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Reproductive Biology and Adaptability of the Invasive Alien Freshwater Amphipod Crangonyx floridanus (Crustacea: Amphipoda, Crangonyctidae)

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We studied the reproductive biology and adaptability of the alien freshwater crangonyctid amphipod Crangonyx floridanus, currently inhabiting a large portion of Japan, both in the field and under controlled laboratory conditions. In the Chikuma River population of this alien amphipod, egg-bearing individuals were found throughout the year. In terms of egg maturation cycle, egg development (during embryogenesis), and egg count per ovipositional cycle, these amphipods display a very efficient reproductive system. This study also established their adaptability to a wide range of water temperatures (primarily 4–20°C, however in some cases, these individuals are able to survive at up to 30°C). C. floridanus’s strong capacity to adapt to broad and variable environmental conditions is certainly contributing to its high rate of population increase, and rapid dispersion throughout Japan.

Key words: reproduction, freshwater amphipod, Crangonyx floridanus, temperature adaptability, alien species

INTRODUCTION

The crangonyctid amphipod, Crangonyx floridanus Bousfield, 1963, which originates from North America, has invaded the freshwaters of Japan. The invading amphipod was first found in Japan in 1989, in the Furutone-numa oxbow lake of the Tone River, which flows along the Ibaraki-Chiba prefectural boundary (Morino et al., 2004). Throughout the 1990s, the number of recorded sightings of this species significantly increased within the Kanto region. The alien amphipod currently inhabits a large portion of Japan, i.e., 28 prefectures on the islands of Honshu, Shikoku and Kyushu (Kanada et al., 2007). Despite many reports of the distribution and expansion of C. floridanus and a comprehensive review by Kanada et al. (2007), little is known of its ecological and reproductive features, and of its potential for a large-scale invasion. Japanese freshwater amphipods specifically occupy habitats characterized by good water quality and cold temperatures (e.g. headwaters, spring-fed waterways, and creeks rich in submerged vegetation) (Kusano, 2001; Kusano and Ito, 2003). Thus, they have been treated as indicator organisms of oligosaprobic waterways (Kusano, 2001). However, a variety of alien amphipods have significantly broader habitats than do native ones, and can inhabit a wide range of environments, even in relatively high water temperature. They readily inhabit the oligosaprobic waterways originally inhabited by native species (Masaki et al., 2003). It has been reported that many introduced and established alien species have rapidly become widely distributed in Japan, and exhibit both high environmental adaptability and fecundity [e.g., the red swamp crayfish Procambarus clarkii, the common slider turtle Trachemys scripta elegans (The Ecological Society of Japan, 2002), and the mudsnail Potamopyrgus antipodarum (Zaranko et al., 1997)]. We have noted that, in a number of these cases, introduced alien species share a set of similar traits that ensures their robust adaptability and relative reproductive superiority, accounting for the rapid and enormous expansion of alien species.

The introduction of alien amphipods has frequently resulted in serious disturbances to freshwater ecosystems,
native amphipods, and other biota. Although alien amphipods have become a serious problem in recent years, ecological, ethological, and reproductive research into these species has thus far been minimal.

In the present study, we conducted ecological research into the alien amphipod *C. floridanus*, with special reference to its reproductive biology, and evaluated its ability to adapt to freshwater environments in Japan.

**MATERIAL AND METHODS**

**Study site**

The initial discovery of *Crangonyx floridanus* in the Chikuma River was made on 14 October 2005, in the Awasa area (Awasa site, Chikuma City, Nagano Prefecture, Central Japan; 36°32' N, 138°07' E, 358 m altitude), during a monthly field sampling for an unrelated research project of the ‘Chikuma River Ecology Research Group’ (Tojo et al., 2007). These alien amphipods live at high densities (i.e., a few hundred to several thousand individuals per m²) and breed in an area (Fig. 1) in which no native amphipods incidentally inhabit the Chikuma River. In the Awasa area, the Chikuma River Office (Hokuriku Regional Development Bureau, Ministry of Land Infrastructure and Transport) operates a river environmental observatory. We have used their 2006 water temperature data in this study (Fig. 2).

