Received: 6 April 2020 | Accepted: 22 June 2020
DOI: 10.1111/jfb.14442

JOURNAL OF FISH BIOLOGY fsbi

BRIEF COMMUNICATION

First observations of intersex development in paddlefish *Polyodon spathula*

Jason D. Schooley[1] | Adam Geik[2] | Dennis L. Scarnecchia[3]

[1]Oklahoma Department of Wildlife Conservation, Jenks, Oklahoma
[2]Montana Fish, Wildlife & Parks, Great Falls, Montana
[3]University of Idaho, Moscow, Idaho

Correspondence
Jason D. Schooley, Oklahoma Department of Wildlife Conservation, P.O. Box 1201 Jenks, OK 74037.
Email: jason.schooley@odwc.ok.gov

Funding information
Oklahoma Department of Wildlife Conservation

Abstract

This study reports the first cases of intersex (abnormal development of both male and female reproductive tissues in a gonad) paddlefish *Polyodon spathula*, an Acipenseriform species of large rivers in the central U.S. and in aquaculture worldwide. Despite a large, multi-decadal data set in Oklahoma, Montana, and North Dakota, intersex development was not observed until 2019, when two individuals were harvested from the Grand Lake/Neosho River stock in Oklahoma. This suggests that intersex development in mid-water, zooplanktivorous paddlefish is rarer than in bottom-dwelling sturgeons for which intersex development is regularly observed. Although contaminants are implicated in causing intersex development in other Acipenseriformes, more investigation is needed.

KEYWORDS
contaminants, hermaphroditism, intersex, paddlefish, pollution, sturgeon

Intersex gonadal development (the presence of both male and female gonadal tissue) has been observed and reported for diverse taxa of fishes, including sturgeons (Acipenseridae: Feist *et al*., 2005). Intersex development has been characterized both as a naturally occurring phenomenon and as a pathological result of endocrine disruption associated with various contaminants (Bizarro *et al*., 2014; Jobling *et al*., 1998; Vajda *et al*., 2008; Vigano *et al*., 2001), though the literature often notes insufficient evidence to implicate direct causes of the condition in wild stocks (Bahamonde *et al*., 2013).

Among Acipenseriform fishes, gonochorism is typical, with intersex development "rare and unusual" (Van Eenennaam and Doroshov, 1998, p. 632). Several species of sturgeon have shown intersex development, both in Eurasia (sterlet, *Acipenser ruthenus*: Williot *et al*., 2005; Russian sturgeon, *Acipenser gueldenstaedtii*: Jackson *et al*., 2006) and in North America (Atlantic sturgeon, *Acipenser oxyrinchus*: Van Eenennaam and Doroshov, 1998; white sturgeon, *Acipenser transmontanus*: Feist *et al*., 2005; shovelnose sturgeon, *Scaphirhynchus platorynchus*: Columbo *et al*., 2007; June, 1977; Moos, 1978; Schwarz *et al*., 2006).

In the Missouri and Mississippi River drainages of the central U.S., observations of intersex development of shovelnose sturgeon are observed in low frequency within stocks but with some regularity across different wild stocks and localities. Hamel *et al*. (2015) identified rates of intersex occurrence of 1.6%–7% for wild shovelnose sturgeon. In their own field observations, they described intersex development as "female gametes imbedded within testicular tissue" (p. 601) in fish spatially restricted to one 6-km stretch of the Platte River, Nebraska, where species abundance was high. Harshbarger *et al*. (2000) found 29% of a small sample of shovelnose sturgeon in the Mississippi River to be intersex, suggesting locally high levels of organochlorine compounds as a likely cause. Similarly, Koch *et al*. (2006) associated intersex development in shovelnose sturgeon individuals caught from the middle Mississippi River with higher levels of organochlorine compounds.

As the last survivor of the family Polyodontidae, with the declaration of the Chinese paddlefish *Psephurus gladius* as extinct (Zhang *et al*., 2020), a comparison of the American paddlefish *Polyodon spathula* to its nearest sturgeon relatives within order Acipenseriformes is the next logical step (Grande and Bemis, 1991). In contrast to shovelnose sturgeon, intersex specimens have not been reported for paddlefish, despite their greatly overlapping geographical ranges, similar reproductive development and many similar life-history

attributes such as potadromy, slow maturation and long lifespan (Russell, 1986; Keenlyne, 1997; Scarnecchia *et al*., 2007, 2011; Tripp *et al*., 2009).

Sex and reproductive status, through measurements of gonad and gonadal fat (fat deposits adjacent to the gonads of female and male paddlefish) weights, is identified for each paddlefish as it is processed as part of the free fish-cleaning service provided by the Oklahoma Department of Wildlife Conservation's (ODWC) roe donation programme at the Paddlefish Research Center (PRC) in northeast Oklahoma. More than 12,000 male and 15,000 female paddlefish harvested by anglers from Grand Lake O′ the Cherokees (Grand Lake) and its headwaters during the period 2008–2019 have provided data. In addition, ODWC has examined smaller numbers of complete gonads of paddlefish from other Oklahoma reservoirs. In all, more than 28,000 paddlefish were examined over the 11-year period. Sampling this quantity of fish had resulted in the observation of several uncommon reproductive anomalies, including sterility, atresia, tumour-like growths and extreme asymmetry in size of the ovaries or testes. Nonetheless, no intersex paddlefish had been observed. The only comparable data set is from the Yellowstone-Sakakawea stock of Montana and North Dakota, where, since 1991, paddlefish gonads have been inspected and weighed exactly as for the Oklahoma PRC fish. Of more than 50,000 fish examined over that time, from fish often twice the age of Oklahoma fish (Scarnecchia *et al*., 2007, 2011), no intersex fish were observed (D. Scarnecchia, unpubl. data).

