Natural Propagation and Growth of the Endangered Japanese Bagrid Catfish \textit{Pseudobagrus tokiensis} in an Ecological Test Pond

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Abstract: The propagation and rearing of \textit{Pseudobagrus tokiensis} were performed in an ecological test pond from 2004 to 2007 in order to collect baseline data for habitat restoration and restocking purposes. Over the four-year period of the study, spawning in \textit{P. tokiensis} occurred between the end of August and September. \textit{Pseudobagrus tokiensis} exhibited sexual dimorphism with respect to growth with males tending to be larger. In females, maximum standard length (SL) was approx. 150 mm and individuals 120 mm< were rare, while males with SL exceeding 200 mm, were often observed. The relation between SL and the mass of both males and females, could be expressed by \(y=0.133x^{2.482}\) (\(R^2=0.981\)) and \(y=0.0431x^{2.775}\) (\(R^2=0.948\)), respectively, the logarithms of which were significantly different (\(t\)-test, \(P<0.001\)).

Successful propagation of the catfish during the study period resulted in a remarkable increase in their numbers in the test pond. They can, to a certain degree, inhabit artificial, concrete-lined streams, provided that a stable supply of spring water can be ensured, large natural stones are arranged along the side walls, and variously sized pebbles are used to line the stream bottom.

Key words: Cut-tailed bullhead; Ex-situ preservation; Artificial stream; Biotope

\textit{Pseudobagrus tokiensis} is a freshwater catfish belonging to the family Bagridae (Fig. 1). The present species is distributed throughout eastern Honshu, primarily along the Pacific Ocean from the Mabuchi River system in Aomori Prefecture in the north, to the Nakamura River system in Kanagawa Prefecture in the south; and also along the Sea of Japan from the Yoneshiro River system in Akita Prefecture in the west to the Kuzuryu River system in Fukui Prefecture, in the east (Tashiro and Hatta 1974; Miyadi et al. 1976; Hosoya 1993; Maeda and Taki 1995).

While the habitat preference of this species generally extends from upper to middle river reaches, the habitat available to this species has come under threat in recent years due to degradation of these river environments. This prompted the Ministry of the Environment to designate this species as “Vulnerable” (Ministry of Environment 2003). Extensive destruction of suitable habitat in Kanagawa Prefecture has resulted in a remarkable decrease in the range of this species in recent years. For example, the catfish no longer occurs in either the Nakamura River (Tashiro and Hatta 1974) or the Sagami River (Kudou and Matsuda 1982) where it had been observed previously. The prefectural Red Data Book of Kanagawa classifies the species as “Critically Endangered”, and there is concern that the species may already be extinct (Suguro and Senou 2006). The Inland Water Experimental Station of the Kanagawa Prefectural Fisheries Technology Center (hereinafter referred to as the experimental station) has been developing hatchery production techniques to preserve genetic diversity of \textit{P. tokiensis} in captivity, and the success of rearing...
P. tokiensis populations from the Tama and Tsurumi River systems has already been demonstrated (Suguro 2000). Pseudobagrus tokiensis from the Tama River system were bred in an artificial stream system (hereinafter referred to as the test pond) at the station, and their natural propagation was confirmed in November 2003. The present study aim to collect baseline biological data for application to future habitat restoration measures by investigating the propagation and growth of P. tokiensis from 2004 to 2007 in the test pond.

**Materials and Methods**

**Ecological test pond and fish**

The test pond at the station consists artificial stream lined with concrete and filled with pebbles, small stones, Arakida-soil and sand on the bottom, in that order. It has an approximately area of 400 m² and 98.1 m length (Fig. 2). The test pond is comprised of a main stream and “the Japanese bitterling pond” and is fed by underflow from the Sagami River. Water in the system was circulated by a submerged pump (river underflow 380 l/min, circulated water 500 l/min, and total flow of 880 l/min, inclination of the river 12.5%). The main stream area is divided into four sections (A to D) by weirs, starting from the upstream reaches of the system (Table 1). In the autumn of 1999, the test pond was stocked with P. tokiensis yearling (0+, n=50) from the Tama River system that had been bred at the station. Their natural propagation was confirmed in 2003. In addition to P. tokiensis, the test pond was stocked with ten other fish species, including Japanese bagrid catfish.

