



Paternal Care Behavior Increases in the Presence of Conspecific Females in the Giant Water Bug, *Kirkaldyia deyrolli* (Belostomatidae: Heteroptera)

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Abstract The males of the giant water bug, *Kirkaldyia deyrolli*, care for egg masses on the emergent vegetation above the water surface in aquatic environments. A previous study reported that attending males supply the eggs with water and guard them against mature females (infanticide) until hatching. That study additionally concluded that males staying on the egg mass for longer than necessary may be a counterstrategy against females considering infanticide. In the present study, laboratory experiments were performed to evaluate the effect of conspecific females on paternal care during the egg-attending period using “with-” and “without-female” treatments. The hatching rates of eggs guarded by males with or without the presence of females were not significantly different. However, the proportion of males on the egg mass was significantly higher when the females were present than when not. We conclude that male behavior may be a counterstrategy against infanticidal females, in which males avoid and reduce the risk of detection by climbing out of the water when females are present.

Keywords Aquatic insect · egg mass destroying behavior · lethocerinae · parental care · sexual conflict

Introduction

Although animal eggs and larvae are at risk of predation, parental care is believed to increase the survival of offspring (Clutton-Brock 1991; Costa 2006). Parental care has defensive functions of protecting eggs from predators, feeding larvae and creating trophic eggs (the function is not reproduction but nutrition for offspring hatched from viable eggs), protecting eggs from fungus, and supplying oxygen to eggs in aquatic animals (Costa 2006; Machado and Trumbo 2018). Protection of offspring by males is well known in several fish and amphibians but is extremely rare in arthropods (Costa 2006). This phenomenon is, however, known in Heteroptera, thrips, sea spiders, millipedes, and harvestmen (Requena et al. 2013).

In the giant water bug (Belostomatidae), lethocerinae females lay an egg mass on the vegetation or branch above the water surface (Cullen 1969; Ichikawa 1989; Smith and Larsen 1993; Smith 1997; Macías-Ordóñez 2003). The male of *Kirkaldyia deyrolli* (formerly known as *Lethocerus deyrollei* or *Lethocerus deyrolli*) periodically supplies egg mass with water until hatching (Ichikawa 1988; Ohba 2002), shields the egg

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mass from direct sunlight if the sunshine during daytime and defence against ants (Ohba and Maeda 2017), and takes care of the hatchlings for approximately half a day (Ohba et al. 2006). In addition, females of this species are known to search for males during the breeding season and, if the males they find are protecting egg masses, they destroy them to lay their own eggs (Ichikawa 1990, 1991). Males who care for egg masses resist attacks by “egg-mass-destroying females” when they are detected (Ichikawa 1990), but two-thirds males were destroyed his egg mass by the females (Ichikawa 1991). However, considering the egg-mass-destroying behavior does not occur if the males cannot be found, it has been considered that the male’s strategy is to position itself on the egg mass rather than in the water at night to avoid detection (Ichikawa 1995). However, it has been pointed out that this observation has not been subjected to appropriate experimental control; it would be useful to test whether males spend more time out of the water when they detect the presence of females than when they do not (Costa 2006). It is necessary to compare the behavior of males engaged in egg mass protection in the presence and absence of females to determine if the behavior of males spending time on egg masses is a counterstrategy against females (Costa 2006).

In the Lethocerinae, male care increases as the egg period progresses (Ichikawa 1995). It has been suggested that male watering behavior is important for egg development (Ichikawa 1988) because an increase in the frequency of male ascents and total duration of staying on the egg mass has been observed at night (Ichikawa 1995). However, there have been no observations of this mechanism during the daytime, when eggs tend to dry out, and such whole-day observations are essential for understanding male behavior in lethocerine species.

The purpose of this study was to determine: (1) whether the male behavior of egg mass protection changes immediately after oviposition until hatching by conducting a controlled experiment with and without the presence of a female near the egg mass, and (2) whether male protective behavior increases as the egg period progresses, both at night and during the day.

Materials and Methods

Insects

Adult male and female *K. deyrolli* were collected from western Hyogo Prefecture, Japan, and reared under laboratory conditions (semi-natural day length in Nagasaki Prefecture, Japan; about 16L:8D cycle hours). Considering this species is listed on the national Red List of Japan, it was difficult to collect enough adults before the reproductive season from a single population. Therefore, emerged-adults in 2020 originated from different egg masses (laboratory-reared adults) and wild adults collected in spring of 2021 from same population were used. Therefore, we expected that adults used here may not have high genetic relatedness. The adults were reared individually in rearing containers (16.7×24.4 cm×10.0 cm), which were filled with activated charcoal (JEX Tyo-Otokuyo, Yashigara Activated Charcoal, GEX Co. Ltd., Osaka). Dechlorinated tap water was added to a depth of 2–3 cm. Goldfish *Carassius auratus auratus* (2–3 cm in standard length) or loach *Misgurnus anguillicaudatus* (5–10 cm in standard length) were fed to adult *K. deyrolli* every other day. The water in the rearing containers was changed every other day. The study was performed in compliance with the ethical provision of Nagasaki University and the current laws of Japan.