Two intersex paddlefish were observed in 2019, both of which displayed testis-ova. On 26 March, the first fish was harvested by an angler in upper Grand Lake and brought to the PRC. This fish (Fish 1862) was 108 cm in body length (front of eye to fork of caudal fin; Ruelle and Hudson, 1977) and weighed 19.2 kg. The fish resembled a male in body form, *i.e*., leaner and with a lower weight than a female fish of the same length. It contained one testis of typical appearance embedded in gonadal fat. Rupturing from the second testis, however, was a small ovary with undersized eggs of approximately 1.5 mm diameter (Figure 1a). Normal, mature roe of Grand Lake paddlefish typically range 2.0–2.2 mm in diameter (ODWC, unpubl. data); nonetheless, it is not known whether the undersized roe were immature. Roe colour was dark grey, the most common colour from the stock. Viability of the roe was not determined, and no samples were retained. The second testis appeared otherwise normal in density and vascularization, although possibly undersized. The ovary was excised

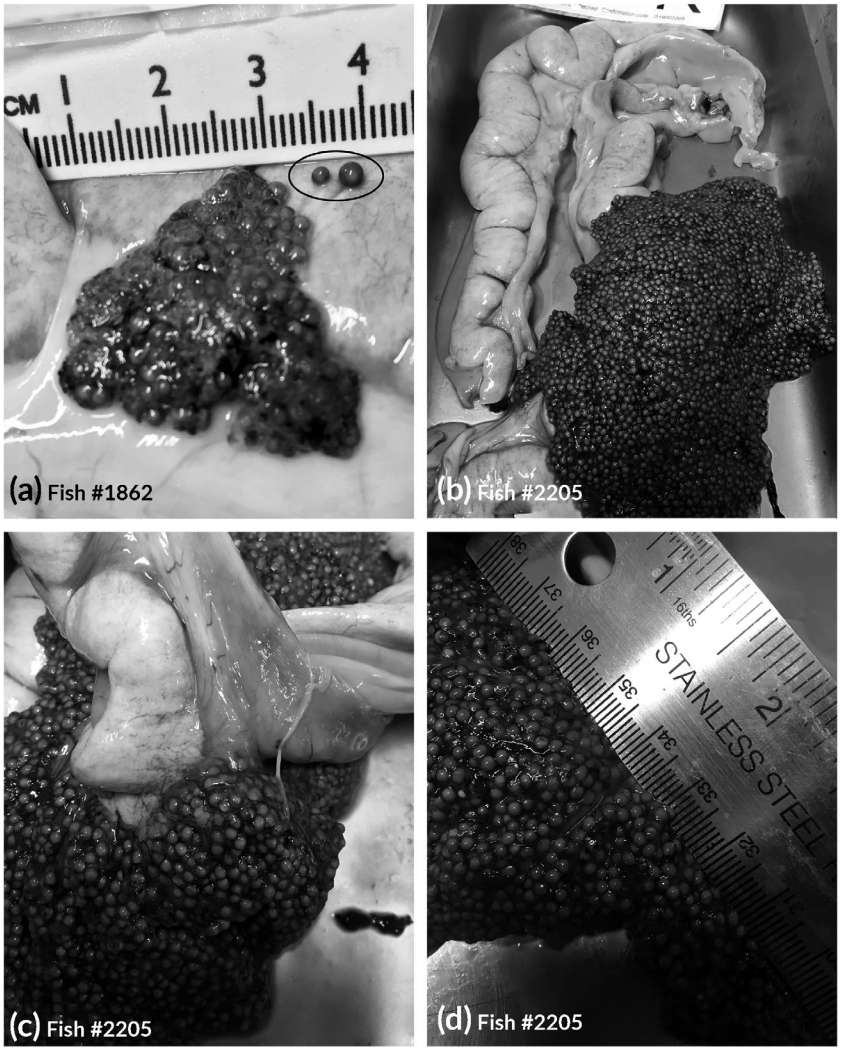

FIGURE 1 Intersex gonads of two paddlefish *Polyodon spathula* harvested from the Grand Lake, Oklahoma, USA, stock in 2019. Pictured are vascularized testicular tissues surrounded by gonadal fat deposits with a small ovary rupturing from one testis. Undersized ovum of intersex paddlefish 1862 (a) is compared to a typical-sized ovum from normal fish (indicated by the circle). The much larger ovary of paddlefish 2205 (b) with the testis-ova transitional area (c) and typical size of ova (d) are also illustrated

from the testis with surgical scissors prior to its manual separation from attached gonadal fat for independent weight determination (Table 1). Roe weight was unfortunately not recorded.