**Experiment on duration of the reproductive period**

Material was collected at study sites near the river’s shore where vegetation hangs down into the water, using a D-frame hand net (300 mm in width, with 1 mm mesh). Sampling was conducted approximately monthly from January to December 2006 (sampling dates were 9 Jan., 15 Feb., 16 Mar., 6 Apr., 17 May, 7 Jun., 30 Jul., 18 Aug., 10 Sep., 18 Oct., 28 Nov., and 19 Dec.). The first 100 individuals collected each month were fixed with 70% ethanol. The body length of each individual, excluding antennae and caudal fans, was measured to the nearest 0.1 mm using a binocular microscope with an objective micrometer. The number of egg-bearing females was counted to determine the percentage of brooding individuals.

**Average egg number per oviposition cycle of copulated females:**

The number of eggs (i.e., the number of eggs per oviposition) of brooding females was counted, and the relationship between egg number and female body size was recorded. This data was handled discretely each month, so as to eliminate any effect of seasonal variance.

**Egg developmental period**

To record the developmental period of *C. floridanus* eggs, we used eggs oviposited in the laboratory under careful observation, ensuring the accurate verification of 0 hour of embryogenesis. After mating, females were individually incubated in 60 mm diameter petri dishes in about 20 ml deionized water and a small piece (about 10 × 50 mm) of dead leaf (mainly the Poaceae plant *Phragmites japonica*) collected in the field as a food source. Eggs cradled by their female parent were incubated at 4 ± 1°C, 10 ± 1°C, or 20 ± 1°C. It was difficult to verify the exact time of hatching, as the process occurs within the egg brood pouch (the “oostegite” or...
"marsupium"), we treated the period up to the mother’s release of her offspring (i.e., 1st instar juveniles) as the "egg incubation period."

**Temperature adaptability**

Brooding females were separately incubated at 10 ± 1°C. Early instar juveniles (about 1 week after hatching) were studied to assess their success, adaptability, and ability to survive at a range of habitat temperatures. Hatched offspring were brooded in a 5 ml Petri dish with 3–4 ml deionized water, and provided with a small piece (about 5 × 5 mm) of dead leaf collected in the field as a food. This one-week period following hatching was used to acclimatize the juveniles prior to the period of experimental observation, when groups of amphipods were observed individually at one of a range of assigned temperatures. During the period of acclimatization, each group of juveniles was reared at a temperature exactly halfway between temperature of their incubation during the embryonic stage (10 ± 1°C) and the group’s assigned final experimental temperature. After acclimatization, ten offspring were selected ran-

**Fig. 2.** Seasonal change of water temperature at the Chikuma River study site (Awasa site, Chikuma City, Nagano Prefecture, Central Japan) in the research period (from 1 January to 31 December, 2006). Daily water temperature (maximum, minimum, and mean), based on data recorded at the Chikuma River Observation site (Kuiseke, Chikuma City) by the Ministry of Land Infrastructure and Transport. Mean ± S.D. of annual water temperature was 13.4 ± 7.4°C. The particularly low temperature in the summer season was due to the impact of unusually heavy rainfall on July 18–19, 2006.

**Fig. 3.** Monthly recordings of body size for the first 100 randomly sampled individuals (*Crangonyx floridanus*) randomly collected each month from January to December 2006, at the Chikuma River study site (Awasa site, Chikuma City, Nagano Prefecture, Central Japan). Solid dark shading represents the number of egg-bearing females.
randomly and used for the observation. The groups’ experimental temperatures were adjusted to 4 ± 1°C, 10 ± 1°C, 15 ± 1°C, 20 ± 1°C, 25 ± 1°C and 30 ± 1°C, respectively. Twelve females contributed 10 offspring at each temperature. Thus, 120 specimens were observed for each temperature level. The number of surviving specimens at each temperature was recorded every day for 20 days. Water and food plants were changed every two or three days; about half of the water was replaced at each change.

**SEM observation**

To prepare samples for scanning electron microscopy (SEM), embryos were dissected from the fixed specimen’s brood lobe, sonicated for a few seconds with an ultrasonic cleaner, dehydrated in a graded ethyl alcohol series, transferred to t-butyl alcohol, and then dried in a freeze drier (VFD21, Shinku Device, Ibaraki). Dried embryos were directly observed without coating under a low vacuum SEM (Miniscope TM-1000, Hitachi High-Technologies, Tokyo).

**RESULTS**

The annual fluctuations of the site’s water temperature during 2006 are shown in Fig. 2. The particularly low temperatures in the summer season resulted from unusually heavy rainfall on July 18–19, 2006.