**Table 1. Average ± standard deviation (minimum-maximum) of environmental condition in ecological test pond**

<table>
<thead>
<tr>
<th></th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>Average</th>
</tr>
</thead>
<tbody>
<tr>
<td>Area (m²)</td>
<td>19.2</td>
<td>20.1</td>
<td>39.0</td>
<td>37.1</td>
<td>28.9</td>
</tr>
<tr>
<td>Current Length (m)</td>
<td>21.3</td>
<td>13.0</td>
<td>26.5</td>
<td>19.9</td>
<td>20.2</td>
</tr>
<tr>
<td>Width (cm)²</td>
<td>ave. 55.5 ± 45.2</td>
<td>115.0 ± 86.0</td>
<td>139.8 ± 70.5</td>
<td>124.8 ± 139.0</td>
<td>108.8</td>
</tr>
<tr>
<td></td>
<td>max. 65.0</td>
<td>210.5</td>
<td>380.0</td>
<td>650.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>min. 20.0</td>
<td>22.5</td>
<td>30.5</td>
<td>45.5</td>
<td></td>
</tr>
<tr>
<td>Depth (cm)²</td>
<td>ave. 25.3 ± 8.8</td>
<td>43.0 ± 2.9</td>
<td>37.8 ± 7.8</td>
<td>28.9 ± 12.8</td>
<td>33.8</td>
</tr>
<tr>
<td></td>
<td>max. 43.5</td>
<td>48.0</td>
<td>54.5</td>
<td>44.5</td>
<td></td>
</tr>
<tr>
<td>Current (cm/s)²</td>
<td>ave. 17.2 ± 9.0</td>
<td>3.5 ± 4.2</td>
<td>4.1 ± 4.5</td>
<td>6.3 ± 9.7</td>
<td>7.8</td>
</tr>
<tr>
<td></td>
<td>max. 29.3</td>
<td>15.5</td>
<td>13.1</td>
<td>27.1</td>
<td></td>
</tr>
<tr>
<td>Bottom (%)³</td>
<td>rock (&gt;500 mm)</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
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<tr>
<td></td>
<td>big stone (250-500mm)</td>
<td>16.0</td>
<td>12.7</td>
<td>8.5</td>
<td>7.3</td>
</tr>
<tr>
<td></td>
<td>stone (50-250mm)</td>
<td>84.0</td>
<td>76.1</td>
<td>46.9</td>
<td>58.0</td>
</tr>
<tr>
<td></td>
<td>pebble (4-50mm)</td>
<td>0.0</td>
<td>5.6</td>
<td>0.8</td>
<td>4.0</td>
</tr>
<tr>
<td></td>
<td>mud (&lt;4mm)</td>
<td>0.0</td>
<td>5.6</td>
<td>43.8</td>
<td>30.7</td>
</tr>
<tr>
<td>Cover (%)⁴</td>
<td>5.0</td>
<td>40.0</td>
<td>20.0</td>
<td>60.0</td>
<td>31.3</td>
</tr>
</tbody>
</table>

¹ Measured per 1.0 m.
² Measured per 1.0 m².
³ Takemon et al. 1993.
⁴ The percentage of Potamogeton oxyphyllus and Egeria densa on bottom.

**Fig. 1. Pseudobagrus tokiensis**, an endangered Japanese bagrid catfish from the Tsurumi River. Upper, Male (SL = 125 mm); Lower, Female (SL = 105 mm).
fatminnow *Rhynchocypris lagowski* and metropolitan bitterling *Tanakia tanago*.

**Sampling and data analysis**

The water temperature was measured by a data logger (TR-52s, Shiro Industry Co. Ltd., Japan); the water temperature in river section B was measured automatically at hourly intervals. The pH and dissolved oxygen (DO) were measured once every ten days by a Multi-function Water Quality Monitoring System (U-11, Horiba Ltd., Japan).

