Journal of Marine Animals and Their Ecology

Vol 6, No 2, 2013
Printed in Canada

Effects of crushed conspecifics and starvation periods on the foraging behavior of the marine gastropod sea snail *Nassarius fraterculus*

Yue Zhang[1], Seiji Goshima[1]
[1]Graduate School of Fisheries Sciences, Hokkaido University, Hakodate, Hokkaido

Abstract

The effects of different starvation periods and of crushed conspecifics on the foraging behavior of the marine gastropod sea snail known as *Nassarius fraterculus (N. fraterculus)* were examined. In the first experiment, the starvation tolerances of different-sized males and females were determined. In the second experiment the foraging behaviors, including feeding percentage, searching time, eating time, and eating frequency were examined after different starvation periods (3, 6 and 12 days) in two groups: one exposed to chemical cues from crushed mussels (its normal prey) and another exposed to chemical cues from crushed conspecifics. The starvation tolerance was significantly affected by body size but not sex. Cues from crushed conspecifics caused a significant decrease in the percentage of *N. fraterculus* that fed and a significant increase in the time spent searching for food. As the starvation periods increased, these two effects subsided. The time spent eating and the eating frequency were affected only slightly by crushed conspecifics in all starvation periods. These results show that chemical cues from crushed conspecifics inhibited some foraging behaviors.
[JMATE 2013;6(2):20-27]

Keywords: Scavenger, starvation tolerance, predation risk, feeding percentage, searching time

Introduction

Decisions made by animals while foraging are influenced by both internal factors such as hunger and health status and external factors such as the presence of predators and prey (4, 22). For example, starving organisms can improve their energy intake using various methods such as increasing their foraging speed and increasing their foraging time (1, 14, 29). These behaviors can increase both the foraging efficiency and the animal's fitness (15).

While foraging, organisms must also defend against predators (18). Important cues that can be used to detect a predator include chemical cues from the predator, as well as cues from damaged or dead conspecifics that have been preyed on (12, 14). If an animal judges the risk of predation to be higher than the risk of starvation, it might choose to give up foraging. But if the risk of starvation is greater, it might choose to forage even if predators are present (22). So chemical cues from both predators and prey are important when an animal decides if and when it will feed (21).

Chemical cues are typically released during a predation event and, thus, can be important ecological indicators of predation risk (8, 25). Many species display anti-predator behavior in response to injury-released chemical cues from conspecifics (10). An example is the scavenger whelk (*Buccinanops globulosum*), which will stop feeding and leave its food when it detects chemical cues from crushed conspecifics (7). When these species detect cues from a conspecific, they must weigh the risk of predation against the need to feed, which will depend on how well-fed the animal is. So the feeding state of an animal will presumably influence its decision to forage or not.

Nassarius fraterculus (N. fraterculus) is an intertidal scavenger snail that is widely distributed in the northwest Pacific including Japan and has a high starvation tolerance (11). Field investigations have shown it forages on crushed mussels (*Septifer virgatus*) and can quickly and accurately identify the location of food (5, 27). Because of its ability to detect chemical cues, *N. fraterculus* is an excellent model for investigating how organisms adopt a foraging strategy in the presence of different carrion.

The purpose of the present study was to determine if the starvation tolerance of the *N. fraterculus* is affected by body size and sex and if the presence of crushed conspecifics influences its foraging behavior.

Materials and Methods

Two experiments were conducted as follows. The survival of starving animals can be affected by physical characteristics such as body size and sex (16, 26). To account for this, in the first experiment, how long

Received January 24, 2014; Accepted February 25, 2014
Correspondence: Yue Zhang
Graduate School of Fisheries Sciences, Hokkaido University, 3-1-1 Minato-cho, Hakodate, Hokkaido 041-8611, Japan
Email: nmtlzy@126.com ; Phone: +81-138-40-5549

different-sized males and females could survive without feeding was examined. Based on the results of this experiment, and to minimize foraging behavioral differences between individuals because of body size and sex, a second experiment was conducted using only large males and females to observe their foraging behavior when offered either crushed conspecifics or crushed mussels following three different starvation periods. Details regarding the experiments are as follows.