On 7 April, an angler harvested a second intersex paddlefish from the Neosho River (a headwater river of Grand Lake). This fish (Fish 2205) measured 99.2 cm in body length and weighed 13.2 kg. As with the first fish, the second fish contained one apparently normal testis. Nonetheless, the ovary and roe rupturing out of the second testis were more substantial in size than that of the first fish (Figure 1b,c). The second testis appeared otherwise normal in density, vascularization and size. Egg size (approximately 2.0 mm in diameter) and colour (dark grey) were typical for Grand Lake paddlefish (Figure 1d). Roe weight comprised 42% of total gonad weight (Table 1).

The dentary bone of each fish was collected, sectioned and the ages estimated (Adams, 1942) as described in Scarnecchia *et al*. (2006). Fish 1862 was estimated as 19 years (2000 cohort) and Fish 2205 estimated as 20 years (1999 cohort). As slight underestimation of ages sometimes occurs with paddlefish of this age (Scarnecchia *et al*., 2006), it is more likely that both fish were from the strong 1999 cohort, which dominated the harvest over the period 2008–2018 (Scarnecchia *et al*., 2011; Schooley *et al*., 2014).

Each fish appeared to have undersized ovaries rupturing from only one testis. Therefore, they were compared to normal males. The calculated male gonado-somatic index (GSI = gonad weight/live weight) of 0.009 for Fish 1862 was lower than expected for the estimated age (Table 1). In contrast, the GSI of 0.016 for Fish 2205 was higher than expected for the estimated age (Table 1). The intersex ovaries of both fish were diminutive in comparison to typical ovary size of normal females of age 19 or 20 in the Grand Lake stock (Table 1). Gonadal fat body weight (GFB = gonad fat weight/live weight) was 0.024 for Fish 1862 and 0.006 for Fish 2205; both were substantially lower than expected for their respective ages (Table 1) which may indicate that the intersex fish were investing resources to roe development instead of gonadal fat reserves – consistent with lower gonadal fat weights of typical mature females in the stock (Scarnecchia *et al*., 2011).

The exact cause of these two intersex fish from Oklahoma remains unknown. The rarity of the occurrence makes attributing any specific cause problematic; nonetheless, one possibility is contaminants. Grand Lake is formed by the combined headwaters of the Neosho, Spring and Elk Rivers (listed respectively by size from largest to smallest). Tar Creek, a tributary to the Neosho River, drains the area surrounding Picher, Oklahoma, a site of long-term zinc-lead mining from the late 19th to mid-20th centuries (Gibson, 1972; Stewart and Fields, 2016; Weidman, 1932). The area, now known as the Tar Creek Superfund Site (Beattie *et al*., 2017; EPA, 2019b), has been highly impacted by contaminants. The legacy has been documented contamination of air, soil (Beattie *et al*., 2017), surface water and groundwater, sediment, wells, and harm to terrestrial plants, fish (Oklahoma Department of Environmental Quality, 2008), other aquatic life, birds (Beyer *et al*., 2004), other wildlife and human health (Manders and Aber, 2014; Suggs, 1986). Heavy metals have been associated with endocrine disruption (*e.g*., Paschoalini *et al*., 2019) and intersex fish (Grieshaber *et al*., 2018) of other fish species elsewhere, although direct causal links are difficult to establish. Other contaminant problems have also occurred associated with Grand Lake in the preceding decades, including industrial chemical spills in the Spring River near Baxter Springs, Kansas (EPA, 2011). Water quality in the Elk River has been primarily impacted through increases in agricultural land use, industrial animal farming and the associated effluents (Smith *et al*., 2007). All three headwater rivers have been listed as impaired by the U.S. Environmental Protection Agency under the Clean Water Act section 303(d) due to a variety of causes, including pathogens, toxic compounds, pesticides, nutrients, metals (chromium, lead, thallium, zinc) and pH (EPA, 2019a). In several cases, paddlefish reproductive development has been shown to be susceptible to environmental factors such as water pollution or contamination with known endocrine disruptors, including dichlorodiphenyltrichloroethane (DDT) and polychlorinated biphenyls (PCBs) (Adolfi *et al*., 2019; An and Hu, 2006; Gundersen *et al*., 1998; Huang *et al*., 2003). If contaminants are the cause of these intersex fish, it will be worthwhile to continue monitoring the harvest for the presence of other intersex fish and any increase in the frequency of occurrence. Paddlefish in Grand Lake are known to utilize all three headwater rivers (Neosho, Spring and Elk Rivers) opportunistically, based on hydrology (for spawning migrations) and on forage availability (ODWC, unpubl. data). Therefore, retrospectively associating a source river of these and other intersex fish may only be possible through life-history reconstruction using dentary or otolith microchemistry (Whitledge *et al*., 2019).