**Duration of the reproductive period**

Fig. 3 shows the body length frequency distribution of *C. floridanus* for the monthly collection period. Amphipods of various sizes were collected throughout the year. Seasonal fluctuations of the median and deviation of each sample’s body size are shown in Fig. 4. No significant seasonal trend or variance was observed in the frequency distribution of body length (Figs. 3, 4). All monthly samples included egg-bearing females (Fig. 3; mean ± S.D. of all months in 2006 was 14.1 ± 7.8%; maximum 32.0% in August, minimum 5.0% in April; these values were calculated using all 100 collected individuals, including males and juveniles). A chi-square test of each month’s frequency data for egg-bearing females revealed no significant differences (p = 0.260). In a multiple test (Tukey test) of egg-bearing females, although significant month-to-month differences were revealed for a few of combinations (e.g., p < 0.001 in April vs. August), seasonal reproductive trends were not clearly revealed.

**Average egg number per ovipositional cycle**

The average egg number per egg-bearing female (i.e., the number of eggs per oviposition cycle) was 35.5 ± 12.2 (mean ± S.D.; 12–83 eggs, minimum-maximum; n = 128). The actual number of egg-bearing females was 168, but some eggs were lost due to mishandling during collection or fixing; data for such specimens was excluded. In the few months that the number of brooding females was very low, data from other collected egg-bearing females from another voluntary quantitative sampling of 100 individuals were added (n = 46 in total). The relationship between body size and the egg number showed a positive correlation (r² = 0.478, n = 128) (Fig. 5). No seasonal effect on the number of eggs was apparent (Fig. 5).

**Egg developmental period**

The period of egg development was 72.7 ± 4.19 days [mean ± S.D.; 67–78 days, shortest-longest; n = 7 (i.e., n = 7 is the number of observed batches)]. Furthermore, embryogenesis, hatching, and release of offspring were almost completely synchronous in individual batches, and no significant variation in egg development period was observed (Fig. 1D, E) at 4°C, 25.3 ± 4.15 days (19–30 days; n = 7), 10°C, 10.6 ± 0.57 days (10–11 days, n = 15) at 20°C, and 8.75 ± 0.44 days (8–9 days, n = 12) at 25°C. The period of embryogenesis was strongly correlated to water temperature.

**Temperature adaptability**

The results for the 20-day temperature adaptability...
Fig. 6. The survival graph from the study of temperature adaptability of *Crangonyx floridanus* (mean ± S.E.). Twelve unique females contributed 10 offspring 6 times. These were observed at each of 6 temperature levels: 4°C, 10°C, 15°C, 20°C, 25°C, and 30°C, and are shown in Fig. 6. The highest survival rate was observed at 10°C (95.8 ± 6.69%; mean ± S.D., n = 12), followed by 4°C (86.7 ± 22.7%, n = 12), and then 15°C (74.2 ± 32.9%, n = 12). The survival rate both at 20°C and 25°C decreased to around 40% (20°C: 43.3 ± 8.1%, n = 12; 25°C: 35.8 ± 16.8%, n = 12). At 30°C, a large number of the specimens died shortly after the start of the experiment commenced; about 50% in the first day and about 80% in 6 days, resulting in a very low overall survival rate. However, about half of those individuals that survived the initial period, survived the full duration of the experiment at 30°C. The overall survival rate was nearly 10% (7.5 ± 13.6%, n = 12) of all specimens examined.

**DISCUSSION**

The duration of the reproductive period

We did not observe seasonal reproductive trends in the Chikuma River population of *Crangonyx floridanus*. In the graphs of body size (Fig. 3), two distinct peaks are seen in stably low water temperature, such as a large spring-fed agate throughout the year, especially in habitats with a (Kusano, 2001). The native anisogammarid amphipods (Anisogammaridae, Gammaridae, Pongogeneidae) tend in throughout the year. Japanese freshwater amphipods (e.g., *Jesogammarus jesoensis*, sea-sonal fluctuations in batch size (i.e., the number of eggs per batch) are considered to be related to food supply. For example, when their habitat is rich in salmon carcasses, they tend to oviposit significantly more eggs (Kusano and Ito, 2005). Our research on the alien amphipod *Crangonyx floridanus*, however, showed no season-related fluctuations in batch or egg size.

