J. Mar. Biol. Ass. U.K. (2006), **86**, 1401–1405
Printed in the United Kingdom
doi: 10.1017/S0025315406014433

Combined effect of risk type and activity rhythm on anti-predator response of the shore crab *Gaetice depressus* (Crustacea: Grapsidae)

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The response of the shore crab *Gaetice depressus* to predation risk stimuli of either the octopus *Octopus vulgaris* or crushed conspecifics was examined at two different time phases in the activity rhythm (active and inactive period) by laboratory experiments. When octopus chemical stimuli were introduced to the experimental aquarium, the crab activity decreased in the active period (night) but not in the inactive period (daytime). When the chemical stimuli of crushed *G. depressus* were introduced, the activity of the crabs increased in the inactive period although the stimuli decreased the activity in the active period. This indicates that *G. depressus* adjust their anti-predator response according to a combination of the type of predation risk and also the activity rhythm.

INTRODUCTION

Prey organisms have been shown to respond to predators by increasing their use of refuge, foraging for food at different times and changing activity levels (Wahle, 1992; Burrows & Gibson, 1995). In aquatic environments, prey organisms typically detect predators by chemical stimuli released either by the predator itself (Chivers et al., 1996; Mima et al., 2003) or by damaged conspecifics (Hazlett, 1994). For example, Mima et al. (2003) demonstrated that the hermit crab *Pagurus filholi* change the preference for shell type to superior protective shells under the stimuli of predatory crab. The crayfish, *Orconectes virilis*, ceased all movement in response to alarm stimuli emanating from conspecifics (Hazlett, 1994).

Intertidal and estuarine animals are also known to have a temporal activity rhythm, which might be determined by the diurnal light:dark cycle (Ratchford & Eggleston, 1998) and/or synchronize with the ebb and flow of the tide (Forward et al., 2005). Animals change foraging activity and alter their habitat use according to their activity rhythm. Activity rhythm may also affect the response to predation risk. However, there are few studies that have examined the combined effects of activity rhythm and predator chemical stimuli on the anti-predator response.

Gaetice depressus is a common shore crab in Japan, inhabiting intertidal rocky shores, and the common octopus *Octopus vulgaris*, a predator for *G. depressus*, is often found in the same habitat with the shore crab along the coast of Kyushu, southern Japan. We hypothesized that *G. depressus* may show a behavioural response to the predation threat of *O. vulgaris* chemical stimuli and/or the stimuli emanated from crushed conspecifics. *Gaetice depressus* have clear activity rhythms in males although females do not show a clear pattern; activity time of males is highest in the night and lowest in the daytime (Sakamoto, 2005). The activity rhythm might affect the anti-predator response in the shore crab. Here we examine the response of the male shore crab *G. depressus* to chemical stimuli of the predatory octopus *Octopus vulgaris* and crushed conspecifics at two different time phases (active and inactive period) in the laboratory.

MATERIALS AND METHODS

Male *Gaetice depressus* (15.0–16.9 mm in carapace width (CW)) were collected from the rocky coast of Nokama Island (32°34′N 130°23′E), Ariake Bay, western Japan from late August to early October 2004 to examine the response of crabs to predator risk stimuli in the night and daytime. Prior to each experiment, crabs were kept in a large container (41×31×10 height, cm) with running seawater (26–29°C) for 1–7 days under natural light condition at the Aitsu Marine Station, Kumamoto University. Crabs were not fed during the holding period. We used a female of *Octopus vulgaris* (760 g) as a predator. Prior to the experiment, the octopus was kept in a container with running seawater in the laboratory and was fed *ad libitum* with the oyster *Crassostrea gigas*.

The experiment was conducted in two sets of two plastic aquaria (30×18×24 height, cm) with running seawater. Seawater flowed into the two aquaria through an upstream head tank (30×18×24 height, cm) at 600 ml/min. The head tank and the aquaria were connected with vinyl hose. The bottom of both aquaria was covered with sand (3 cm depth), and a shelter made of a brick (10×8×8 height, cm), was placed in the centre of each aquarium. A small plastic piece (3 cm diameter, 1.5 cm height) was placed under the shelter to make an effective crevice for the crabs. The two aquaria were covered with black poly-

