

# Early maturation in males of the giant water bug, *Appasus major* Esaki (Heteroptera: Belostomatidae)

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これまでの研究では、比較的冷涼な湿地に住む、オオコオイムシ *Appasus* (= *Diplonchus*) *major* の新成虫のオスは、野外において翌年の春まで卵塊を保護しないことを強調している。しかしながら、本研究は水温が高いハビタットでは新成虫のオスが羽化し、越冬する前に卵塊を保護することを示した。本調査地は、以前の野外研究が行われていた生息地よりも水温が高く、そのことが幼虫の発育を早め、初期（7月）に羽化した個体が成熟したものと考えられる。本研究は、オオコオイムシのオスの新成虫が、野外において卵塊を保護していた初めての報告である。

Previous studies have show that the newly emerged adults of the giant water bug, *Appasus* (*Diplonchus*) *major* Esaki, do not reproduce until the following spring in cool habitats. We observed the life cycle of *A. major* in a warm water environment. Our results suggest that at least some new generation males carry egg masses (encumbered) successfully before overwintering. The encumbered adult males of the new generation appeared between July and August (new males/total males: 1/31 in July; 8/26 in August). We hypothesize that the warmer environment enhances the speed of development. Thus early emerging adult males are able to mature prior to winter because of the long-day photoperiod in July. This is the first report documenting the presence of encumbered new generation *A. major* males in their natural habitat before winter.

**Key words :** *Appasus major*, Belostomatidae, New generation males, Eggs, Water temperature,

Overwintering

## Introduction

The giant water bug, *Appasus major* Esaki, inhabits shallow waters in mountainous regions, including areas dominated by traditional rice paddies (Ichikawa, 1996). Populations of this species are declining in many regions in Japan. Because of the decline, *A. major* is included in the local edition of the red data list in 10 of 47 prefectures (Association of Wildlife Research & EnVision, 2007). Despite the decline in population numbers, little is known regarding the life cycle of this species.

Some studies (Ichikawa, 1996; Mukai and Ishii, 2007) show that *A. major* adults overwinter and reproduce in June and July. The newly emerged adults then appear from late July onwards. *Appasus major* prefers cooler waters than the congeneric species, *A. japonicus* Vuillefroy (Okada and Nakasuji, 1993). Previous studies suggest that *A. major* has a strict univoltine life cycle in their natural habitat (Ichikawa, 1989, 1993; Mukai and Ishii, 2007). Conversely, *A. japonicus* is able to produce 1-2 generations in a single year (Okada and Nakasuji, 1993). The difference may be related to environmental factors such as temperature. *Appasus japonicus* lives in warmer water which is likely to increase the rate of development whereas *A. major* live in cooler water which may lengthen the developmental period (Okada and Nakasuji 1993). In the present study, a field work using mark and recapture censuses was carried out in a warmer wetland than in previous studies (Okada and Nakasuji, 1993; Ichikawa, 1993), in order to reveal the life cycle of *A. major* under different thermal conditions.

## Materials and Methods

The study was conducted in a fallow rice field in Misaki (34°58' N, 133°58' E), Okayama prefecture between April and October, 2006. We measured the seasonal abundance of *A. major* by conducting field surveys. We conducted the surveys in a 19.8 m<sup>2</sup> area of a ditch located along the ridge of the fallow rice field. The ditch received direct sunshine during the day, and was filled with water (5-15 cm depth) throughout the study period.

We captured adults using a D-frame dipnet (28 cm



in width, 3 mm mesh). Each adult was individually numbered on the thorax using paint markers (Paint Marker®, Mitsubishi) and were subsequently released into the ditch. We recorded the individual number, age (overwintered or new generation adults), sex, and the numbers of eggs on the back of the males prior to release. New generation adults were easily recognized by their clean wings and/or soft body. We then surveyed the study site on a weekly basis throughout the study period, twenty six times in total. In addition, we measured water temperature during each survey.

