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# First report of the occurrence of a female Amur sturgeon *Acipenser schrenckii* in advanced stages of oogenesis, off the coast of Mombetsu, Hokkaido, Japan

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**Abstract**— A female Amur sturgeon *Acipenser schrenckii* was captured by a salmon set-net at the coast of Mombetsu, Hokkaido, Japan, on 2 November 2016. The sturgeon was 164 cm in total length, 156 cm in fork length and 24 kg in body weight. The GSI of the sturgeon was 15.8 and the mean diameter of the largest group of oocytes was 2.5 mm and these were at the midvitellogenic stage. This is the first report of a female Amur sturgeon in advanced stages of gametogenesis from coastal Japan.

**Key words:** *Acipenser schrenckii*, Acipenseriformes, oocyte development, reproduction, migration, bycatch, Hokkaido

## Introduction

The order Acipenseriformes, *i.e.*, sturgeons and paddlefishes, is known as an ancient group of actinopterygian fishes (for a detailed review of fish phylogenetic relationships, see Inoue et al. 2003). These fish have been tentatively classified as potamodromous, freshwater amphidromous or anadromous, based on their life history pattern (Bemis and Kynard 1997). All acipenseriform species were CITES-listed in 1997 because of widespread declines in abundance due to habitat degradation, overfishing and illegal trade in caviar (Pikitch et al. 2005, Doukakis et al. 2012, Ye and Valbo-Jørgensen 2012). Consequently, the accumulation and application of knowledge about sturgeon biology has become an important pursuit as it will aid in the conservation of stocks.

Amur sturgeon *Acipenser schrenkii* is a member of the Family Acipenseridae, which is found in the Amur River basin in China and Russia. The species can reach a maximum length of more than 3 m and weigh more than 190 kg, and the age of such fish exceeds 65 years (Krykhtin and Gorbach 1994). First spawning of female Amur sturgeon in the middle part of the Amur River occurs after reaching 105 cm TL and 6 kg BW (Ren 1981, Zhang 1985). Spawning takes place mostly during May and June in the main river channel of the Amur at 2–3 m depth (Zhang 1985, Krykhtin and Svirskii 1997, Wei et al. 1997), where spawners deposit dark-colored demersal eggs that average 3.27 mm in diameter, spanning a range between 3.15–3.37 mm (Liu et al. 2000). Koshelev et al. (2014) regarded this species as anadromous since extensive spawning runs from the estuary to the river were observed in autumn and spring, though Bemis and Kynard (1997) classified the species as potamodromous. Moreover, the fact that Omoto et al. (2004) and Azuma et al. (2017) reported a few individuals of Amur sturgeon from along the coast of Hokkaido, northern Japan, suggests the possibility of a long-distance oceanic migration in this species. Omoto et al. (2004) observed ovaries of a female Amur sturgeon captured at coastal Hokkaido and it contained only immature white-colored oocytes (as opposed to large, pigmented, dark-colored oocytes that represent advancing development) that measured 0.73 mm in diameter.

The migration ecology and reproductive physiology of Amur sturgeon remain unknown because ocean-migrating sturgeon have been caught only incidentally. In this study, we describe the body size, condition factor and developmental stage of ovaries of a female Amur sturgeon in advanced stages of oogenesis, captured by an inshore salmon set-net at the coast of Hokkaido, Japan.

## Materials and Methods

A sturgeon was captured on 2 November 2016 near Mombetsu, Hokkaido, Japan, by a set-net for chum salmon *Oncorhynchus keta* and pink salmon *Oncorhynchus gorbuscha* (Fig. 1). The latitude, longitude and depth of the capture site were recorded. The sturgeon was kept in a seawater pond at Mombetsu city for use as broodstock in future seed pro-