Collection and maintenance of animals

Experiments were conducted during October-November 2012 and April-May 2013 using *N. fraterculus* collected from the shore at Shinori, Hakodate, Hokkaido, Japan (41°45′N, 140°49′E), where these snails live buried in sand 1-5 cm below the surface at the low-water, neap-tide level. Crushed mussels (*S. virgatus*) were used as food to draw *N. fraterculus* to the surface, where they were collected with forceps. The snails were transported to the Graduate School of Fisheries Sciences, Hokkaido University in Hakodate, maintained in a tank supplied with circulating, filtered seawater with a salinity of 34 psu at 10°C, and fed crushed mussels *ad libitum* once a day for at least one week before the start of the experiment.

Experiment 1: Starvation tolerance

During October-November 2012, an experiment was conducted to determine how long starved *N. fraterculus* could survive. The snails were acclimated for one week before the start of the experiment, and after the final feeding, they were measured (total length of the shell) and divided into three groups based on their body size (mean body size ± SD): small (S, 3.55 ± 0.17 mm), medium (M, 6.71 ± 1.17 mm) and large (L, 11.61 ± 2.00 mm). Each size group comprised five males and five females. Each individual was separately placed in a transparent plastic box (length × width × height = 150 mm × 100 mm × 110 mm) containing static artificial seawater with a salinity of 34 psu at 10°C, which was replaced daily, and exposed to a 12:12 h light-dark cycle (day: 8:00 am - 8:00 pm; night: 8:00 pm - 8:00 am). Then these snails were not fed and during the 51-day starvation experiment (the time period it took for all the snails to die), the snails were observed daily and removed if they died.

Experiment 2: Comparison of foraging behavior using crushed mussels or crushed conspecifics as food

Based on the results of Experiment 1 large *N. fraterculus* with a mean body size of 13.6 ± 1.33 mm in both sexes were used to examine the effects of chemical cues from crushed conspecifics and from crushed mussels on the foraging behavior of these snails. Factors measured included the feeding percentage (the percentage of snails that fed), searching time (the time it the snails to reach the food), eating time (how long the snails fed), and eating frequency (the number of times the snails fed during the 30 minutes after it first started feeding).

N. fraterculus were kept in a tank (length × width × height =600 mm × 300 mm × 500 mm) for one week and fed crushed mussels *ad libitum* every day. The mussels used in this experiment were frozen at -20°C for preservation and thawed two hours before feeding. A total of 240 *N. fraterculus* were then placed individually in translucent plastic cases (length × width × height =130 mm × 90 mm × 70 mm) containing static artificial seawater with a salinity of 34 psu at 10°C, which was changed daily. They were fed crushed mussels daily for one more week until the start of the experiment. The 240 snails were then divided into two feeding conditions to observe their behaviors – one fed crushed mussels and another fed crushed conspecifics. Each feeding condition was divided into three groups, each comprising 40 individuals that were starved for 3, 6 or 12 days. During the acclimatization period, it was noted that *N. fraterculus* normally fed about once every 3 days, so a 3-day period was chosen to simulate normal feeding conditions. The two longer periods (6 and 12 days) are less than half of the starvation tolerance of these snails (discussed below), so they were chosen to observe the effects of starvation without harming the animals.

After these starvation periods, the feeding experiments began. In each experiment, a snail was moved to one corner of its box containing static seawater. Food (either crushed mussels or crushed conspecifics) was put in the opposite corner, and then the feeding percentage, searching time, eating time, and eating frequency were observed visually. The snails that did not feed within 30 minutes after the food was introduced were classified as "not feeding" and were not observed further (this time period was judged as a sufficient for chemical cues from the food to diffuse throughout the container and for the snail to then decide whether or not to feed). The snails that did feed were then observed for another 30 minutes for a total observation time of 1 hour. therefore, sixty minutes (1 hour) after the start of the experiment, the snails were classified as either "feeding" or "finished feeding".

Data analysis

In Experiment 1, results from the three size groups (10 snails in each group) and from both sexes (15 of each sex) were analyzed using the Kaplan-Meier method of survival analysis to determine the mean survival tolerance (in days) and to determine if the mean survival tolerance was affected by body size or sex (28). Moreover, pairwise comparisons of body size were performed using log rank test statistics (χ^2).