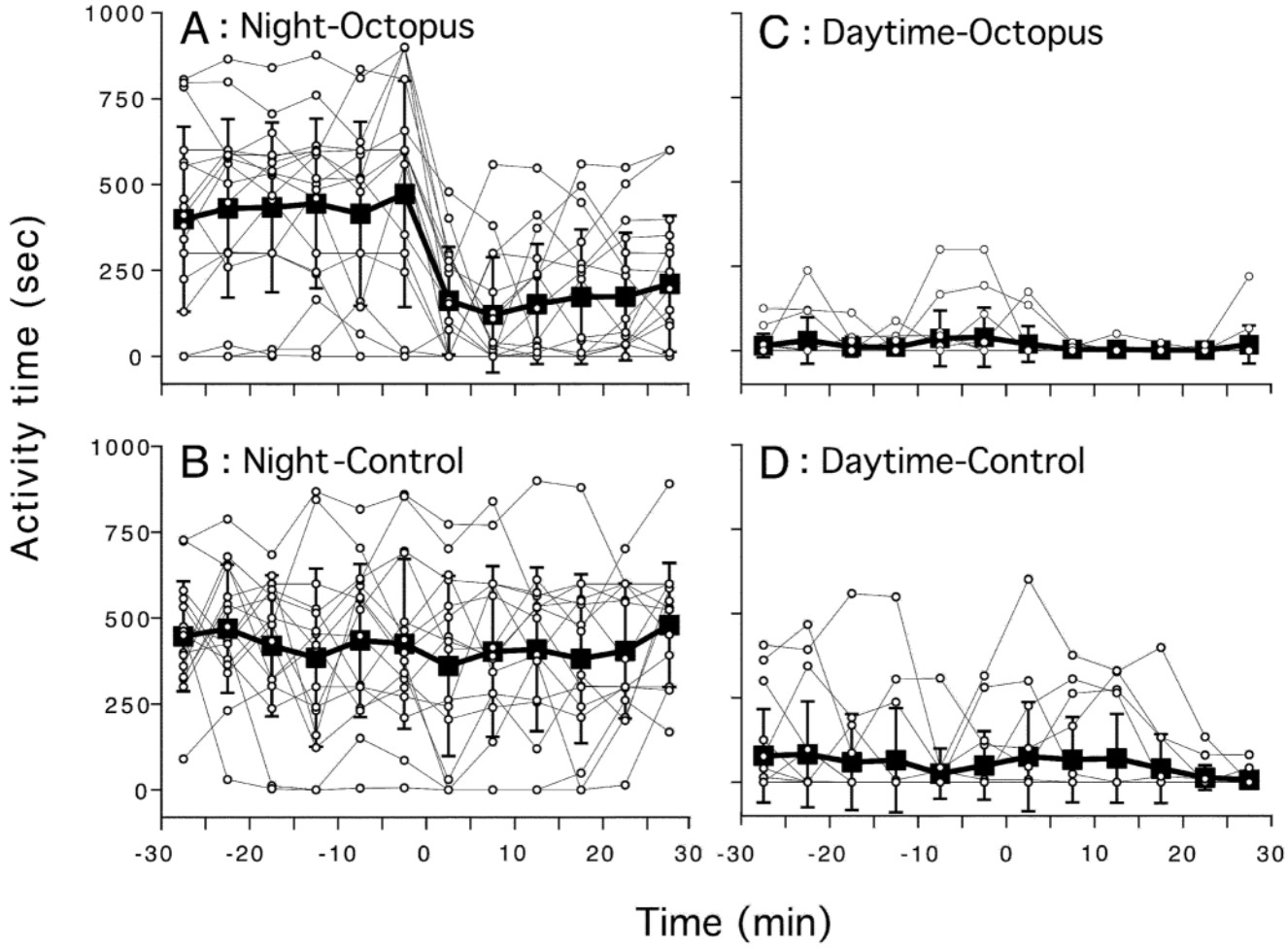

Figure 1. Temporal change of activity time for 30 min before and after initiating chemical stimuli of octopus and control mesh net during night (A, B) and daytime (C, D). Open circles and closed squares indicate total activity times of three shore crabs *Gaetice depressus* for five minutes in each aquarium and the mean of total activity time of all replicates, respectively. Each chemical stimulus was introduced at time 0. Error bars mean standard error.

ethylene plastic sheets during the experiment to reduce any external disturbance.