Egg number per ovipositional cycle of copulated females

In the present study, we showed a significant positive correlation between body size and egg number (Fig. 5). During the egg-bear period following oviposition, the subsequent egg batch is simultaneously maturing in this amphipod. Although young mature females oviposit a comparatively small number of eggs, they successively produce more eggs proportionate to their growth.

In the native amphipod *Jesogammarus jesoensis*, season-al fluctuations in batch size (i.e., the number of eggs per batch) are considered to be related to food supply. For example, when their habitat is rich in salmon carcasses, they tend to oviposit significantly more eggs (Kusano and Ito, 2005). Our research on the alien amphipod *Crangonyx floridanus*, however, showed no season-related fluctuations in batch or egg size.

Egg developmental period

In this study, for the sake of convenience, we have referred to the entire period up to the release of offspring by the female parent as the “egg period (egg incubation period).” This “egg period” is strongly correlated to water temperature, which seems to be a general characteristic of a wide range of arthropods. As mentioned in the results section, *C. floridanus* exhibits approximately monthly breeding cycles at 10°C. At 20°C, the period of embryogenesis was extremely shorter (10.6 ± 0.55 days), and the next batch of eggs became ready for oviposition much more quickly. This results in a very efficient ovipositional cycle, and presumably explains the high rate of population increase of this alien species, and its rapid spread throughout Japan.

The reproductive cycle of *C. floridanus* can be repeated several times throughout its life, as observed in some breeding pairs. When incubated at 10°C, at the end of an egg-bearing cycle, mature eggs for the next reproductive cycle are already clearly observable through the translucent cuti-cle. As soon as offspring are hatched and separate from their female parent, the female molts and mates (within one day in some cases; cf. Fig. 1A), and then oviposits the eggs of the next cycle. In many cases, the whole process ceases within several hours after mating (cf. Fig. 1B). In the case of incubation at 20°C, because the egg incubation period is very short, the maturation of the next reproductive cycle’s eggs will not complete by the time offspring of the current cycle are released. Maturation takes about two more weeks to complete, meaning that their breeding cycle is only slightly faster than that of individuals incubated at 10°C. In addition, we have often observed in the field that egg-breeding females carry mature eggs of the next breeding cycle, additional evidence that this species is a very efficient breeder.
Temperature adaptability

Until now, no experimental research into the temperature adaptability of the alien amphipod *C. floridanus* had been conducted, which is also true for Japanese native freshwater amphipods. This is likely because native amphipods inhabit specific regions characterized by good water quality and cold temperatures, such as headwaters, spring-fed waterways, and creeks (Kusano and Ito, 2003). Also many Japanese amphipods breed during periods of low water temperature (Kusano, 2000). However, the range of habitats suited to *C. floridanus* is significantly broader than that of native amphipods. At the Chikuma River site, alien amphipod *C. floridanus* continue to survive even during summer when water temperatures reach nearly 30°C, and the minimum temperature remains higher than 25°C for several days (Fig. 2).

Our study of the adaptability of *C. floridanus* juveniles, also suggests a tolerance of high temperatures. Indeed, at water temperatures of 20°C, 25°C, and 30°C, the survival rates of *C. floridanus* were lower than those at lower temperatures. At 30°C, a large number of individuals died within several days after the start of the experiment, resulting in a very low overall survival rate. However, most individuals that survived the initial period continued to survive, and nearly 10% of specimens examined survived the full duration of the experiment. This experiment used a step change between only two water temperature levels, and it provided only a limited opportunity for adaptation. But these results should prompt further investigation into the rapid expansion of this alien amphipod’s distributional range. More gradual acclimatization may result in higher initial survival rates.

Adaptability of *C. floridanus*

The robust adaptability to variations in water temperature and the reproductive efficiency of *C. floridanus* are evident, as shown by the present study. In another notable case of accidental introduction from Florida to California, *C. floridanus* rapidly established itself and expanded its population within its new habitat (Zhang, 1997; Toft, 2000; Toft et al., 2002, 2003). This intra-North American spread is considered to have occurred via the ballast water of ships, and/or ornamental water plants (e.g., water hyacinth and water pennywort). The origin of this alien amphipod’s introduction into Japan is still not understood. The abovementioned intra-North American case, and the case of the closely related species *C. pseudogracilis*, which was also introduced into Europe from North America and rapidly established itself and expanded its distribution, may provide a good point of reference and opportunities for comparative study.

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