In Experiment 2, the Kaplan-Meier method and correlation analysis (Pearson correlation coefficient) were used to analyze the data, but survival time was replaced in turn by searching time, eating time and eating frequency. The factors were food type and starvation periods. An independent samples 't' test (t) was used to analyze the variance of feeding percentage at a fixed starvation period and food type.

In all data analyses, the significance level was set at $P \leq 0.05$.

Results

Effects of body size and sex on survival during starvation

The mean lengths of survival were 37 ± 1.5 days in the small group, 42 ± 2.0 days in the medium group, and 41 ± 1.7 days in the large group. The lengths differed significantly among groups ($\chi^2 = 7.32$, $P = 0.026$), and pairwise comparisons between body size groups showed that the small (S) group differed significantly from both the medium (M, $\chi^2 = 4.20$, $P = 0.041$) and large (L, $\chi^2 = 3.88$, $P = 0.049$) groups. Survival decreased faster in the S group than in the M and L groups (Figure 1a), while the M and L groups did not differ significantly ($\chi^2 = 1.06$, $P = 0.304$). The mean length of survival was 40 ± 1.5 days for both males and females ($\chi^2 = 0.05$, $P = 0.816$). The survival curves of males and females were similar (Figure 1b).

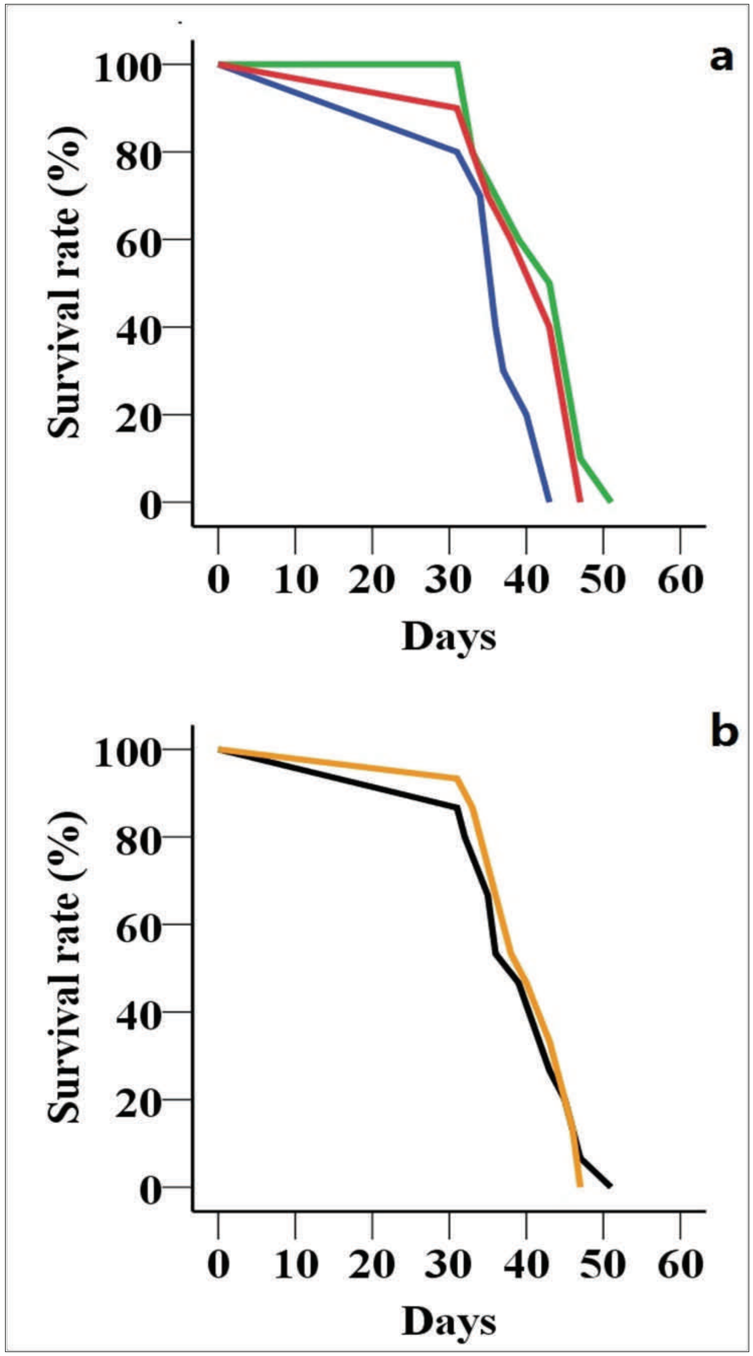

Figure 1: Survival curves of starved *N. fraterculus*. (a) Three size group (S = 3.55 ± 0.17 SD mm; M = 6.71 ± 1.17 mm; L = 11.61 ± 2.00 mm). Each group comprised ten *N. fraterculus*. Blue line: small size group; green line: middle size group; red line: large size group. (b) Males vs females. Each sex comprised fifteen *N. fraterculus*. Black line: male group; orange line: female group.

Effects of crushed conspecific and starvation period on foraging details