The experiment involved presentation of the octopus stimuli condition and the conspecific stimuli condition, and each condition had a control condition. We carried out 16 replicates for the octopus condition and 12 replicates were conducted in the conspecific condition. The control replicates were carried out simultaneously with the experimental replicates and therefore the number of replicates was the same as the corresponding experimental conditions. Under the octopus condition, the octopus entered into a mesh net and was placed in the head tank at night (2020–0430) or daytime (0640–1820). An empty mesh net and was placed in the head tank of another set as the control condition. Under the conspecific condition, a crushed male crab *G. depressus* (15.0–16.9 mm in CW) was wrapped with gauze, which was made from cotton, and placed in the head tank, and gauze without a crushed crab was placed under control condition at night or daytime. Newly crushed crabs were used for each trial. The octopus or the crushed crab was removed from the head tank after one hour. The whole experimental set, including the aquaria, the head tank, mesh nets and gauze, was washed and rinsed with running seawater after each trial.

Since the activity time of individual crabs largely varied in our preliminary observation, we used three crabs in each experimental aquarium and measured the total activity time of the three crabs to reduce the effects of the variation in activity time. Three crabs were marked on their carapace using fingernail polish for individual identification. The shore crabs were monitored over a 24 h period with a video camera set (Sony DCR-TRV310K: sensitive in the visible and infrared spectra) placed above the experimental aquaria. During the night-time, two red light sources (25W and 40W) were used to observe the crab similar to other studies (e.g. Spanier et al., 1998). The behaviour of the crabs was recorded on a VHS recorder (Victor model HR-B9).

To examine the anti-predator response in the shore crab, activity time of crabs was recorded every five minutes for 30 min before and after placing either the predator or control stimulus. The activity time was defined as the summed time that the three crabs spent outside the shelter in the aquarium. When the crabs remained motionless for over 30 s outside the brick shelter, we excluded the duration from the activity time. We used two-way repeated-measures analysis of variance (ANOVA) to compare the activity time under control and experimental conditions with time as the within-subject factor and