Fig. 2. Schema of the ecological test pond at the Inland Water Experimental Station of the Kanagawa Prefectural Fisheries Technology Center. Arrows indicate the direction of water flow. Black bars indicate weirs.
In order to assess the condition of the biota of the test pond, species evaluations were conducted by collecting aquatic organisms including fish, using an electrical fisher (Type-12, Smith-Root Co., USA) on 1 August 2006.

Surveys were also conducted in the test pond on 17 occasions between 26 May 2004 and 19 December 2007. During collection, the circulation pump was turned off and one person operating an electrical fisher moved from the downstream section D towards the upstream section A, followed by a group of two to four people who collected the stunned \textit{P. tokiensis} with dip nets (100 × 70 cm) and spoon nets (24 × 18 cm). Thirty minutes after completion of the initial sweep of the test pond, the process was repeated. The survey took place over the six hours from 9:00 to 15:00. The captured \textit{P. tokiensis} were then anesthetized with eugenol (FA-100, Mitsubishi-Tanabe Pharmacy Co., Ltd, Japan) to determine their SL, weight and sex, before being immersed in 0.5% salt water for one hour and then released back into the stream section from which they were captured. We analyzed growth and frequency, population of each section and sexual difference with standard length and body weight.

Assessments of the degree of propagation in \textit{P. tokiensis} were complementary undertaken between 12 August 2005 and 12 October 2005, 26 June 2007 and 25 August 2007 using water glasses and spoon nets.

**Results**

\textit{Temperature and biota of the test pond}

The monthly average water temperature of the test pond was $17.8 \pm 2.6^\circ C$ (average ± standard deviation (SD)), ranging from 11.6 to 24.2°C (minimum to maximum). Average water temperature peaked in September 2004, 2006, and 2007, and in August 2005, while the lowest temperatures were recorded in February 2005 and 2007, and in March 2006 (Fig. 3). The average

![Fig. 3. Changes in the water temperature and seasonal change in the condition factor of female \textit{Pseudobagrus tokiensis} of the ecological test pond. Bars indicate the mode of the water temperature and solid circles indicate average of water temperature for each month and diamond marks indicate average of BMI.](image-url)
Fig. 4. Frequency distribution of standard length on *Pseudobagrus tokiensis*.
pH was 7.8 ± 0.2, ranging from 7.5 to 8.2, and the DO was 9.8 ± 0.4 mg/l, ranging from 9.1 to 10.6 mg/l. The ten fish species maintained in the system such as *P. tokiensis*, silver crucian carp, topmouth gudgeon *Pseudorasbora parva*, Japanese fatminnow, metropolitan bitterling, Asian weather loach *Misgurnus anguillicaudatus*, Japanese eightbarbel loach *Leфua echigonia*, medaka *Oryzias latipes*, common freshwater goby *Rhinogobius* sp. OR and Japanese sculpin *Cottus pollux*, as well as crustaceans, nuka shrimp *Paratya compressa improvisa* and two mollusks, kawanina *Semisulcospira libertina* and freshwater pearl mussel *Margaritifera laevis*. The most abundant species in sections B and C were topmouth gudgeon and silver crucian, respectively.

**Collection survey**

In 2004, 35 individuals of *P. tokiensis* (83.0 ± 52.9 mm, average standard length (ASL) ± SD) were collected on May 26, 24 (88.3 ± 51.5 mm) on July 30, 31 (94.4 ± 43.2 mm) on August 25, 48 (76.7 ± 46.1 mm) on October 7, 30 (88.1 ± 53.2 mm) on November 4, and 28 (65.6 ± 32.3 mm) on December 6. Yearling (0+, 25.7 ± 1.7 mm) were collected on October 7, followed by seven yearling (34.3 ± 3.2 mm) on November 4, and then 11 yearling (36.0 ± 4.3 mm) on December 6 (Fig. 4). These yearling were only collected from sections B and C in the test pond (Fig. 5). Judging from their appearance, no yearling were considered to have been propagated in July or August of 2004; however, seven yearling (29.8 to 40.8 mm SL) appeared for the first time in October. Furthermore, since yearling with approximately the same size was observed in September, spawning is estimated to have occurred in the same month. Although yearling measuring approximately 30 mm (SL) had been collected in May, it was estimated that it had overwintered from the previous year, had then been collected before increasing in size.