Significantly fewer *Nassarius fraterculus* fed on crushed conspecifics than on crushed mussels (Figure 2a, t = 6.05, P < 0.01). Those fed conspecifics had a longer searching time than those fed mussels (Figure 2b, $\chi^2 = 19.62$, P < 0.01). The eating times for snails offered crushed conspecifics and for those offered crushed mussels did not differ significantly (Figure 2c, $\chi^2 = 0.56$, P = 0.46). The eating frequency was slightly lower in snails that fed on crushed conspecifics than in those that fed on crushed mussels, but the difference was not significant (Figure 2d, $\chi^2 = 3.04$, P = 0.81).

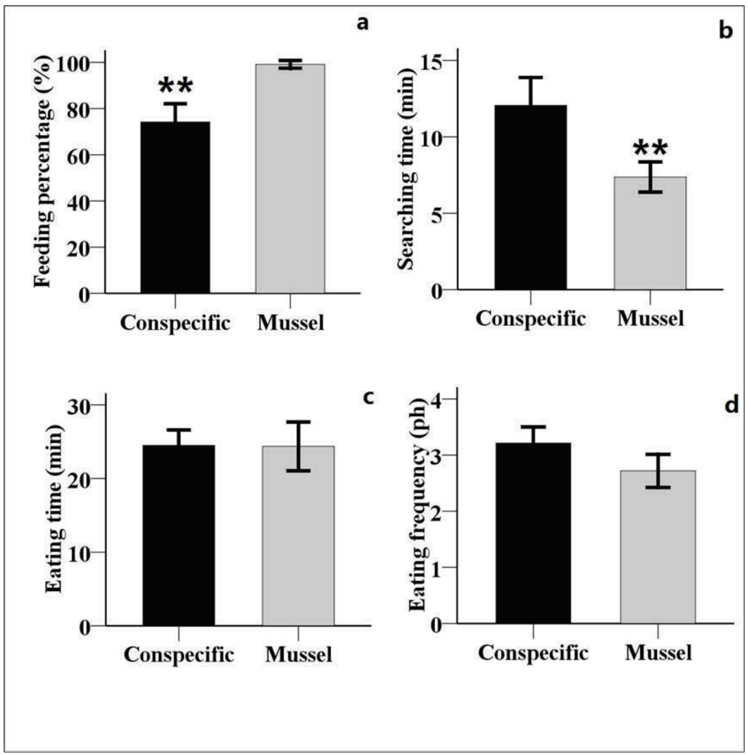

Figure 2: Comparisons of (a) feeding percentage (% of *N. fraterculus* that fed), (b) searching time, (c) eating time, and (d) eating frequency (ph=per hour) for *N. fraterculus* fed conspecific and mussels. Data were analyzed for about 120 replicates in each food type group. Error bars indicate the 95% confidence interval. Black bar: conspecific group; grey bar: mussel group; **P < 0.01.

For snails offered crushed mussels, the feeding percentage was nearly 100% for each starvation group. But for those offered conspecifics, the percentages in the three starvation groups ranged from 55 to 88% (Figure 3a) and was lowest in the 3-day starvation group (Figure 3a, t = 5.71, P < 0.01). Snails offered conspecifics spent a significantly longer time (17.7 minutes) searching for prey than those offered mussels (6.2 minutes) (Figure 3b, $\chi^2 = 28.75$, P < 0.01). The eating times for snails offered crushed conspecifics and for those offered crushed mussels did not differ significantly in any starvation group. In the 3 and 6-day starvation groups, the eating time for snails offered mussels, was slightly longer than for those offered conspecifics. However, in the 12-day starvation group, the eating time was shorter in snails that fed on mussels (Figure 3c). As the starvation period increased, the difference in eating frequency between the crushed mussel group and the crushed conspecific group decreased (Figure 3d).