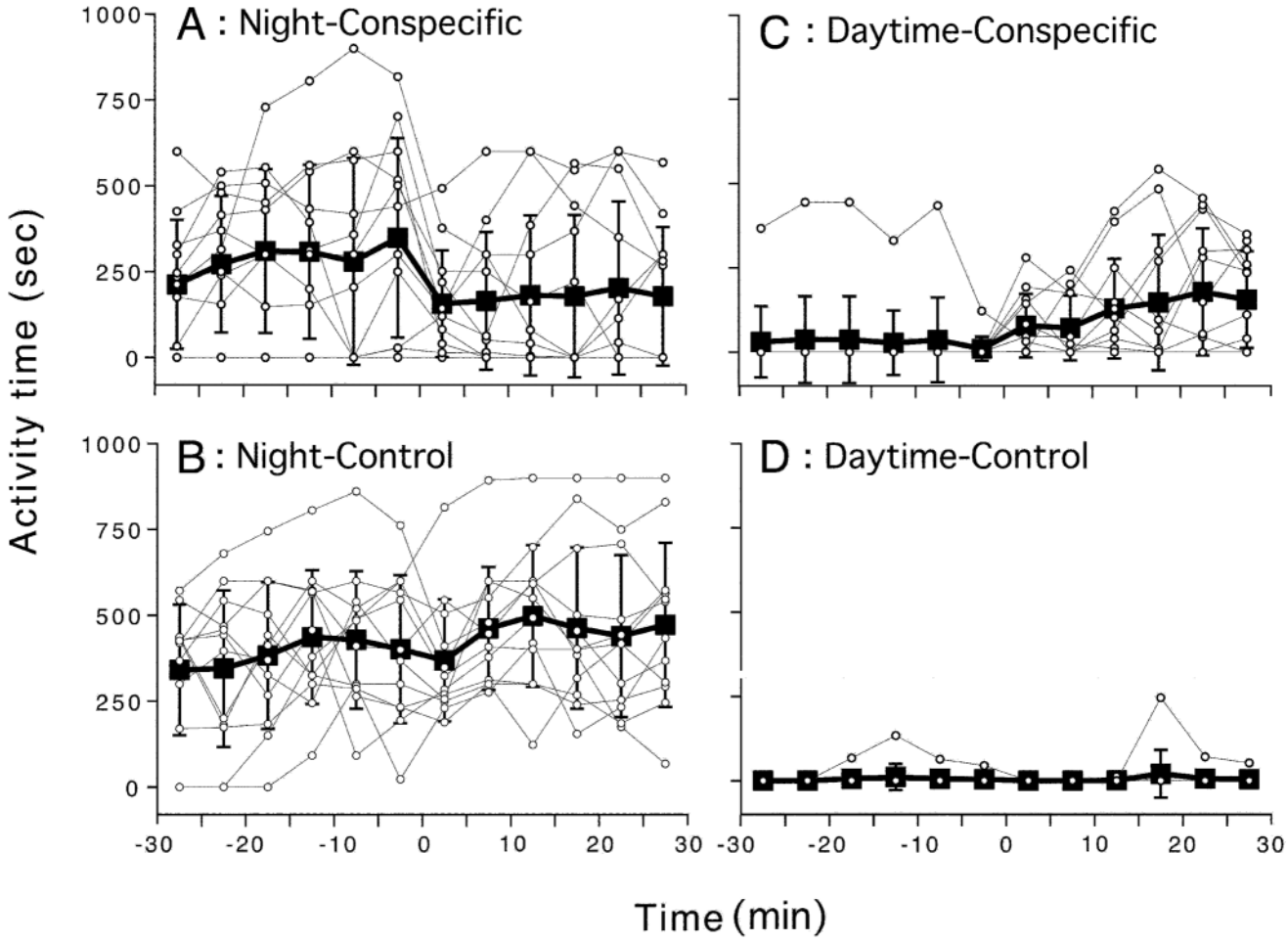

Figure 2. Temporal change of activity time for 30 min before and after initiating chemical stimuli of crushed conspecifics and control gauze during night (A, B) and daytime (C, D). Open circles and closed squares indicate total activity times of three shore crabs *Gaetice depressus* for five minutes in each aquarium and the mean of total activity time of all replicates, respectively. Each chemical stimulus was introduced at time 0. Error bars mean standard error.

condition (control vs experimental) as the between-subject factor. Homogeneity of variance was confirmed with Cochran's test prior to analysis. Data of daytime experiments were logarithmically transformed to meet the assumption of equal variance. The activity times for ten minutes before and after the stimuli were tested by Wilcoxon's signed-rank test to confirm that the addition of chemical stimuli affected the activity time.

RESULTS

Figures 1 and 2 show the temporal changes in activity times for thirty minutes in each aquarium before and after initiating the chemical stimuli of octopus and crushed conspecific, respectively. There was a significant difference in temporal change between octopus stimuli and control conditions during the active period of night (two-way repeated-measures ANOVA, $F_{11,330}=7.06$, $P \leqslant 0.0001$) but not during the inactive period of daytime (two-way repeated-measures ANOVA, $F_{11,330}=1.01$, $P=0.43$) (Table 1). The activity time decreased after the predatory stimuli of the octopus stimuli condition during night-time (Figure 1A). Significant differences in temporal change were also found for the crushed conspecific stimuli (two-way repeated-measures ANOVA, night: $F_{11,242}=2.10$, $P=0.02$, daytime: $F_{11,242}=5.24$, $P<0.0001$) (Table 1). However, the temporal pattern of the night experimental condition was a contrast to those of daytime. While the activity time decreased after adding the chemical stimuli of the crushed conspecific during night-time (Figure 2A), it increased during daytime (Figure 2C).

When we compared the activity times for ten minutes before and after the stimuli by Wilcoxon's signed-rank test, the activity time significantly decreased after the introduction of octopus stimuli at night (Wilcoxon's signed-rank test, N=16, $T=4.5$, $P<0.01$). However, no significant difference was detected under the octopus condition in the daytime (Wilcoxon's signed-rank test, N=16, $T=36.5$, $P>0.05$). The activity time decreased significantly after the introduction of crushed crab stimuli at night (Wilcoxon's signed-rank test, N=12, $T=10.5$, $P<0.05$). On the other hand, the activity time increased in eight of 12 replicates under the conspecific condition in the daytime. Although no significant difference was detected under this condition (Wilcoxon's signed-rank test, N=12, $T=15$, $P>0.05$), the activity of one replicate was extremely high before the introduction of chemical stimuli (Figure 2C). We found a significant increase in the activity time when the value of this trial replicate was excluded from the statistical dataset as an outlier

Table 1. *Repeated-measures ANOVA table for examining the temporal change between control and experimental conditions.*

		Octopus				Conspecific			
		Daytime*		Night		Daytime*		Night	
Source	df	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*
Treatment	1	1.360	0.2527	2.811	0.1040	25.832	<0.0001	8.701	0.0074
Time	11	2.132	0.0179	10.015	<0.0001	5.406	<0.0001	1.026	0.4241
Treatment×Time	11	1.012	0.4348	7.061	<0.0001	5.244	<0.0001	2.102	0.0209

*, logarithmically transformed.

