Life history of an invasive freshwater shrimp *Neocaridina davidi* (Bouvier, 1904), (Decapoda: Caridea: Atyidae) in the Tomoe River, the Boso Peninsula, eastern Japan

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Abstract.— We studied the life history of *Neocaridina davidi* (Bouvier, 1904), native to mainland China and Korea, invading the Tomoe River, in the Boso Peninsula of eastern Japan. Sampling was carried out monthly from November 2012 to October 2013. Recruitment occurred from July to September, and the spawning season was from April to September. Large males with a carapace length more than 6.0 mm were rarely collected or absent from July to August, and, similarly, large females with a carapace length more than 7.0 mm were rarely collected or absent from September to October. Evidence from the current study suggests life span for this species is approximately 10–15 months. The life history of *N. davidi* is very similar to that of a congeneric species, *N. denticulata*, reported in previous studies.

Key words: *Atyidae*, *invasive species*, *life history*, *Boso Peninsula*

■ Introduction

Neocaridina shrimp are commonly used as fishing bait or kept in aquaria as pets, and, as a result, have spread into various localities around the world (Englund & Cai, 1999; Niwa, 2010; Klotz *et al.*, 2013). *Neocaridina davidi* (Bouvier, 1904) is a landlocked species native to mainland China and the Korean peninsula (Liang, 2004). Klotz *et al.* (2013) claimed that *Neocaridina heteropoda heteropoda* Liang, 2002 and *Neocaridina denticulata sinensis* (Kemp, 1913) are junior synonyms of *N. davidi*. Englund & Cai (1999) reported that *N. davidi* (as *N. denticulata sinensis*) inhabited Oahu in Hawaii. Klotz *et al.* (2013) also reported that *N. davidi* was found in the Gillbach River in western Germany. Fujita *et al.* (2011) reported that *N. davidi* (as *N. denticulata sinensis*) inhabited three rivers in western Japan; the Gono River in Shimane Prefecture, the Saba River in Yamaguchi Prefecture, and the Kako River in Hyogo Prefecture. In these three rivers, *N. da-*

vidi and *N. denticulata* (De Haan, 1844) co-inhabited locally (Fujita *et al.*, 2011). On the other hand, it should be noted that *N. denticulata* is naturally distributed in areas of western Japan, mainland China, Taiwan, and the Korean peninsula (Kubo, 1938; Kamita, 1970; Suzuki & Sato, 1994; Cai, 1996; Hamano *et al.*, 2000; Liang, 2004; Hayashi, 2007; Suzuki & Naruse, 2011; Toyota & Seki, 2014).

We surveyed 281 sites of 27 rivers in the Boso Peninsula to investigate the freshwater shrimp fauna. Among these sites, we preliminarily surveyed 7 sites in the Tomoe River on July 30, 2012 (Sites 1 to 7 in Fig. 1) and collected *Neocaridina* species at 4 sites. We collected 1 specimen from Site 1, 5 specimens from Site 2, 40 specimens from Site 3, and 11 specimens from Site 4. Mitsugi *et al.* (2017) compared the morphological characteristics and mitochondrial DNA sequence variation of the specimens they have collected in 2013 and 2014 at the Site 3 of the Tomoe River with those of other *Neocaridina* species and con-

cluded that the specimens from the Tomoe River were *N. davidi*. The time of invasion and invasion route of *N. davidi* to the Tomoe River are unknown. However, since the ornamental carp fish (Nishiki-goï) *Cyprinus carpio* (Linne, 1758) is frequently observed in the Tomoe river, *N. davidi* may have invaded the Tomoe River through intentional disposal or accidental escape from aquaria (Mitsugi *et al.*, 2017).

The life history of *N. davidi* has not been studied previously either in its natural distribution areas or in the invasion areas. In contrast, the life history of *N. denticulata* was investigated by several studies, with some studies reporting that the life span of this species was 11–16 months (Ogawa *et al.*, 1987; Sato *et al.*, 1994; Oh *et al.*, 2003). Niwa and Hamano (1990) recognized two life history groups from the same study area. One is a group with a life span of approximately 12 months, and another has a life span of approximately 2 months.

Neocaridina denticulata does not have a natural distribution in eastern Japan, and is not found in the Tomoe River (Kamita, 1970; Suzuki & Sato, 1994; Hamano *et al.*, 2000; Hayashi, 2007; Suzuki & Naruse, 2011; Toyota & Seki, 2014). In the present study, we investigated the life history of *N. davidi* in Site 3 of the Tomoe River where many specimens were collected in the preliminary survey on July 30, 2012. And we compare the life history of the Tomoe River population of *N. davidi* with that of *N. denticulata* reported in other areas in the previous studies, with the purpose of examining the differences in life history between the two species.

Materials and Methods

Study site

The Tomoe River is 9.2 km in length and flows through the Boso Peninsula to the Pacific

Fig. 1. Map of the Tomoe River and the location of the study sites.

Ocean. The study area is located at a distance of 3.2 km from the estuary, at an altitude of 15 m (Site 3 in Fig. 1), and with a river width at the study area of approximately 3 m. The bottom sediment was composed of sand and gravel. At the study site, *N. davidi* inhabited a shallow area (water depth of approximately 20 cm) where the emergent plant *Typha latifolia* (Linne, 1753) was abundant.