We used the Jolly-Seber method (Jolly, 1965; Seber, 1965) to estimate the number of adults present during each survey. To evaluate the relative abundance of *A. major* nymphs, the D-frame dipnet was swept 20 times along a 50 cm path across the water surface. This procedure was repeated 3 times. We counted the number of nymphs from each instar. Data from published literature was used to compare water temperature of the present study with other study to other study sites (Okada and Nakasuji, 1993; Ichikawa, 1993).

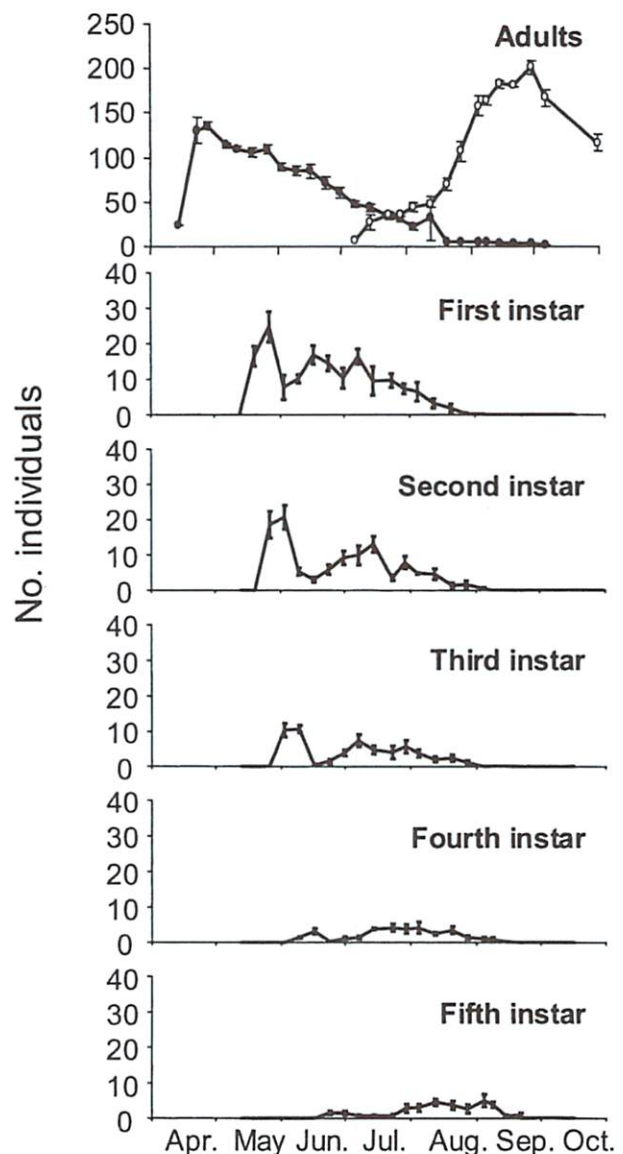
We compared the number of eggs carried by overwintered and new generation males using Student's *t*-test. Water temperature was compared using the Wilcoxon signed rank test, with month as a replicate. Before analysis, monthly mean water temperature was calculated using daily data for each month. Comparisons in which  $P < 0.05$ , were considered statistically significant. All statistical tests were conducted using JMP software (JMP version 7.0, SAS Institute, 2007).

## Results and Discussion

Overwintered adults were most abundant in April (Fig. 1). Encumbered males appeared at the study site between April and August. Nymphs appeared from mid May onwards, and new generation adults appeared between July and October. Encumbered new generation males appeared between July and August (new generation males/total males: 1/31 for July, 8/26 for August, Fig. 2). All encumbered new generation males had emerged in July. Overwintered males carried significantly more eggs than new generation males (overwintered males:  $77.13 \pm 10.94$  (mean  $\pm$  SE), 31-115 (range); new generation males:  $47.71 \pm 3.09$ , 31-56; *t*-test:  $df = 13$ ,  $t = 2.431$ ,  $P = 0.030$ ). We observed egg shells among the clutch of two new

generation males in August, suggesting that hatching was successful. The water temperature was significantly higher at the present study site than at study sites in previously published research (Table 1).