This species is univoltine and reproduces between late May and early August, during the East Asian rainy season (Hasizume and Numata 1997). Our observations were conducted from June 10 to August 3, 2021. To obtain the egg mass, a stick (25 mm diameter, 20 mm long) was set up in the reproductive containers (75 cm×45 cm×35 cm) as oviposition sites. A pair of *K. deyrolli* was then introduced into each reproductive container. The female was removed immediately after oviposition. Sticks with the egg masses and the attendant males were each transferred to the plastic container for behavioral observations, ready for observation by 3:00 p.m. The observations were carried out for 21 clutches during the egg developmental period until hatching.

Experiment and Observation

To investigate the effect of females against the paternal behavior of *K. deyrolli*, two treatments were set

up—“with female” ($n=10$) and “without female” ($n=11$). For the “with female” treatment, females barring the mother and with mature eggs (judging by her greenish and swollen abdomen) were used. The plastic container ($23 \times 32 \times 28$ cm, 5 cm deep) containing food (five goldfish), and a cage (insect cage square, $20 \times 12 \times 9$ cm, DAISO) were prepared for behavioral observation (Figs. 1, S1). The water was replaced every three days with dechlorinated tap water. When a female was added to the “with female” treatment, a goldfish was also placed in the cage as food, and additional goldfish were added as required. The plastic containers were set in a room at room temperature at 25°C and irradiated with a reptile light (Reptile UVB 150, JECS Corporation). Daylight hours were set at 16 h (5:30–21:30). The insects were illuminated with a red LED light during night-time (21:30–5:30) (CLEAR LED600, JECS Corporation) to observe their behavior. We assumed that the red lights did not alter insect behavior. The behavioral observations were filmed every 30 min using an interval recorder Recoro (King Jim Co., Ltd.).

The behavior of the attending males (above the water surface on the egg mass or under the water surface) were recorded every 30 min from oviposition until hatching (see Ohba and Maeda 2017). The egg hatchability was recorded once the nymphs hatched from the egg masses.

A generalized linear mixed model (GLMM, the *glmmML* package) with a binomial distribution was used to examine the effect of females, incorporating the number of occurrences of the attending male on the egg mass as a response variable. Each male brooding an egg mass was considered as a random effect. Treatment (with female or without female), light and dark conditions (daytime or night), days before hatching, and their interactions were

incorporated into the GLMM explanatory variables. All the models were compared and ranked according to the Akaike’s Information Criterion (AIC) in the *MuMIn* package in R version 3.6.2 (Barton and Barton 2013). This method compares the explanatory ability of each model using Akaike weights. This may be interpreted as the probability that a given model is the most likely description for the observed data (Burnham and Anderson 2002). The model comparisons were based on their delta AIC, which is the difference between the AIC for each model and the lowest observed AIC value (delta AIC=0 indicated the ‘best’ model). The models with AIC values differing by less than two were considered equivalent (Burnham and Anderson 2002). The egg hatchability in the “with” and “without female” treatment was compared using Exact Wilcoxon rank sum test.

Results

Two models were selected as the best ones based on the AIC (Table 1). In each of the best models (Table 2), days before hatching (DBH), light and dark (LD) condition, treatment (T), DBH*T interaction and LD*T interaction in model 1, and LD, T, DBH*LD interaction, and LD*T interaction in model 2 did not include zero in the 95% confidence interval (CI).