Sampling methods

Sampling was carried out monthly from November 2012 to October 2013, using a hand net with a 25 cm-wide mouth (mouth area of approximately 420 cm² mouth area and mesh size of 1.0 mm). All samplings were performed by the same person (MM) by sweeping the hand net through the water 15 times. The water temperature was measured by sinking the stick thermometer (Shinwa Rules, Sanjo, Japan; Item Code: 72508) to the bottom of the water at each sampling. Collected specimens were fixed and preserved in 80% ethanol.

Measurements and method of observation of specimens

Neocaridina davidi is distinguished from other *Neocaridina* species by the following combination of characters; the rostrum reaches near to, or is slightly beyond, distal end of the third segment of the antennular peduncle, the propodus of the third pereopod is more incurved in males than in females and the endopod of the first pleopod of the male is oval-shaped, and is 1.2–1.5 times as long as broad (Liang, 2004). The sex was determined by the shape of the endopod of the first pleopod (Cai, 1996; Englund & Cai, 1999; Liang, 2004; Klotz *et al.*, 2013; Toyota & Seki, 2014), which was indistinguishable in specimens with a carapace length less than 3.0 mm; hence, such specimens were defined as juveniles. Oviparous condition in females was also recorded.

We measured the carapace length (from the

postorbital margin to the posterior margin of the carapace) in all specimens. In addition, the maximum body length (from the postorbital margin to the end of the telson) was recorded for males and females. For measurements and observation, we used a stereomicroscope (M50, Leica Microsystems, Wetzlar, Germany) equipped with an eyepiece micrometer.

Statistical analysis

Size-frequency histograms were constructed using R 3.3.1 software (R Development Core Team, 2016), to analyze cohort structure, recruitment season, spawning period, and life span. First, in order to enable data analysis in R 3.3.1, data was tabulated every month for males, females and juveniles using the basic function “data.frame” implemented in R 3.3.1. Size-frequency histograms were then constructed with 0.5 mm carapace length size intervals using the generic function “hist” implemented in R 3.3.1.

Results

Overview of collected specimens and water temperature

A total of 1235 specimens were collected, including 319 juveniles, 463 males and 453 females (427 non-oviparous females and 26 oviparous females) (Table 1). The sex ratio (male : female) was 1 : 0.98. Carapace length of the collected specimens was in the range 0.8–7.7 mm. The largest male was 6.4 mm (carapace length) and 25.5 mm (body length), collected in March 2013; while the largest female was 7.7 mm (carapace length) and 30.0 mm (body length), collected in May 2013. The highest water temperature recorded at the study site was 24.0°C in August 2013, while the lowest was 6.0°C in February 2013 (Fig. 2).

Population fluctuation

Size-frequency histograms for juveniles, males and females are shown in Fig. 3. In No-

Table 1. Comparison of the life history of *Neocaridina davidi* in the Tomoe River and *Neocaridina denticulata* in previous studies.

Reference	This study	Ogawa <i>et al.</i> (1987)	Niwa & Hamano (1990)	Sato <i>et al.</i> (1994)	Oh <i>et al.</i> (2003)
Species	<i>Neocaridina davidi</i>	<i>Neocaridina denticulata</i>	<i>Neocaridina denticulata</i>	<i>Neocaridina denticulata</i> *	<i>Neocaridina denticulata</i> *
Locality	Tomoe River, Chiba Prefecture, Japan	Ashida River, Hiroshima Prefecture, Japan	Sugou River, Hyogo Prefecture, Japan	Manose River, Kagoshima Prefecture, Japan	Young-am, Korea
Recruitment season	July–September	Early July, late September	Early June–early September	June–September	July–August
Spawning period	April–September	Early May–early September	Early May–late September	Late April–early October	May–September
The period that large shrimps were very scarce collected or not collected	July–October	August	June–August	June–September	October
Life span	10–15 months (?)	11, 14 months	2, 12 months	12 months	15.6–16.8 months
Maximum size of specimens	Male: 6.4 mm** (25.5 mm***) Female: 7.7 mm** (30.0 mm***)	Male: 24.3 mm*** Female: 29.9 mm***	Male: 24.1 mm*** Female: 28.6 mm***	Male: 27.1 mm*** Female: 29.0 mm***	Male: 7.0–7.5 mm** Female: 9.38 mm**
Minimum size of ovigerous females	5.0 mm**	16.6 mm***	15.4 mm***	20.2 mm***	5.2 mm**

* *Neocaridina denticulata* in original paper.

** Carapace length.

*** Body length.

Fig. 2. Seasonal change in water temperature at Site 3.

vember 2012, a small number of juveniles with carapace length greater than 2.0 mm were captured. Assuming that the same reproductive process occurred in 2013, juveniles collected in November 2012 are considered to be born 2012. Juveniles with a carapace length equal to or less than 2.0 mm were not collected from November 2012 to June 2013 because recruitment didn't occur this period. From April 2013, ovigerous females appeared. From July to September 2013, juveniles with a carapace length equal to or less than 2.0 mm were abundant. Juveniles collected between July and October 2013 were considered to be born 2013. In

October 2013, many individuals were mature, a small number of juveniles with carapace length greater than 2.0 mm were captured.