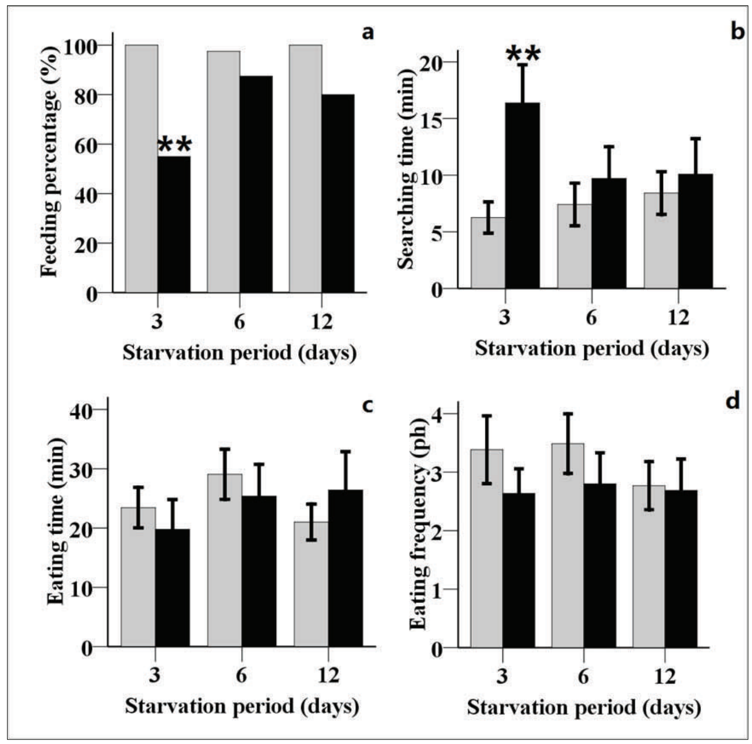

Figure 3: Comparisons of (a) feeding percentage (% of *N. fraterculus* that fed), (b) searching time, (c) eating time, and (d) eating frequency (ph=per hour) between *N. fraterculus* fed crushed conspecific and those fed crushed mussels follows three different starvation periods. Data were analyzed for about 40 replicates in each treatment group. Error bars indicate the 95% confidence interval. Black bar: conspecific group; grey bar: mussel group; **P < 0.01.

The feeding percentage of *N. fraterculus* was correlated positively with starvation period (Figure 4a, Pearson correlation = 0.125, P = 0.05). The searching time showed no clear trend with increasing starvation period (Figure 4b, Pearson correlation = -0.82,

P = 0.21). Also no correlation was observed between the eating time and starvation period (Figure 4c, Pearson correlation =0.00, P = 0.99). There was a negative but insignificant correlation between eating frequency and starvation period (Figure 4d,
Pearson correlation = -0.12, P = 0.09).