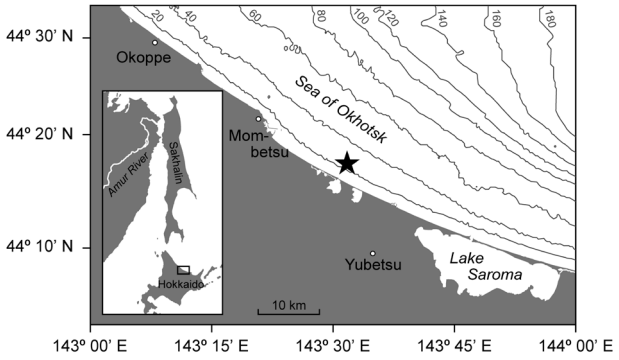


**Fig. 1.** Map of the collection area and its location within Hokkaido (inset). The solid star indicates the collection site where the Amur sturgeon *Acipenser schrenckii* was captured.

duction, but the fish died 10 days after transfer. The fish was immediately frozen *post obitum* and transferred to the Graduate School of Fisheries Sciences, Hokkaido University, Hakodate city. The total length (TL, cm) and fork length (FL, cm) were measured to the nearest cm and the body weight (BW, kg) was weighed to the nearest 0.1 kg. The gonads were removed and weighed to the nearest 0.1 kg (GW, kg). Morphometric data were used to calculate two indices, as follows: condition factor (K), $K=10^6\ BW\ FL^{-3}$; gonadosomatic index (GSI), $GSI=100\ GW\ BW^{-1}$.

Species identification using molecular genetic markers was carried out according to Azuma et al. (2017), because morphological characters were not distinctive enough to discriminate between pure Amur sturgeon and hybrids of kaluga *Huso dauricus* and Amur sturgeon, which have been found both in the Amur River (2–5% of larvae, Krykhtin and Svirskii 1997) and at the inshore Hokkaido coast (Omoto et al.

