



Notes on reproductive ecology of *Pseudaspius nakamurai* in the Agano River

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Received: 25 June 2024 / Revised: 22 July 2024 / Accepted: 22 July 2024
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The cyprinid fish *Pseudaspius nakamurai* (Doi and Shinzawa 2000; Sakai et al. 2020) is endemic to Japan and inhabits several large rivers that flow into the Sea of Japan (Honma 1991; Sakai et al. 1991). Owing to the difficulty of conducting field studies in large rivers, their life history has been reported in fragments and remains largely unknown. This species is known as a piscivorous (Honma and Matsumoto 2001; Matsumoto et al. 2004) and can grow up to a total length ≥ 80 cm (Honma 1991). Their distribution ranges from estuaries and lagoons to the upper reaches of large rivers (Onodera and Honma 1976; Honma and Matsumoto 2001). Although the genus *Pseudaspius* is exceptionally known as anadromous within the Cyprinidae family, previous studies have suggested the loss of sea-migration traits in *P. nakamurai* based on the analysis of the otolith Sr/Ca ratios (Sakai and Imai 2005; Imai et al. 2008). In the late spring of 2024, we observed the spawning of *P. nakamurai* in a tributary of the Agano River system, Niigata Prefecture. Here, we describe the spawning habitat and the morphology of eggs and juveniles of this species.

On 29 April 2024, we found a spawning aggregation of *P. nakamurai* in a tributary of the Agano River system (the detailed location was withheld for habitat protection). The spawning aggregation was observed during the time in a day from 1745 to 1830, and was partially recorded using an underwater camera (Olympus Tough TG-6, Japan). After observation, 40 eggs attached to the riverbed were collected and transferred to the laboratory. On 6 May, to confirm

whether spawning was ongoing, we searched for the spawning aggregation of *P. nakamurai* while walking along the riverbank and measured environmental factors (depth, water velocity, substrate, and water temperature) at five points along a crossline in the spawning habitat. Water velocity and water temperature were measured using an electromagnetic velocity meter (LP-30, KENEK Corporation, Japan) and a digital thermometer (DT-15N, GEX, Japan), respectively. The collected eggs were kept in an aerated aquarium (11 × 21 cm with a height of 13 cm), and half of the water was changed daily. The photoperiod and water temperature were maintained under natural conditions (approximately 14L:10D, 16–20 °C). After eight days of hatching, fish were fed fish pellets (Hikari Baby & Baby, Kyorin, Japan) and frozen baby brine shrimp (Vitacrine Baby Brine, Kyorin, Japan). The long diameters of the eggs were measured using the ImageJ software (Rasband 1997–2018), with photographs of 26 eggs randomly selected for measurement.

During the April observation, an aggregation consisting of approximately 15 *P. nakamurai* with nuptial coloration was found (ca. 1 × 1 m) where a shallow branched flow entered the mainstream. Several individuals sometimes moved to a shallow place near the outlet of the shallow branched flow into the main stream (Fig. 1a, b) and displayed spawning-like behavior, causing water to splash. Numerous eggs were found attached to the riverbed (Fig. 2a). The spawning site was characterized by slower water velocity (mean \pm SD = 0.008 \pm 0.006 m/s) and shallower depth (17 \pm 7.6 cm) compared visually to the centerline of the mainstream. The dominant substrate was pebbles (4–64 mm, slightly modified from Bain et al. 1985) and no silt accumulation on the riverbed was observed. The water temperature of the spawning habitat was 16.4°C.

The size of the collected eggs and yolk sacs was mean 3.42 \pm 0.42 mm (range, 2.40–4.07; $n = 26$) and 2.25 \pm 0.44 mm (1.49–3.05; $n = 26$), respectively. The egg size in this study was slightly larger than that reported for eggs obtained by artificial fertilization in *P. nakamurai* from the

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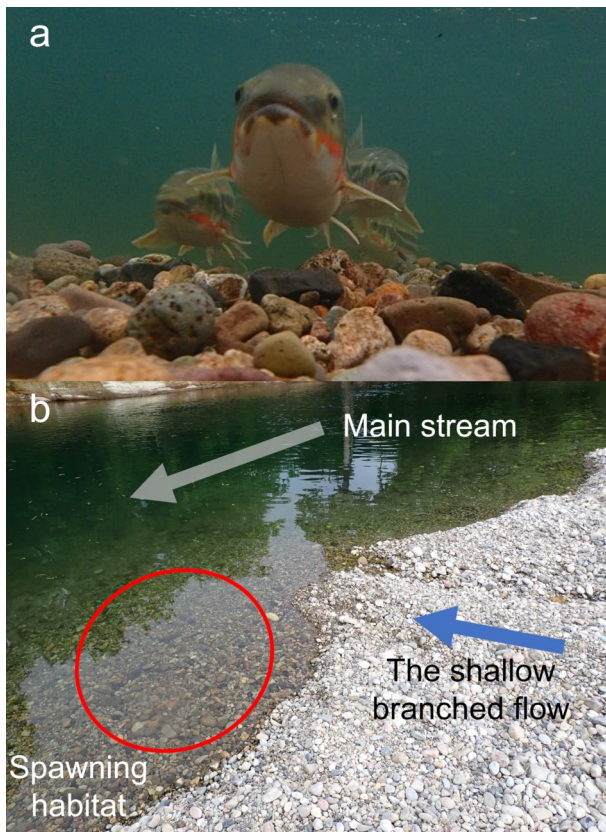