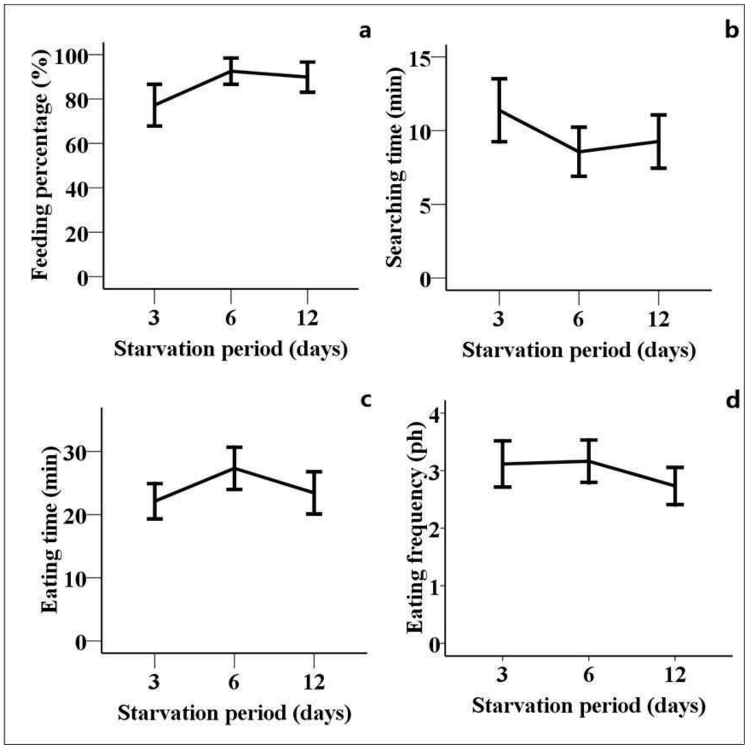

Figure 4: Comparisons of starvation period and (a) feeding percentage (% of *N. fraterculus* that fed), (b) searching time, (c) eating time, and (d) eating frequency (ph=per hour) for *N. fraterculus* fed both crushed conspecifics and mussels. Data were analyzed for about 80 replicates in each starvation period. Error bars indicate the 95% confidence interval.

The feeding percentage in the crushed conspecific group starved for 3 days was significantly lower than in the group starved for 6 days (Figure 5a, t = 5.71, P < 0.01) and 12 days (Figure 5a, t = 3.13, P < 0.01). For snails offered crushed mussels, however, the feeding percentage varied little among starvation groups (Figure 5a, t = 0.98, P = 0.33). The searching time for snails offered crushed mussels increased with increasing starvation period, but the correlation was also insignificant (Figure 5b, Pearson correlation = 0.11, P = 0.32). However, the searching time for snails offered crushed conspecifics and starved for 3 days was significantly longer than that for snails starved for 6 days (Figure 5b, $\chi^2 = 10.67$, P = 0.001) and 12 days (Figure 5b, $\chi^2 = 6.94$, P = 0.008). In the groups fed crushed mussels, the eating time for snails starved for 12 days (21 minutes) was significantly shorter than for those starved for 6 days (29 minutes) (Figure 5c, $\chi^2 = 8.28$, P = 0.004), but in groups fed crushed conspecifics, the eating time did not differ significantly among starvation groups (Figure 5c, $\chi^2 = 3.97$, P = 0.14). For snails that fed on crushed mussels, the eating frequency in the group starved for 12 days (2.76 per hour) was significantly lower than in the group starved for 6 days (3.56 per hour) (Figure 5d, $\chi^2 = 4.40$, P = 0.03).

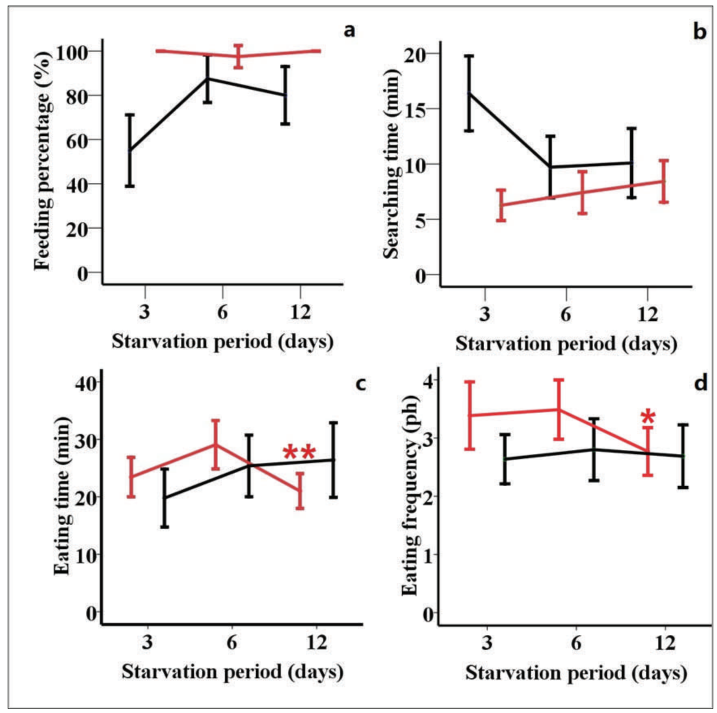

Figure 5: Comparisons of starvation period and (a) feeding percentage (% of *N. fraterculus* that fed), (b) searching time, (c) eating time, and (d) eating frequency (ph=per hour) between *N. fraterculus* fed crushed conspecific and those fed crushed mussels follows three different starvation periods. Data were analyzed for about 40 replicates in each treatment group. Black line: conspecific group; red line: mussel group; *P < 0.05; **P < 0.01.