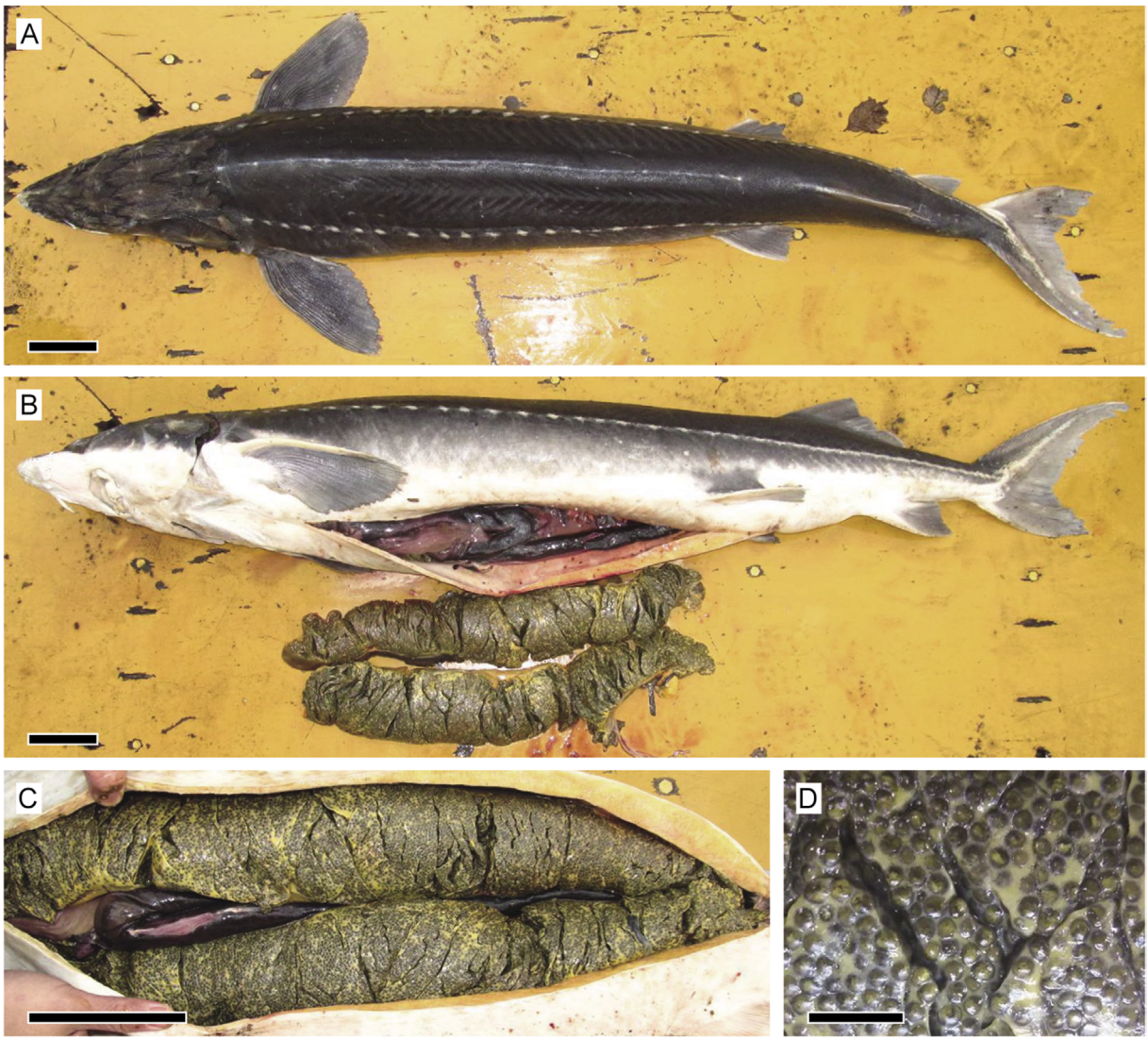


**Fig. 2.** Female Amur sturgeon *Acipenser schrenckii*, captured off the Mombetsu coast, Japan. A: Dorsal view of the sturgeon. B: Lateral view of the sturgeon after removal of ovaries. C: Ventral dissection of the sturgeon. D: Close-up of an ovary; ovarian follicles are readily visible to the naked eye. Scale bar indicates 10 cm for A, B, C, and 1 cm for D.

2004, Azuma et al. 2017). Two regions of mtDNA, *i.e.*, Cytb and Cox1, and a single region of nDNA, *i.e*, a partial sequence of RAG1, were amplified and direct-sequenced using Sanger chemistry. Sequences from mtDNA were compared to the data of wild and cultured Amur sturgeon, and SNPs in RAG1 were identified using the electropherogram. A short tandem repeat (STR) locus, *An_20*, was amplified by PCR, and the allele size was measured in the manner described by Azuma et al. (2017).

The sex was scored as female by visual inspection of gonad morphology. Two pieces of ovarian tissue were sampled for isolation of ovarian follicles and subsequent photography using a digital camera DFC290 (Leica) under a stereomicroscope MZ16 (Leica). The mean diameter of the largest group of oocytes (OD) was calculated by averaging the diameters of 16 randomly selected dark-colored oocytes measured by Image J (Schneider et al. 2012). Another piece of ovarian tissue was fixed in Bouin solution for 2 days and then transferred to 70% ethanol, dehydrated in an ascending series of graded ethanol concentrations, and embedded in paraffin. Sections of 6 $\mu$m thickness were prepared and stained with hematoxylin and eosin. Sections were observed using an optical microscope ECLIPSE 80i (Nikon) and photographed using a digital camera DXM1200F (Nikon).

## Results and Discussion

The mtDNA sequence was identical to that of an Amur sturgeon caught in Shibetsu in 2013 (Azuma et al. 2017). Furthermore, the specimen only had a cytosine in position 62 of the RAG1 marker, and the allele sizes of the *An_20* marker were 125, 137 and 149 bp. These characters provide strong support for the idea that the dissected specimen was a pure Amur sturgeon (see Table 3 in Azuma et al. 2017).

The latitude, longitude and depth of the capture site were 44°17′08″N, 143°31′58″E and 23 m (Fig. 1). The capture location was approximately 1000 km south-southeast of the estuary of the spawning river of the species, the Amur River.