*Appasus major* tended to develop more rapidly in the present study than in previous studies (Okada and Nakasuji, 1993; Mukai and Ishii, 2007). Each instar was observed about 1 month earlier than in the previous



**Fig. 1** Abundance and phenology of *Appasus major*. The data represent the mean and variance (calculated by Jolly-Seber method) or standard error for adults and nymphs, respectively. The overwintered (black circles) and newly emerged (white circles) adults are shown separately.

studies. We also observed egg masses on new generation males (Fig. 2). Because *A. major* males attempt to copulate before receiving eggs to assure paternity, these new generation males were likely reproductively mature (Ichikawa, 1989). Moreover, the presence of hatched egg shells indicates that the eggs were viable. However, we cannot rule out the possibility that the eggs were fertilized by sperm from another male stored in the females spermatheca (Smith, 1979a).

Our observations of the encumbered new generation males before winter differ from previous reports, which documented univoltinism in this species (Ichikawa, 1989, 1993; Okada and Nakasuji, 1993; Mukai and Ishii, 2007). We hypothesize that environmental factors are responsible for the difference in the life cycle at this study site. The combination of warm water temperatures and a long-day photoperiod during July likely contribute to the

early maturation of new generation males (Table 1). The warm water temperatures appear to hasten growth of the nymphs and favor early emergence of young adults in July. Photoperiod is also likely to play a key role in reproductive success. For example, short-day photoperiods during late summer and autumn induce reproductive diapause in new *Kirkaldyia* (*Lethocerus*) *deyrolli* (Belostomatidae) adults (Hasizume and Numata, 1997). Assuming that day-length also plays a role in inducing diapause in *A. major*, the new generation males may have emerged early enough (July) at our study site such that reproduction was not affected by day-length. Conversely, males emerging in August or later, are likely to enter reproductive diapause due to the short-day photoperiod.

New generation males carried significantly fewer eggs than overwintered males. We speculate that this

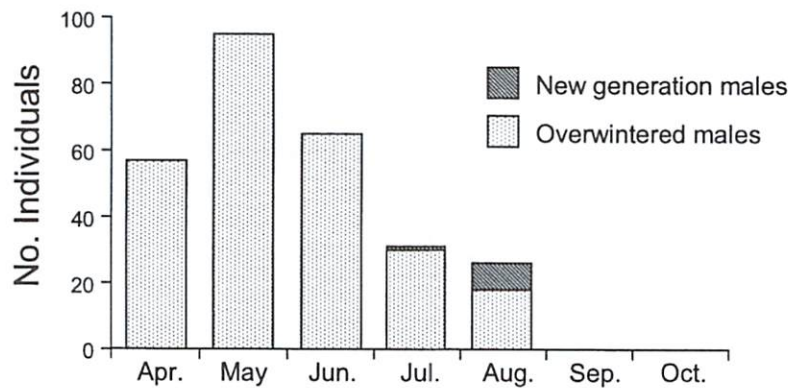


Fig. 2 Seasonal fluctuation in the abundance of encumbered males.

Table 1 Comparison of water temperature in the study site with that of previous studies

Mean water temperature <sup>1</sup>	Study site	Reference
25.6	Misaki, Okayama	Present study
21.4 *	Western Hyogo	Ichikawa (1993)
16.9 *	Tsuboi, Okayama	Okada and Nakasuji (1993)
19.0 *	Kanayama, Okayama	Okada and Nakasuji (1993)
18.1 *	Kuko, Okayama	Okada and Nakasuji (1993)

1) From April to September

\* The water temperature in all previous study site was significantly lower than in Misaki ( $P < 0.05$ , Wilcoxon signed rank test).



may be due to selection for the older males by females (Smith 1979b). Although we were able to document reproduction in young males prior to overwintering, we were not able to determine the reproductive success of young females.

In summary, our results show that environmental conditions can play a role in determining the life cycle of *A. major*. In warm water and under long day-length photoperiods bivoltinism is likely to be favored. Conversely, it appears that cool water temperatures favor univoltinism. However, further research is needed to determine the fitness of offspring carried by the younger males.

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