This is the first documentation on intersex development in paddlefish. Its presence in only 2 of more than 78,000 paddlefish examined by Montana, North Dakota and Oklahoma suggests that it is unlikely to be found in the small samples of paddlefish typically inspected in most other studies. The rate of occurrence in paddlefish (<0.01%) is evidently considerably rarer than in shovelnose and

TABLE 1 Measured or mean values (with 95% C.I.) of reproductive characteristics, including gonado-somatic index (GSI) and gonadal fat bodies (GFB)

Fish	Roe weight (kg)	Testes weight (kg)	Fat weight (kg)	GSI	GFB
#1862 age 19		0.178	0.466	0.009	0.024
#2205 age 20	0.156	0.214	0.085	0.016	0.006
Females age 19–20 (*n* = 307)	5.868 (5.706–6.029)		0.123 (0.107–0.140)	0.237 (0.234–0.241)	0.005 (0.004–0.006)
Males age 19 (*n* = 254)		0.194 (0.176–0.212)	0.810 (0.729–0.890)	0.011 (0.010–0.012)	0.050 (0.044–0.056)
Males age 20 (*n* = 23)		0.261 (0.220–0.302)	1.554 (1.079–2.029)	0.012 (0.011–0.013)	0.071 (0.038–0.104)

other sturgeons, where intersex fish have been found in frequencies that are orders of magnitude greater than those for paddlefish (2.1%: Lake Oahe, South Dakota: June 1977; 2%: middle Mississippi River: Columbo *et al*., 2007; 7%: lower Missouri River: Wildhaber *et al*., 2005). It is possible that the difference is related to life-history differences between sturgeons and paddlefish. Unlike shovelnose sturgeon (and other extant Acipenseriform species), which are bottom-dwellers feeding suctorially (Hochleithner and Gessner, 2012; Miller, 2004; Weisel, 1979), the paddlefish has evolved (Grande and Bemis, 1991) into a zooplanktivore (Eddy and Simer, 1929; Rosen and Hales, 1981) with a morphology adapted for mid-water filter-feeding (Imms, 1904; Miller, 2004; Weisel, 1979). Gupta *et al*. (2009) found that sediments in the Ganges River accumulated heavy metals, particularly zinc and lead, and noted that sedimentary (*i.e*., bottom-dwelling and feeding) fish species exhibit higher levels of heavy metals than pelagic fishes. The role of sediments in endocrine disruption chemicals in fish in field situations is just beginning to be investigated (Müller *et al*., 2020).

The extreme rarity of intersex paddlefish observed within two actively studied and heavily sampled harvest fisheries may render these two observations of intersex paddlefish from Grand Lake of only minimal concern to overall wild stock status. Koch *et al*. (2006) reported that intersex development in shovelnose sturgeon was influenced by organochlorine compounds (from pesticides) before or during sexual differentiation. If intersex development is a result of environmental influences at paddlefish early life stages, this condition would likely be observed in more fish from Grand Lake than what have been seen to date – especially for the 1999 cohort. Alternatively, it may also be that only as the Grand Lake fish of the strong 1999-year class have gotten older (*i.e*., about 20 years) has the intersex condition manifested. If the intersex condition is an artefact of old age or senescence in paddlefish, when reproductive dysfunction may be more common (Reznick *et al*., 2002; Scarnecchia *et al*., 2007), the decreasing dominance of the 1999 cohort in the stock (from harvest and natural mortality) may create little cause for concern about overall stock status as the older cohorts are replaced by younger ones (Schooley *et al*., 2014). If intersex gonadal condition is, in part, an artefact of senescence, then this condition would be expected to increase in frequency within the 1999 cohort as it nears its maximum lifespan. In the Yellowstone-Sakakawea stock, the great age of some paddlefish in this northern stock (>50 years) relative to the Grand Lake stock (Scarnecchia *et al*., 2007, 2011) would seem to allow considerable time for bioaccumulation of contaminants. Yet no intersex fish have been found there. Despite the rarity of intersex fish, however, its occurrence may be only one, perhaps more obvious, response of fish to endocrine disruption. Other physiological effects (McMaster, 2001) or behavioural changes (Söffker and Tyler, 2012) affecting reproduction associated with endocrine disruption may be more difficult to detect with simple visual observations. Inasmuch as paddlefish and other Acipenseriforms are most commonly recruitment limited (Scarnecchia *et al*., 2019), any effects detrimental to successful reproduction and recruitment are worth monitoring.

The abundance of the Grand Lake paddlefish also reduces any potential effects of occasional intersex fish on stock status. In rare species or in aquaculture, where intersex individuals may be more abundant, self-fertilization may increase the frequency of intersex development and decrease the reproductive output of the stock (Hamel *et al*., 2015). Henne *et al*. (2006) found self-fertilization success rate (9%) to be higher than that of cross-fertilization (2%) for endangered, intersex shortnose sturgeon *A. brevirostrum*. Nonetheless, fry survival was lower for self-fertilized offspring (1%) than for cross-fertilized (11%), indicating a potential two-fold impact on wild recruitment for this rare species when intersex individuals are present. In rare stocks undergoing restoration, where genetic diversity is a concern to managers, a risk of self-fertilization may be notable if intersex fish were commonly observed. Nonetheless, paddlefish are generally abundant in Oklahoma.