The Amur sturgeon showed none of the morphological abnormalities frequently found in aquacultured individuals (*e.g.*, abnormalities in head shape, curvature of body and tail, absence of a septum between the nostrils, abnormalities of the olfactory rosettes, underdevelopment of eyes, underdevelopment of pectoral fins and/or pelvic fins, shortened opercula; Chebanov and Galich 2013) (Fig. 2A, B). The fish was 164 cm TL, 156 cm FL and weighed 24 kg, which seems to be substantially bigger than the biological minimum size for spawning, known to occur beyond 105 cm TL and 6 kg BW in the Amur River (Ren 1981, Zhang 1985). Indeed, the body size of the fish was within the size range of Amur sturgeon in spawning condition in the Amur River, surpassing the mean size of such spawners (95–207, 139.7±0.8 cm FL; Koshelev et al. 2014). The condition factor of the sturgeon was 6.3 and the GSI was 15.8. The sturgeon had gonads that contained dark-colored, rather well-developed oocytes (Fig. 2B, C, D), which measured ~2.5 mm in diameter. The oocytes were clearly more developed than the immature white-colored oocytes (0.73 mm in diameter) of previously captured Amur sturgeon from the coast of Hokkaido (Omoto et al. 2004), although somewhat smaller than fully developed and ovulated eggs (mean diameter 3.27 mm, range 3.15–3.37 mm, Liu et al. 2000). The largest group of oocytes was identified as being in the midvitellogenic stage by histological observation, though the oocytes were badly damaged as a result of needing to go through a freeze-thaw cycle (Fig. 3). Based on our experience of artificial reproduction, Amur sturgeon having midvitellogenic oocytes is considered likely to spawn within one or two years. As far as we know, Amur sturgeon having such well-developed oocytes have never been captured in Japan, and the present report thus is the first to document on the occurrence of such a specimen at inshore coastal Japan.

It is unknown whether these far-dispersed sturgeon will return and migrate upstream into the Amur River to spawn, or die without homing and reproducing. Additionally, there is the possibility that such fish make their upstream journey into a river other than the Amur to spawn. The current description of the occurrence and reproductive characteristics of the sturgeon may help to understand the migration and reproduction of acipenserid species. Moreover, Amur sturgeon, a cold water-adapted species, is considered a new aquaculture fish in northern Japan; hence, the description of the oo-

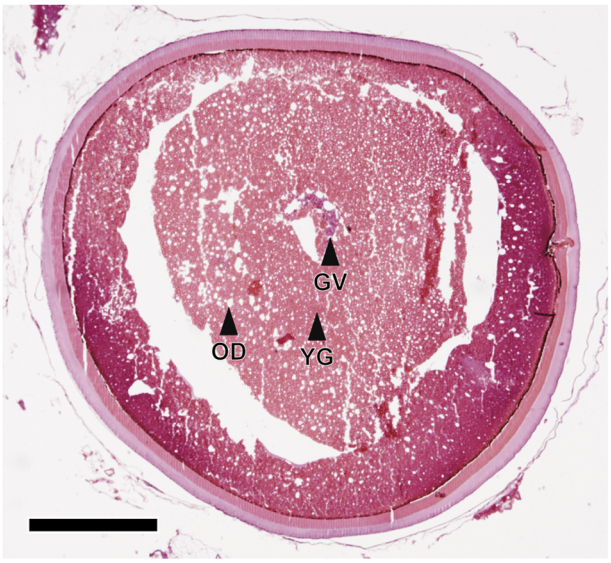


**Fig. 3.** Light micrograph of ovarian tissue from female Amur sturgeon *Acipenser schrenckii*, illustrating an oocyte of the largest group in the midvitellogenic stage. The oocyte was badly damaged because of a freeze-thaw episode. GV: germinal vesicle; OD: oil droplet; YG: yolk globule. Scale bar indicates 500 $\mu$m.

cyte developmental state of Amur sturgeon captured at the coast of Hokkaido can be applied to contribute to sturgeon broodstock management and to aquaculture development in Japan.

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