The proportion of males on the egg masses was higher in the “with female” treatment, regardless of the time of day (Fig. 2). The proportion of males on the egg masses increased as the days before hatching decreased (closer to hatching), and males were observed on the egg masses more significantly at night than during daytime. As indicated by the DBH*T interaction, the difference between the “with” and “without female” treatments increased

Fig. 1 Experimental aquarium for behavioral observation

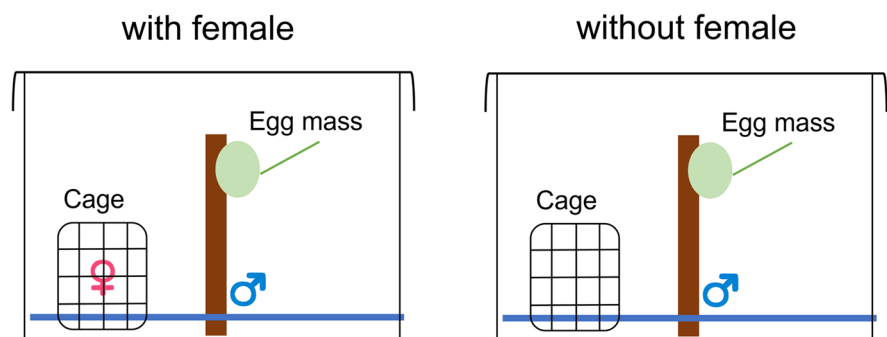


Table 1 Ranking of statistical models that explain paternal care behavior in *Kirkaldyia deyrolli*

Model rank	Model	k^a	AIC ^b	Delta AIC ^c	Akaike weight ^d
1	Day before hatching (DBH) + Light and dark (LD) + Treatment (T) + DBH*T + LD*T	56	2,000.1	0.00	0.49
2	Day before hatching (DBH) + Light and dark (LD) + Treatment (T) + DBH*LD + DBH*T + LD*T	64	2,002.0	1.83	0.20
3	Day before hatching (DBH) + Light and dark (LD) + Treatment (T) + DBH*T	24	2,002.8	2.61	0.13
4	Day before hatching (DBH) + Light and dark (LD) + Treatment (T) + DBH*LD + DBH*T + LD*T + DBH*LD*T	128	2,002.8	2.67	0.13
5	Day before hatching (DBH) + Light and dark (LD) + Treatment (T) + DBH*LD + DBH*T	32	2,004.6	4.47	0.05

The best model to fit the observed data (whose $\Delta AIC \leq 2.0$) is indicated in bold within the top 5 models

^aThe number of parameters

^bAkaike’s Information Criteria (AIC)

^cDifference in the AIC value between model and the model of rank 1

^dAIC weight-relative strength of support for the model

Table 2 Results of the GLMM on the paternal care behaviour in *Kirkaldyia deyrolli*

	Model rank	Model	Estimate	95% C.I.
[†] The coefficient indicates the relative effect of light compared with dark ^{††} The coefficient indicates the relative effect of ‘without female’ compared with ‘with female’ Factors shown in bold do not include 0 in the 95% CI	1	(Intercept)	0.800	(−0.576, 2.176)
		Day before hatching (DBH)	−0.054	(−0.099, −0.009)
		Light and dark (LD)[†]	−4.112	(−4.362, −3.862)
		Treatment (T)^{††}	3.277	(1.144, 5.410)
		DBH*T	−0.266	(−0.337, −0.195)
		LD*T	−0.402	(−0.769, −0.034)
	2	(Intercept)	0.772	(−0.612, 2.156)
		Day before hatching (DBH)	−0.048	(−0.101, 0.006)
		Light and dark (LD)[†]	−4.047	(−4.441, −3.652)
		Treatment (T)^{††}	3.272	(1.135, 5.408)
		DBH*LD	−0.266	(−0.337, −0.195)
		DBH*T	−0.015	(−0.086, 0.055)
		LD*T	−0.404	(−0.772, −0.036)

as the days before hatching progressed, especially during the night-time observations. In addition, as demonstrated by the LD*T interaction, there were no differences in the proportion of males on the egg masses during the daytime, with some days having large differences between treatments and others having small differences. On the other hand, the proportion of males on the egg masses in the “with female” treatment was always higher than that in the “without female” treatment at night.