Additional information on intersex fish and sex determination would benefit aquaculture activities. Based on the conservation value of sturgeons as imperilled with simultaneous commercial value as a source of caviar (Williamson, 2003), much ongoing research is related to artificial and environmental cues for sex determination in captive and wild stocks (Hassanzadeh *et al*., 2019; Keyvanshokooh and Gharaei, 2010; Muller *et al*., 2018; Webb and Doroshov, 2011). In paddlefish aquaculture, chromosomal manipulation to produce females (for caviar production) has been successful using external and chemical stimuli (Mims and Shelton, 1998; Shelton *et al*., 2019). Aquaculture of paddlefish in the U.S. is utilized for restoration (Schwinghamer *et al*., 2019) and commercial (Shelton *et al*., 2019) ends, whereas paddlefish aquaculture abroad in Europe (Jarić *et al*., 2019) is purely for commercial caviar and food production, and production in China additionally supplies the aquarium trade (Ji and Li, 2019).

More investigation and monitoring are needed to understand paddlefish intersex development and its causes in the wild. Additional observations of intersex paddlefish in Oklahoma or elsewhere will be required before additional hypotheses could be formulated. Early identification of intersex individuals by ODWC during the examination process may provide additional opportunities to collect blood samples (for hormonal assays), genetic samples (for sex chromosome analyses) or flesh and organ samples (for bioaccumulated contaminants).

ACKNOWLEDGEMENTS

The authors thank Oklahoma Department of Wildlife Conservation colleagues B. Brown, K. Cunningham, K. Kuklinski and A. Nealis for thoughtful feedback on the manuscript.

ETHICAL STATEMENT

The care and use of paddlefish complied with Oklahoma laws, guidelines and policies as approved by Oklahoma Department of Wildlife Conservation under the authority of state statutes.

ORCID

Jason D. Schooley https://orcid.org/0000-0003-1726-0602

REFERENCES

Adams, L. A. (1942). Age determination and rate of growth in *Polyodon spathula*, by means of the growth rings of the otoliths and dentary bone. *American Midlands Naturalist*, *28*, 617–630.

Adolfi, M. C., Nakajima, R. T., Nobrega, R. H., & Schartl, M. (2019). Intersex, hermaphroditism, and gonadal plasticity in vertebrates: evolution of the Mullerian duct and Amh/Amhr2 signaling. *Annual Review of Animal Biosciences*, *7*, 1–24.

An, W., & Hu, J. (2006). Effects of endocrine disrupting chemicals on China's rivers and coastal waters. *Frontiers in Ecology and the Environment*, *4*, 378–386.

Bahamonde, P. A., Munkittrick, K. M., & Martynuik, C. J. (2013). Intersex in teleost fish: are we distinguishing endocrine disruption from natural phenomena? *General and Comparative Endocrinology*, *192*(25–35), 25–35.

Beattie, R. E., Henke, W., Davis, C., Mottaleb, M. A., Campbell, J. H., & McAliley, L. R. (2017). Quantitative analysis of the extent of heavy metal contamination in soils near Picher, Oklahoma, within the Tar Creek superfund site. *Chemosphere*, *172*, 89–95.

Beyer, W. N., Dalgarn, J., Dudding, S., French, J. B., Mateo, R., Meisner, J., … Spann, J. (2004). Zinc and lead poisoning in wild birds in the tri-state mining district (Oklahoma, Kansas, and Missouri). *Archives of Environmental Contamination and Toxicology*, *48*, 108–117.

Bizarro, C., Ros, O., Vallejo, A., Prieto, A., Extebarria, N., Cajaraville, M. P., & Ortiz-Zarragoitia, M. (2014). Intersex condition and molecular markers of endocrine disruption in relation with burdens of emerging pollutants in thicklip grey mullets (*Chelon labrosus*) from Basque estuaries (south-East Bay of Biscay). *Marine Environmental Research*, *96*, 19–28.

Columbo, R. E., Garvey, J. E., & Wills, P. S. (2007). Gonadal development and sex-specific demographics of the shovelnose sturgeon in the middle Mississippi River. *Journal of Applied Ichthyology*, *21*, 420–427.

Eddy, S., & Simer, P. H. (1929). Notes on the food of paddlefish. *Transactions of the Illinois State Academy of Science*, *21*, 59–68.

EPA (2011). Orval Kent Food Company, Inc., to Pay $390,000 Civil Penalty for Industrial Wastewater Discharges at Baxter Springs, Kan. United States Environmental Protection Agency Newsroom. Available from: https://archive.epa.gov/epapages/newsroom_archive/newsreleases/34c76c6978b657c88525782900716667.html [November 14, 2019].

EPA. (2019a). Online Searchable Database of Clean Water Act Section 303(d) Listed Impaired Waters and their Causes of Impairment from All Years. United States Environmental Protection Agency. Available from: https://iaspub.epa.gov/apex/waters/f?p=131:9 [November 14, 2019].

EPA. (2019b). Tar Creek Superfund Site Strategic Plan: Cleanup Progress and Plans for the Future. United States Environmental Protection Agency, Region 6. Dallas, Texas. p 47. Available from: https://semspub.epa.gov/work/06/100017221.pdf [November 14, 2019]

Feist, G. W., Webb, M. A. H., Gundersen, D. T., Foster, E. P., Schreck, C. B., Maule, A. G., & Fitzpatrick, M. S. (2005). Evidence of detrimental effects of environmental contaminants on growth and reproductive physiology of white sturgeon in impounded areas of the Columbia River. *Environmental Health Perspectives*, *113*, 1675–1682.