In 2005, 32 individuals (75.3 ± 53.9 mm) were collected on May 25, 21 (89.6 ± 46.2 mm) on July 26, and 84 (50.7 ± 39.0 mm) on October 14. The first yearling (*n*=47; 25.5 ± 3.1 mm) were collected on October 14 from sections B and C.

In 2006, 53 individuals (60.2 ± 29.8 mm) were collected on February 14, 38 (67.4 ± 32.6 mm) on August 1, 101 (67.1 ± 31.2 mm)
on November 24, and 82 (68.5 ± 29.6 mm) on December 25. The first yearling ($n = 13$, 28.2 ± 5.6 mm) were collected from sections A, B and C on November 24, followed by nine yearling (29.7 ± 3.8 mm) on December 25 from B and C. Although this was the first year that yearling were found in section A, most of the yearling fish were distributed in B and C.

In 2007, 101 individuals (70.3 ± 29.0 mm) were collected on January 30, 65 (76.9 ± 33.2 mm) on June 29, 143 (63.7 ± 42.9 mm) on September 13, and 117 (72.6 ± 29.1 mm) on December 19. The first 48 yearling were collected in sections A, B and D on September 13, followed by ten on December 19 from sections A and D. In the same year, yearling were distributed throughout all of the sections in the test pond and were collected from all of the stream sections, except C.

The relation between the SL (mm) and the weight (mg) of male and female specimens, could be expressed by $y = 0.133x^{2.482}$ ($R^2 = 0.981$) and $y = 0.043x^{2.775}$ ($R^2 = 0.948$), respectively (Fig. 6), with the logarithms of these curves used to assess statistical differences in these relationships ($\hat{\sigma}^2$, $\hat{\sigma} = 2.776x-10.05$; $\hat{\sigma}$, $\hat{\sigma} = 2.454x-8.780$; $t$-test; $P < 0.001$). The body-mass indexes (BMI) on the collected adult fish, were 11.92 ± 3.09 for males and 16.30 ± 3.55 for females. With the values for females being significantly higher than those of males (Man-Whitney $U$-test; $P < 0.001$). The BMI values for adult females reached to peak in August, thereafter decreased (Fig. 3).

Comparisons of the body weight and SL in both sexes, therefore excluded data for the months of May to September (breeding season). The results showed a significant difference between males (11.92 ± 3.09) and females (16.30 ± 3.55), suggesting the persistence of sexual dimorphism in this species during the non-breeding season.

Upon finding a mature individual with approximately 70 mm (SL) in the test pond, all the catfish with at least 70 mm were considered to be so matured that they can be analyzed. Observations of males and females in the test pond presented the male: female ratio to be 81:100.

No spawned eggs or hatchlings were observed in 2005. However, in 2007, 13 hatchlings (ASL 13.8 ± 0.6 mm, average body weight 0.03 ± 0.01 g) were collected from section D on August 25. The approximately 5 m² area that these hatchlings were collected from had almost no flow, but Brazilian Elodea *Egeria densa* was flourishing. Since the growth conditions of the propagated hatchlings were essentially identical, it was estimated that the offspring were derived from the same parents.