Discussion

Starvation tolerances vary among *Nassarius* species. In Hong Kong, the intertidal species known as *Nassarius festivus* can survive 20 days, while the subtidal species known as *Nassarius siquijorensis* can survive only 7 days (6,17). This difference may be related to differences in food availabilities in the two zones (20). In the present study, the intertidal species, *N. fraterculus*, was found to survive about 40 days. This much longer survival period than in the Hong Kong species could be due to differences in temperature between Hong Kong and our study site. Invertebrate poikilotherms have lower metabolic rates in cold temperature, which could prolong the period of starvation tolerance.

The starvation tolerance of animals can be affected by many other factors, such as physiology, body size, age and sex (3). In our study, the average starvation tolerance period for unfed individuals was two days shorter in the small size group than that in the middle and large size groups. This result is consistent with previous research on the whelk *Biomphalaria alexandrina*, in which the lower tolerance of this whelk to starvation was attributed to its smaller body size containing a smaller amount of stored nutrients (9). Small animals also tend to have a larger body surface relative to their mass, so many have a higher metabolic rate than larger animals (known as body surface law) (2). Differences in the amount of stored nutrients and in metabolic rates between the size groups could explain why the starvation tolerance period was shorter in small individuals.

Significantly more *N. fraterculus* fed on crushed mussels than on crushed conspecifics. Many aquatic species, including fishes, amphibians and mollusks, show an alarm or escape response when they encounter chemical cues from the injured tissues of crushed conspecifics, presumably as a response to possible predation (13). Stenzler also reported that three snail species (*N. obsoletus*, *N. vibex* and *N. trivittatus*) respond to crushed conspecifics by leaving food, which could be a defense mechanism against predation (25). Our results suggest *N. fraterculus* also displays this response.

As the starvation periods increased, however, the number of *N. fraterculus* that fed on crushed conspecifics increased, which suggests they became more willing to feeding on conspecifics when there is a shortage of food. Similar results were previously reported in it another similar species *Nassarius dorsatus*, which was found to begin feeding on conspecifics after 10 days of starvation (19). Morton *et al*. similarly showed that the *N. festivus* reacted differently to crushed conspecifics depending on how long it had been starved (23).

In the groups starved for three days, *N. fraterculus* offered crushed conspecifics spent more time searching for prey than individuals offered crushed mussels. *N. fraterculus* offered crushed conspecifics also made a less direct path to the prey than those offered mussels. But as the starvation period increased from 3 to 6 days, the searching time in *N. fraterculus* offered crushed conspecifics decreased significantly from 17.7 to 10.2 minutes. For *N. fraterculus* fed crushed mussels, the searching time was shorter and varied little (7.5 minutes) after the different starvation periods (Figure 2). This suggests that as the risk of starvation increases, it might override the fear of predation, causing them to feed more quickly on conspecifics (22).

Many organisms increase their eating time after a period of starvation (24). However, the opposite result was observed in the *N. fraterculus* which decreased its eating time after 12 days starvation. All three starvation groups consumed similar amount of food, but snails starved for 12 days spent less time eating, indicating that they fed faster. A similar phenomenon has been observed in starved individuals of *N. festivus*, which finished feeding faster than well-fed ones (23).

As noted above, an animal may increase its exposure to predation when it feeds. So when the predation risk is high, an animal might choose to decrease its eating frequency (29). In the 3- and 6-day starvation groups, the eating frequency was lower in those offered conspecifics than in those offered mussels. This could result if *N. fraterculus* perceives the chemical cues from a conspecific as a danger signal.

Acknowledgements

We thank R. Nakayama and the other members of the Benthos Laboratory at the Graduate School of Fisheries Sciences, Hokkaido University, for their assistance. It was always a great pleasure to work with them. The professional guidance, critical input, and teaching of S. Wada and J. Bower were greatly appreciated.