Gibson, A. M. (1972). *Wilderness Bonanza: The Tri-State District of Missouri, Kansas and Oklahoma*. Norman, OK: University of Oklahoma Press.

Grande, L., & Bemis, W. E. (1991). Osteology and phylogenetic relationships of fossil and recent paddlefishes (Polyodontidae) with comments on the interrelationships of Acipenseriformes. *Journal of Vertebrate Paleontology*, *11*, 1–121.

Greishaber, C. A., Penland, T. N., Kwak, T. J., Pope, W. G., Heise, R. J., Law, J. M., … Kullman, S. W. (2018). Relation of contaminants to fish intersex in riverine sport fishes. *Science of the Total Environment*, *643*, 73–89.

Gundersen, D. T., Krahling, M. D., Donosky, J. J., Cable, R. G., & Mims, S. D. (1998). Polychlorinated biphenyls and chlordane in the gonads of paddlefish, *Polyodon spathula*, from the Ohio River. *Bulletin of Environmental Contamination and Toxicology*, *61*, 650–657.

Gupta, A., Rai, D. K., Pandey, R. S., & Sharma, B. (2009). Analysis of some heavy metals in the riverine water, sediments and fish from river Ganges at Allahabad. *Environmental Monitoring and Assessment*, *157*, 449–458.

Hamel, M. J., Rugg, M. L., Pegg, M. A., Patino, R., & Hammen, J. J. (2015). Reproductive traits of shovelnose sturgeon *Scaphirhynchus platorynchus* (Rafinesque, 1820) in the lower Platte River, Nebraska. *Journal of Applied Ichthyology*, *31*, 1–11.

Harshbarger, J. C., Coffey, M. J., & Young, M. Y. (2000). Intersexes in Mississippi River shovelnose sturgeon sampled below Saint Louis, Missouri, USA. *Marine Environmental Research*, *50*, 247–250.

Hassanzadeh, M. S., Noveiri, S. B., Pourkazemi, M., Yarmohammadi, M., Jalilpour, J., Fashkhami, M. N., & Falahatkar, B. (2019). Masculinization of the gynogenetic juvenile ship sturgeon (*Acipenser nudiventris* Lovetsky, 1828) using 17α-methyl testosterone. *Journal of Applied Ichthyology*, *35*, 1–7.

Henne, J. P., Ware, K. M., Wayman, W. R., Bakal, R. S., & Horvath, A. (2006). Synchronous hermaphroditism and self-fertilization in a captive Shortnose sturgeon. *Transactions of the American Fisheries Society*, *135*, 55–60.

Hochleithner, M., & Gessner, J. (2012). *The sturgeons and paddlefishes of the world: Biology and aquaculture*. Kitzbühel, Austria: AquaTech Publications.

Huang, Y., Twidwell, D. L., & Elrod, J. C. (2003). Occurrence and effects of endocrine disrupting Chemicals in the Environment. *Practice Periodical of Hazardous Toxic and Radioactive Waste Management*, *2003*, 241–252.

Imms, A. D. 1904. Notes on the gill rakers of the spoonbill sturgeon, *Polyodon spathula*. *Proceedings of the Zoological Society of London (part 2)*, 22–35

Jackson, K., Hurvitz, A., Yom Din, S., Goldberg, D., Pearlson, O., Degani, G., & Levavi-Sivan, B. (2006). Anatomical, hormonal and histological descriptions of captive Russian sturgeon (*Acipenser gueldenstaedtii*) with intersex gonads. *General and Comparative Endocrinology*, *148*, 359–367.

Jarić, I., Bronzi, P., Cvijanović, G., Lenhardt, M., Smederevac-Lalić, M., & Gessner, J. (2019). Paddlefish (*Polyodon spathula*) in Europe: An aquaculture species and a potential invader. *Journal of Applied Ichthyology*, *35*, 267–274.

Ji, H., & Li, Y. (2019). Distribution and aquaculture status of north American paddlefish in China. In J. D. Schooley & D. L. Scarnecchia (Eds.), *Paddlefish: Ecological, aquacultural, and regulatory challenges of managing a global resource* (pp. 229–237). Bethesda, Maryland: American Fisheries Society, Symposium 88.

Jobling, S., Nolan, M., Tyler, C. R., Brighty, G. C., & Sumner, J. P. (1998). Widespread sexual disruption in wild fish. *Environmental Science and Technology*, *32*, 2498–2506.

June, F. C. (1977). Reproductive patterns in seventeen species of warmwater fishes in a Missouri river reservoir. *Environmental Biology of Fishes*, *2*, 285–296.

Keenlyne, K. D. (1997). Life history and status of the shovelnose sturgeon, *Scaphirhynchus platorynchus*. *Environmental Biology of Fishes*, *48*, 291–298.

Keyvanshokooh, S., & Gharaei, A. (2010). A review of sex determination and searches for sex-specific markers in sturgeon. *Aquaculture Research*, *2010*, e1–e7.

Koch, B. T., Garvey, J. E., You, J., & Lydy, M. J. (2006). Elevated organochlorines in the brain-hypothalamic-pituitary complex of intersexual shovelnose sturgeon. *Environmental Toxicology and Chemistry*, *25*, 1689–1697.