**Discussion**

The BMI of adult female fish was 21.03 ± 1.21 in July, 19.14 ± 3.58 in August, before falling significantly to 14.98 ± 5.62 in October. This finding also indicated that eggs had been laid at around that time of the year. A yearling with approximately 20 mm (SL) was observed in September by a visual survey, and thus it was estimated that the 2005 spawning season occurred in around September, which was the same as the previous year. In 2006, no propagated yearling appeared in August, but 13 appeared in November. In 2007, no yearling was collected in July, one yearling was observed in a visual survey on August 25, followed by 48 yearling in September. The occurrence of the propagated yearling combined with the BMI data for the adult female fish over the four years of the study revealed that the spawning season in the test pond spanned from the end of August to September.

According to previous studies on the breeding biology of *P. tokiensis*, the spawning season in natural rivers has been reported to extend from June to August (Miyahi et al. 1976; Kimizuka 2006). Spawning migrations in the agricultural water channels of the Naka River system in Tochigi Prefecture have been observed to peak at the beginning of July (Nakamura and Oda 2003a, 2003b), while spawning in aquarium occurred later in July (Suguro 2000). As the test pond uses river underflow, the temperature of the water in spring is relatively similar to that in early summer. The increase in the test pond temperatures lag behind those of natural rivers.

Although no eggs were collected in the pond,
propagated yearling appeared in river sections B and C in 2004 and 2005, followed by the sections A, B and C in 2006, and then sections A, B and D in 2007 (Fig. 5). Since propagated yearling were found in section B with consistently high numbers, it was assumed that this river section also constituted the optimal breeding site in the test pond. Like section B, section C was also considered to compose the optimal habitat; not only because it was stocked, but also because the water was deep and the flow was gentle with muddy substrate among pebbles. The substrate was lined pebbles and aquatic plants, such as Brazilian Elodea and yanagimo Potamogeton oxyphyllus on the river bed of section C, were very similar to those of section B, but the presence of mud in section C is considered unsuitable for optimum habitat for yearling in this species.

*Pseudobagrus tokiensis* in the test pond exhibited marked sexual dimorphism, with males being larger than females. Females generally grow to approximately 150 mm (SL) in maximum, although individuals larger than 120 mm were rare, while males exceeding 200 mm were often observed during the study period. These findings reconfirm the assumption of Nakamura and Oda (2003a) who investigated *P. tokiensis* in the agricultural water channels of the Naka River. Males presented a more elongated shape than females which presented more rotund. This disparity was confirmed by the correlation equations for SL and body weight (Fig. 6), particularly during the breeding season when the abdomens of females increase in size due to maturation of eggs and their BMIs increase.

*Pseudobagrus tokiensis* inhabiting the agricultural water channels of the Naka River, were also observed to have markedly uneven sex ratios, with females far exceeding the males in number (Nakamura and Oda 2003a). Observations on males and females in the test pond showed the male: female ratio to be 81:100; an uneven sex ratio that was similar to that observed in the Naka River, based on the total number of fish collected from May to October.

Regarding habitat preference of *P. tokiensis* in the test pond, the survey conducted on January 30 2007 revealed that 31 yearling were collected from section A, 33 yearling from section B, 20 yearling from section C, and 16 yearling from section D, respectively. The middle reaches (B and C) were originally stocked in 1999.

*Pseudobagrus tokiensis* was thus successfully propagated from 2004 to 2007, with a year-on-year increase both in their population size and distribution throughout the test pond in this study. They are considered to be susceptible to river improvements, and it has been suggested that the apparent disappearance of the catfish in Kanagawa Prefecture is due to significant degradation of river environments, e.g. concrete reinforcement of river banks etc. (Suguro and Senou 2006). To conserve the catfish it will therefore be necessary to restore their habitat and keep existing habitats in good condition. However, the present study also reveal that the species can, to a certain degree, inhabit concrete-lined river channels, provided that water of a suitable quality can be maintained and that large, natural stones are positioned along the side walls and various pebbles in size with current diversity are used to line the concrete bottom. It therefore seems possible that the target species could be reestablished in concrete-lined river sections if the appropriate river substrates were present. Future research will involve working together with river management authorities and the civil engineering departments of local metropolitan councils to restore the habitat of this species in natural river reaches. This will involve expanding this study to that of a restoration study in areas with multiple-nature-type bank protections.

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