Fig. 1 Photographs of the spawning ecology of *Pseudaspius nakamurai*. **a** Spawning aggregates with nuptial coloration. **b** The spawning habitat in this study after the shallow branched flow disappeared, probably due to a decrease in snowmelt water

Mogami River system (mean 2.36 mm; Katsura et al. 1995) and congeneric species (*Pseudaspius hakonensis*, mean 2.89 mm; *Pseudaspius brandtii*, 2.40 mm; *Pseudaspius ezoe*, 2.10 mm; Sakai 1995). The collected eggs hatched 3–4 days after spawning. Juveniles were characterized by the projection of the lower jaw, which is the main diagnostic characteristic of *P. nakamurai* (Honto et al. 1998; Fig. 2b). This provides strong evidence that *P. nakamurai* spawned at the observed location.

Knowledge about the spawning habitat of *P. nakamurai* is limited and vague. Nakamura (1969) reported that *P. nakamurai* spawned in shallow areas of the Tadami River, located in the upper reaches of the Agano River system. It is also known that this species uses the same spawning grounds as *P. hakonensis* (Honma 1991; Katsura 2005). Importantly, microhabitats within the same spawning grounds seem to differ between *P. nakamurai* and *P. hakonensis* without rigorous examination (Katsura 2005). Specifically, *P. hakonensis* spawns in riffles at the end of pools, whereas *P. nakamurai* seems to spawn in drop-offs from riffles to pools (Katsura 2005). These results in *P. nakamurai* are similar to those for the spawning habitat in this study. One week

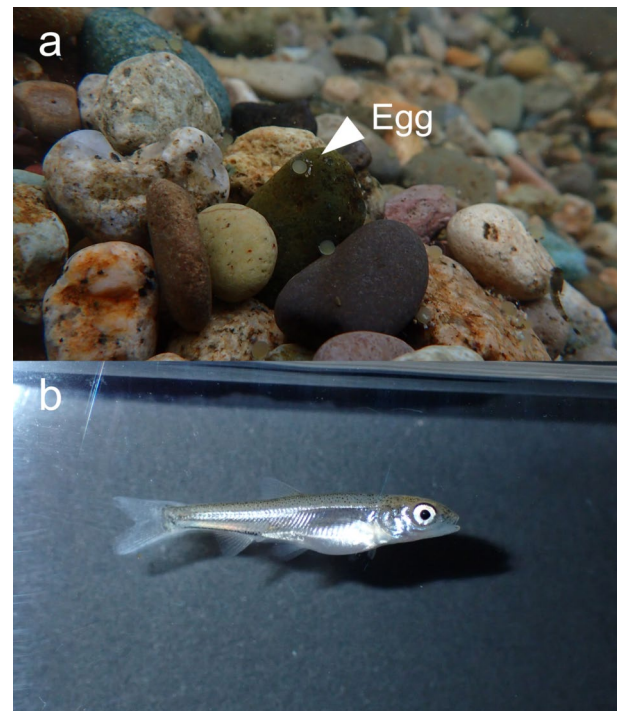


Fig. 2 **a** Collected eggs and **b** juveniles after 43 days of hatching. The juveniles were characterized by the projection of the lower jaw, which is the main diagnostic characteristic of *Pseudaspius nakamurai* (Honto et al. 1998)

after the observation (6 May 2024), the shallow branched flow had already disappeared (Fig. 1b), probably because of a decrease in snowmelt water. Furthermore, although the spawning season is thought to be from April to June (Nakamura 1969; Honma 1991; Katsura 2005), no aggregations or spawning was found in the spawning habitat. Our findings might suggest that the branched flow created by snowmelt shapes the drop-off into a pool, creating a preferred habitat for the spawning of *P. nakamurai* in the Agano River system. Because of the limited observations in this study, future research is needed to clarify the importance of temporary branched flows in the spawning ecology of *P. nakamurai*.

Recent human activities, such as dam construction and river straightening, suppress the flooding process and facilitate the homogenization of river environments (Poff et al. 2007; Ishiyama et al. 2017). These artificial environmental changes negatively impact most species that utilize temporary water areas created by floods as spawning and nursery habitats (e.g., Katano et al. 2003; Onikura et al. 2009; Abe 2012). Considering our findings, such river modifications may result in the loss of the important elements for the spawning habitat of *P. nakamurai*, such as snowmelt water floods and drop-offs from riffles to pools. Therefore, the restoration of the flooding process might be effective

for spawning habitat creation and would be important for conservation of *P. nakamurai*.

Acknowledgments We appreciate the valuable comments of the three anonymous referees on the manuscript. We are grateful to Sayuri Furu-sawa for assistance with rearing the fish, Tomoharu Honma for providing advice on rearing fish, and Kosuke Natsukawa and Yosuke Hirose for help with collecting information and articles for *P. nakamurai*. We also thank the Editage for the English proofreading of the manuscript.

Declarations

Conflicts of interest The authors declare no conflict of interest.

Ethics approval This study complied with the guidelines of the Ichthyological Society of Japan.

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