Manders, G. C., & Aber, J. S. (2014). Tri-state mining district legacy in northeastern Oklahoma. *Emporia State Research Studies*, *49*(2), 29–51.

McMaster, M. E. (2001). A review of the evidence for endocrine disruption in Canadian aquatic ecosystems. *Water Quality Research Journal of Canada*, *36*, 215–231.

Miller, M. J. (2004). The feeding and functional morphology of feeding of north American sturgeons. In G. T. O. LeBreton, F. W. H. Beamish, & R. S. McKinley (Eds.), *Sturgeons and paddlefish of North America* (pp. 87–102). Dordrecht, The Netherlands: Kluwer Academic Publishers.

Mims, S. D., & Shelton, W. L. (1998). Monosex culture of paddlefish and shovelnose sturgeon In D.F. Williamson, G.W. Benz, and C.M. Hoover (Eds.), *Proceedings of the symposium on harvest, trade and conservation of North American paddlefish and sturgeon*. TRAFFIC North America/World Wildlife Fund, Washington, D.C, pp. 67–76.

Moos, R. E. (1978). *Movement and reproduction of shovelnose sturgeon, Scaphirhynchus platorynchus (Rafinesque), in the Missouri River South Dakota*. Doctoral thesis, University of South Dakota, Vermillion). Retrieved from: https://usd-primo.hosted.exlibrisgroup.com/permalink/f/183ctmt/TN_proquest302923339

Müller, A.-K., Markert, N., Leser, K., Kämpfer, D., Crawford, S., Schäffer, A., … Hollert, H. (2020). Assessing endocrine disruption in freshwater fish species from a "hotspot" for estrogenic activity in sediment. *Environmental Pollution*, *257*, 113636.

Muller, T., Ittzes, I., Szoke, Z., Hegyi, A., Meszaros, E., Lefler, K. K., … Kucska, B. (2018). Attempts on artificial induction of sexual maturation of Sterlet (*Acipenser ruthenus*) and identification of late spermatogenesis stage in hermaphroditic fish. *International Aquatic Research*, *10*, 293–297.

Oklahoma Department of Environmental Quality. (2008). Fish tissue metals analysis in the tri-state mining area Follow-up study FY 2006. Oklahoma City.

Paschaolini, A. L., Savassi, L. A., Arantes, F. P., Rizzo, E., & Bazzoli, N. (2019). Heavy metals accumulation and endocrine disruption in *Prochilodus argenteus*. *Ecotoxicology and Environmental Safety*, *169*, 539–550.

Reznick, D. N., Ghalambor, C. K., & Nunney, L. (2002). The evolution of senescence in fish. *Mechanisms of Ageing and Development*, *123*, 773–789.

Rosen, R. A., & Hales, D. C. (1981). Feeding of paddlefish. *Copeia*, *1981*, 441–455.

Ruelle, R., & Hudson, P. L. (1977). Paddlefish (*Polyodon spathula*): growth and food of young of the year and a suggested technique for measuring length. *Transactions of the American Fisheries Society*, *106*, 609–613.

Russell, T. R. (1986). Biology and life history of the Paddlefish—a review. In: J. G. Dillard, L. K. Graham, and T. R. Russell (Eds.), *The Paddlefish: status, propagation and Management* (pp. 2–20). American Fisheries Society, North Central Division, Special Publication 7, Bethesda, Maryland.

Scarnecchia, D. L., Ryckman, L. F., Lim, Y., Power, G. J., Schmitz, B. J., & Riggs, V. (2006). A long-term program for validation and verification of dentaries for age estimation in the Yellowstone–Sakakawea paddlefish stock. *Transactions of the American Fisheries Society*, *135*, 1086–1094.

Scarnecchia, D. L., Ryckman, L. F., Lim, Y., Power, G. J., Schmitz, B. J., & Firehammer, J. A. (2007). Life history and the costs of reproduction in northern Great Plains paddlefish (*Polyodon spathula*) as a potential framework for other Acipenseriform fishes. *Reviews in Fisheries Science*, *15*, 211–263.

Scarnecchia, D. L., Gordon, B. D., Schooley, J. D., Ryckman, L. F., Schmitz, B. J., Miller, S. E., & Lim, Y. (2011). Southern and northern Great Plains (United States) paddlefish stocks within frameworks of Acipenseriform life history and the metabolic theory of ecology. *Reviews in Fisheries Science*, *19*, 279–298.

Scarnecchia, D. L., Schooley, J. D., McAdam, S. O., Backes, K. M., Slominski, A., Lim, Y., & Fryda, D. D. (2019). Factors affecting recruitment of paddlefish: Hypotheses and comparisons with sturgeons. In J. D. Schooley & D. L. Scarnecchia (Eds.), *Paddlefish: Ecological, aquacultural, and regulatory challenges of managing a global resource* (pp. 103–126). Bethesda, Maryland: American Fisheries Society, Symposium 88.

Schooley, J. D., Scarnecchia, D. L., & Crews, A. (2014). Harvest management regulation options for Oklahoma's grand Lake stock of paddlefish. *Journal of the Southeast Association of Fish and Wildlife Agencies*, *1*, 89–97.