(Wilcoxon's signed-rank test, N=11, $T=3$, $P<0.01$). There was no significant difference in all control trials (Wilcoxon's signed-rank test, N=16, 16, 12, 12, $T=51.5$, 93, 39, 33.5, $P>0.05$, respectively).

DISCUSSION

Gaetice depressus exposed to the octopus chemical stimuli decreased the activity time during night-time. Similar results have been reported in other crustaceans (e.g. Hazlett, 1997). The crayfish *Orconectes virilis* decreased movement due to predator stimuli (Hazlett & Schoolmaster, 1998). Palma & Steneck (2001) reported that fleeing might be more hazardous than hiding if the predator is a bird or fish since they use visual signals to locate their food. Boycott & Young (1950) suggested that the movement of prey animals is an important factor to elicit attack behaviour of octopus. Therefore, the decreasing activity time of *G. depressus* at night could be effective to avoid predatory octopus. A species of octopus, *Octopus cyanea*, usually forages once early in the morning and once late in the afternoon (Forsythe & Hanlon, 1997). Although there was no obvious change of activity time in the daytime, it might be considered that the crabs remained inactive under the condition of the octopus stimuli in order to remain hidden from the field of vision of the octopus.

In the crushed crab stimuli experiment, the crabs decreased activity time during the night-time as in the octopus odour experiment. Several crustaceans, such as the crayfish, *Orconectes virilis*, the anomuran *Petrolisthes elongatus* and the brachyuran *Notomithrax ursus*, and *Cyclograpsus lavauxi*, also decrease locomotion when exposed to crushed conspecific odour (Hazlett, 1994, 2000). The decrease of activity in our results can thus be considered as an anti-predator behaviour. However, the response to the crushed conspecific stimuli in activity time was not consistent between night and daytime. During the daytime, we observed that crabs emerged from under the brick and started moving in a similar manner to foraging behaviour. Since *G. depressus* often forage on crushed conspecifics (R. Sakamoto, personal observation), the increased activity of this species may be regarded as a response to the detection of food. The crayfish, *O. virilis* increases activity and decreases use of burrows when chemical stimuli of food are given (Hazlett, 1999). *Clibanaris vittatus* also increases locomotion following introduction of food stimuli (Hazlett, 1996). In contrast, during the night, crabs decreased activity in response to the same chemical stimuli, as stated above. Thus, the stimuli of crushed conspecifics might function as two distinct chemical signals in the two time phases, that is, food stimuli during daytime and predator stimuli during night-time.

Another hypothesis to explain this lack of consistency is a context dependent anti-predator response. Effective anti-predator behaviour (flee or hide) might differ with the type of predator. Although the chemical stimuli of crushed conspecifics would imply the existence of risk, crabs might not be able to specify the type of predator only from the chemical stimuli of conspecifics. In this case, changing the habitat when they detect the chemical signal might be effective to avoid the potential risk. When the crabs were not exposed to any predatory signals, their habitat differed between night and daytime due to the activity rhythm. While crabs moved around or on the brick at night, they were under the brick during the daytime. After detection of crushed conspecifics stimuli, crabs might change their habitat and consequently show a different response to the stimuli between daytime and night.

Not only the biological environment, such as predators and competitors, but also the physical environment, such as the light cycle, tidal cycle and temperature, can change animal behaviour. Our results differed during the night and the daytime in response to the predation risk stimuli. Burrows & Gibson (1995) reported that the expression of behaviour reflects the relative contributions of activity rhythm and endogenous influences (e.g. food and predator stimuli). We suggest that the activity rhythm of an animal could induce a different activity change under predation risk stimuli, which may lead to a better understanding of animal behaviour.

We thank Professor Keiji Baba of Kumamoto University for valuable comments on an early draft and help during this study. We are grateful to Dr K. Nakata and Dr T. Oba of Hokkaido University and Dr C. Norman for their constructive comments on the final draft.

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Submitted 22 May 2006. Accepted 24 October 2006.