Ovigerous females were collected during the period between April and September in 2013. Carapace length of the ovigerous females ranged from 5.0 to 7.7 mm. From April to July 2013, ovigerous females with a carapace length equal to or more than 6.0 mm were collected. Females of this size were collected from November 2012. These females were likely born in 2012, because females born from April to June in 2013 grew to carapace length 5.5–6.5 mm in October 2013. From August to Sep-

Fig. 3. Size-frequency histograms of *Neocaridina davidi* in Site 3 from November 2012 to October 2013. (A) juveniles; (B) males; (C) females. Black bars, ovigerous females; gray bars, juveniles.

tember 2013, ovigerous females with a carapace length equal to or less than 6.0 mm were collected. Females of this size were hardly collected from April to June 2013, but again collected in July 2013. Therefore, these females were likely born in 2013.

The growth rate could not be quantified since the number of collected specimens from December 2012 to June 2013 was small. From July to August 2013, large males with a carapace length more than 6.0 mm were rarely collected or absent from collection. From September to October 2013, large females with a carapace length more than 7.0 mm were rarely collected or absent from collection.

The above-mentioned analysis of the size-frequency histograms suggests that the life span of males is approximately 10 to 13 months and that of females is approximately 12 to 15 months. However, the sample size of shrimp from December 2012 to June 2013 was very small. More samples are needed to accurately estimate life span in this species. In particular, it is difficult to estimate whether large shrimp survive after the second year. However, the maximum size of specimens in the present study was almost the same as the maximum size of the specimens in previous studies (Ogawa *et al.*, 1987; Niwa and Hamano, 1990; Sato *et al.*, 1994). Therefore, we suggest that many of the specimens in the surveyed population reach their end of life in 10–15 months.

Discussion

The life history of *N. davidi* in the present study demonstrates that recruitment occurred from July to September, with the spawning period from April to September. From July to August, large males with a carapace length more than 6.0 mm were rarely collected or absent from collection. From September to October, large females with a carapace length more than 7.0 mm were rarely collected or absent from collection. It was suggested that many of

shrimp reached the end of life in 10–15 months.

Comparisons between the life history of *N. davidi* in the Tomoe River and that of *N. denticulata* as reported in previous studies (Ogawa *et al.*, 1987; Niwa and Hamano, 1990; Sato *et al.*, 1994; Oh *et al.*, 2003) are presented in Table 1. Overall, the life history of *N. davidi* in the present study is quite similar to that of *N. denticulata* reported in previous studies. For example, the recruitment period was from June to September, the spawning season was from April to September, the period that large shrimps were rarely collected or absent from collection was from June to October, the life span is 10–15 months, and the maximum body length is 25.3 ± 1.19 mm in males and 29.4 ± 0.59 mm in females.

Niwa and Hamano (1990) reported the occurrence of a short generation group in the population of *N. denticulata* in the Sugow River, Hyogo Prefecture. The short generation group was recruited in early June, grew rapidly, spawned, and disappeared in August. In the present study, ovigerous females with a carapace length equal to or less than 6.0 mm were collected from August to September. These shrimp may belong to a short generation group. However, there is an additional possibility. These shrimp may belong to a long generation group. Ogawa *et al.* (1987) and Niwa & Hamano (1990) pointed out the possibility that some shrimp in the long generation group that grow rapidly may breed twice, with the first reproductive season in the recruiting year and the second reproductive season in the following year. To clarify this issue, the sampling of shrimp should be done over shorter intervals in summer to more precisely assess population fluctuation.

Both *Neocaridina denticulata* and *N. davidi* inhabited rivers mid-stream, lakes, marshes, and irrigation canals where emergent plants were abundant (Kamita, 1970; Ogawa *et al.*, 1987; Sato *et al.*, 1994; Suzuki & Sato, 1994;

Englund & Cai, 1999; Oh *et al.*, 2003; Liang, 2004; Hayashi, 2007; Suzuki & Naruse, 2011; Toyota & Seki, 2014). This sympatry potentially leads to competition between the two species for habitat.

Nishino (2009) suggested the possibility of interspecific hybridization between *N. denticulata* and *Neocaridina* spp., including *N. davidi* (as *N. heteropoda*), since, in the surveyed area, these species were co-inhabited out of the same local habitat. However, it should be noted that interspecific hybridization between the two species has not yet been demonstrated in breeding experiments. If interspecific hybridization between *N. davidi* and *N. denticulata* is biologically possible, there is a risk of interbreeding in the natural environment, since their life cycles and habitats well overlap with each other, potentially leading to strong competition between them.

Acknowledgements

We thank the late Dr. Y. Usami (Tokyo University of Marine Science and Technology) for useful discussion on the subject. We wish to thank Professor Stephen G. Dunbar (Loma Linda University, USA) for English editing and content review. We are also grateful to the two anonymous reviewers for their valuable comments on this manuscript.

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