Schwarz, M. S., Lydick, C. D., Tillit, D. E., Papoulias, D. M., & Gross, T. S. (2006). A health risk evaluation for pallid sturgeon (*Scaphirhynchus albus*) in the lower Platte River using shovelnose sturgeon (*Scaphirhynchus platorynchus*) as a surrogate. Final Report, U.S. Fish and Wildlife Service, FFS 200260004, Grand Island, Nebraska. p. 105.

Schwinghamer, C. W., Phelps, Q. E., Tripp, S., Yasger, T., & Storts, N. (2019). The role of propagation in paddlefish restoration and conservation: A case study of Missouri. In J. D. Schooley & D. L. Scarnecchia (Eds.), *Paddlefish: Ecological, aquacultural, and regulatory challenges of managing a global resource* (pp. 211–228). Bethesda, Maryland: American Fisheries Society, Symposium 88.

Shelton, W. L., Mims, S. D., Semmens, K. J., & Cuevas-Uribe, R. (2019). Artificial propagation of paddlefish: An overview of developments. In J. D. Schooley & D. L. Scarnecchia (Eds.), *Paddlefish: Ecological, aquacultural, and regulatory challenges of managing a global resource* (pp. 191–210). Bethesda, Maryland: American Fisheries Society, Symposium 88.

Smith, B. J., Richards, J. M., & Schumacher, J. G. (2007). Water and streambed-sediment quality in the Upper Elk River Basin, Missouri and Arkansas, 2004–06: U.S. Geological Survey Scientific Investigations Report 2007–5181, p 53.

Söffker, M., & Tyler, C. R. (2012). Endocrine disrupting chemicals and sexual behaviors in fish – a critical review on effects and possible consequences. *Critical Reviews in Toxicology*, *42*, 653–668.

Stewart, T., & Fields, A. (2016). *Picher, Oklahoma. Catastrophe, memory and trauma*. Norman, OK: University of Oklahoma Press.

Suggs, G. G., Jr. (1986). *Union busting in the tri-state*. Norman, OK: University of Oklahoma Press.

Tripp, S. J., Phelps, Q. E., Colombo, R. E., Garvey, J. E., Burr, B. M., Herzog, D. P., & Hrabik, R. A. (2009). Maturation and reproduction of shovelnose sturgeon in the middle Mississippi River. *North American Journal of Fisheries Management*, *29*, 730–738.

Vajda, A. M., Barber, L. B., Gray, J. L., Lopez, E. M., Woodling, J. D., & Norris, D. O. (2008). Reproductive disruption of fish downstream from an estrogenic wastewater effluent. *Environmental Science and Technology*, *42*, 3407–3414.

Van Eenennaam, J. P., & Doroshov, S. I. (1998). Effects of age and size on gonadal development of Atlantic sturgeon. *Journal of Fish Biology*, *53*, 624–637.

Vigano, L., Arillo, A., Bottero, S., Massari, A., & Mandich, A. (2001). First observation of intersex cyprinids in the Po River (Italy). *Science of the Total Environment*, *269*, 189–194.

Webb, M. A. H., & Doroshov, S. I. (2011). Importance of environmental endocrinology in fisheries management and aquaculture of sturgeons. *General and Comparative Endocrinology*, *170*, 313–321.

Weidman, S. (1932). *The Miami-Picher zinc-lead district*. Norman: University of Oklahoma Press.

Weisel, G. F. (1979). Histology of the feeding and digestive organs of the shovelnose sturgeon, *Scaphirhynchus platorynchus*. *Copeia*, *1979*, 518–525.

Whitledge, G. W., Lance, R. F., Long, J. M., Neely, B. C., & Schooley, J. D. (2019). Applications of emerging fisheries techniques for paddlefish. In J. D. Schooley & D. L. Scarnecchia (Eds.), *Paddlefish: Ecological, aquacultural, and regulatory challenges of managing a global resource* (pp. 127–150). Bethesda, Maryland: American Fisheries Society, Symposium 88.

Wildhaber, M. L., Papoulias, D., Delonay, A. J., Tillit, D. E., Bryan, J. L., Annis, M. L., & Allert, J. A. (2005). Gender identification of shovelnose

sturgeon using ultrasonic and endoscopic imagery and the application of the method to the pallid sturgeon. *Journal of Fish Biology*, *67*, 114–132.

Williamson, D. F. (2003). *Caviar and conservation: Status, Managment and trade of north American sturgeon and paddlefish* (p. 240). Washington, DC: TRAFFIC North America World Wildlife Fund.

Williot, P., Brun, R., Rouault, T., Pelard, M., Mercier, D., & Ludwig, A. (2005). Artificial spawning in cultured sterlet sturgeon, *Acipenser ruthenus* L., with special emphasis on hermaphrodites. *Aquaculture*, *246*, 263–273.

Zhang, H., Jarić, I., Roberts, D. L., He, Y., Du, H., Wu, J., … Wei, Q. (2020). Extinction of one of the world's largest freshwater fishes: lessons for conserving the endangered Yangtze fauna. *Science of The Total Environment*, *710*, 136242.

How to cite this article: Schooley JD, Geik A, Scarnecchia DL. First observations of intersex development in paddlefish *Polyodon spathula*. *J Fish Biol*. 2020;97:919–925. https://doi.org/10.1111/jfb.14442