research goal identified in the national snakehead management plan (ANSTF 2014).

The following protocol can be followed to find testes in a Northern Snakehead. The first cut should begin behind the anus and urogenital opening and extend posteriorly for 50 mm. Care should be taken not to cut away any structures near the urogenital opening. Then a vertical cut should be made toward the dorsum of the fish. The incision can then proceed toward the anterior portion of the fish toward the operculum. The final cut can come down ventrally and end near the base of the pectoral fin, creating a flap than can be removed if desired. If present, the testes will be attached to the urogenital opening and protrude posteriorly into the body cavity. There may be fatty tissue surrounding the testes that will need to be carefully removed for specimen examination.

Acknowledgments. I'd like to thank J. Marcino of MDDNR for his histological analysis of tissue samples. I thank 2 anonymous reviews whose comments greatly improved this note, and J. Love for his assistance in organizing tissue delivery. I am grateful to I. Park and K. Clowes for their help in collecting the Northern Snakeheads from Quantico Creek.

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