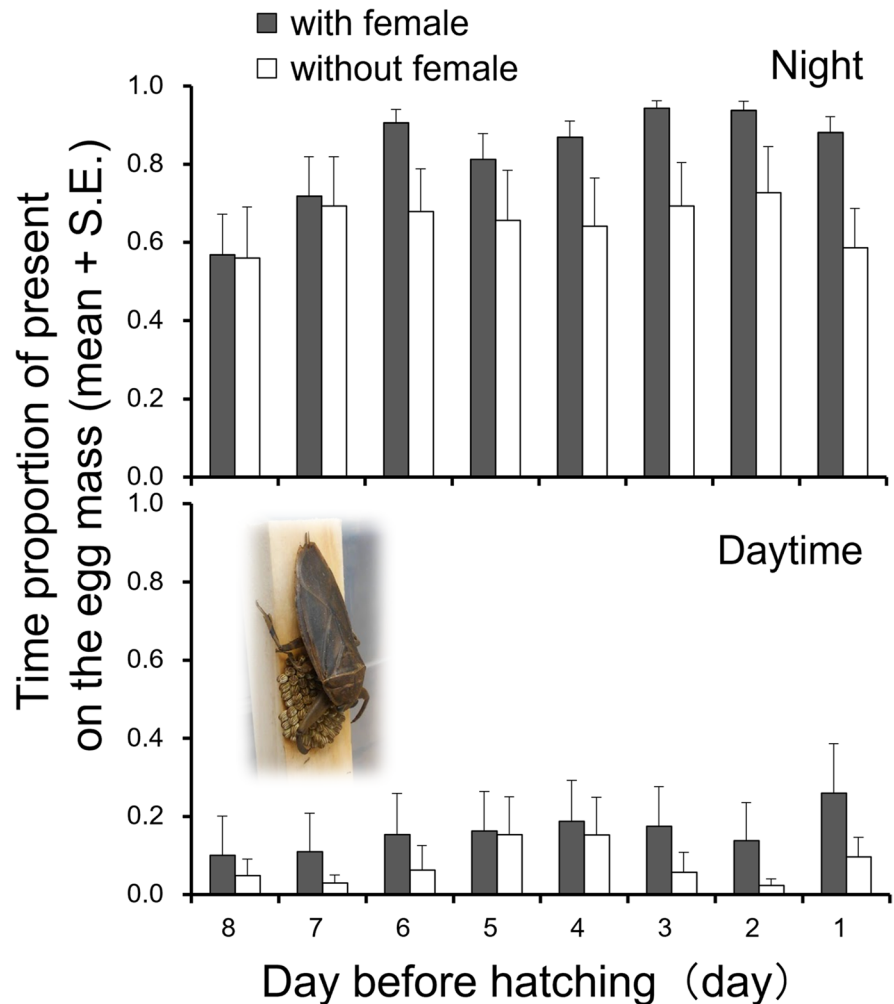
The egg hatchability was $98.0 \pm 4.21\%$ (mean \pm standard deviation, $n=10$) in the “with female” treatment and $93.6 \pm 12.06\%$ ($n=11$) in the “without female” treatment.

There were no significant differences observed between the treatments ($W=45$, $p=0.5439$, Exact Wilcoxon rank sum test).

Discussion

There was no difference observed in the hatchability of the egg masses between the two treatments. However, the proportion of males in the egg masses in the “with female” treatment was higher than that in the “without female” treatment (Fig. 2). It has been previously reported that

Fig. 2 Behavioral comparison of the attending males between the ‘with’ and ‘without females’ treatments



males supply the egg masses with water (Ichikawa 1988) and brood them for longer than necessary (Ichikawa 1990, 1995). However, the results of this study demonstrated that males brood the egg masses for longer than necessary when females are present. In other words, the presence of a female may cause the male to brood the egg mass longer than when the female is not present, thereby reducing the risk of being detected by being in the water. A previous study (Ohba and Maeda 2017) reported that the male brooding behavior did not change by the ant predator approaching; males can protect their egg masses from ants by means of physical and chemical defence. Therefore, conspecific female predators may be more threat than ant predator for male.

Ichikawa (1995) divided the egg brooding period of males into three stages and measured the time males brooded the egg masses by night-time observation. The results demonstrated that the time of brooding the egg mass gradually increased for all the males as egg hatching approached, irrespective of the time. The results of the present study demonstrated a similar trend, and found, for the first time, that the proportion of males on egg masses gradually increase as hatching approaches, even during the daytime, which was not observed by Ichikawa (1995) (Fig. 2). It may be concluded that this behavioral change is due to the increased water demand of the eggs, which increases the frequency of male watering and the time cost of protecting the egg mass, thereby making it more difficult for the female to find the male. Considering this

species is a nocturnal insect, the males are expected to brood the egg masses at night, while the females are more active than during the day. This may be considered a counterstrategy (Ichikawa 1995) for males to avoid detection by climbing out of the water, thereby reducing the risk of detection by females. Additionally, such male behavior shortens the egg period via uniform watering of all eggs, which regulates the hatching synchronization within an egg mass (Ohba 2002) and reduces the risk of egg mass destruction by females.

Author Contribution SO and SK conceived the study; SO designed the methodology; SK collected the data; SO and SK analysed the data, interpreted the results, and wrote the manuscript.

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Data Availability The datasets generated and/or analysed in the current study are available from the corresponding author on reasonable request.

Declarations

Conflict of Interest The authors declare that they have no conflict of interest.

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