References

1. Beecham JA, Farnsworth KD. Animal foraging from an individual perspective: an object orientated model. *Ecological Modelling* 113(1): 141-156. 1998.
2. Blumberg MS. Temperature: a user's guide. In *Body Heat: Temperature and Life on Earth,* edited by Blumberg, MS: Harvard University Press, pp. 33-41. 2002.
3. Borchers P, Hutchings L. Starvation tolerance, development time and egg production of *Calanoides carinatus* in the Southern Benguela Current. *Journal of Plankton Research* 8(5): 855-874. 1986.
4. Brown K M, Alexander Jr JE. Group foraging in a marine gastropod predator: benefits and costs to individuals. *Marine Ecology Progress Series* 112(1): 97-105. 1994.
5. Carlton JT. Introduced marine and estuarine mollusks of North America: an end-of-the-20th-century perspective. *Journal of Shellfish Research* 11(2): 489-505. 1992.
6. Cheung SG. Feeding behavior and activity of the scavenging gastropod *Nassarius festivus* (Powys). In: *The Malacofauna of Hong Kong and Southern China,* edited by Brian Morton, pp. 327-338. 1994.
7. Daleo P, Alberti J, Avaca MS, Narvarte M, Martinetto P, Iribarne O. Avoidance of feeding opportunities by the whelk *Buccinanops globulosum* in the presence of damaged conspecifics. *Marine Biology* 159(10): 2359-2365. 2012.
8. Davenport J, Moore PG. Behavioral responses of the netted dogwhelk *Nassarius reticulatus* to olfactory signals derived from conspecific and nonconspecific carrion. *Journal of the Marine Biological Association of the UK* 82(06): 967-969. 2002.
9. El-Emam MA, Madsen H. The effect of temperature, darkness, starvation and various food types on growth, survival and reproduction of *Helisoma duryi*, *Biomphalaria alexandrina* and *Bulinus truncatus* (Gastropoda: *Planorbidae*). *Hydrobiologia* 88(3): 265-275. 1982.
10. Ferrari MCO, Wisenden BD, Chivers DP. Chemical ecology of predator-prey interactions in aquatic ecosystems: a review and prospectus. *Canadian Journal of Zoology* 88(7): 698-724. 2010.
11. Gillespie GE, Phillips AC, Paltzat DL, Therriault . Distribution of non-indigenous intertidal species on the Pacific Coast of Canada. *Nippon Suisan Gakkaishi* 73(6): 1133. 2007.
12. Gonor JJ. Predator-prey reactions between two marine prosobranch gastropods. *Veliger* 7(4): 228-232. 1965.
13. Hadlock RP. Alarm response of the intertidal snail *Littorina littorea* (L.) to predation by the crab *Carcinus maenas* (L.). *The Biological Bulletin* 159(2): 269-279. 1980.
14. Kats LB, Dill LM. The scent of death: chemosensory assessment of predation risk by prey animals. *Ecoscience* 5(3): 361-394. 1998.
15. Large SI, Smee DL. Type and nature of cues used by *Nucella lapillus* to evaluate predation risk. *Journal of Experimental Marine Biology and Ecology* 396(1): 10-17. 2010.
16. Large SI, Torres P, Smee DL. Behavior and morphology of *Nucella lapillus* influenced by predator type and predator diet. *Aquatic Biology* 16(2): 189-196. 2012.
17. Liu JH, Morton B. Scavenging by the subtidal *Nassarius siquijorensis* (Gastropoda: Nassariidae) in Hong Kong. In: *Proceedings of the third international workshop on the malacofauna of Hong Kong and southern China,* edited by Brian Morton: Hong Kong University Press, pp. 357-375. 1994.
18. Manríquez PH, Elisa JM, Opitz T, Carlos CJ, Lagos NA. Effects of predation risk on survival, behavior and morphological traits of small juveniles of *Concholepas concholepas* (loco). *Marine Ecology Progress Series* 472: 169-183. 2013.
19. McKillup SC, McKillup RV. The decision to feed by a scavenger in relation to the risks of predation and starvation. *Oecologia* 97(1): 41-48. 1994.

20. McKillup SC, McKillup RV. Effect of food supplementation on the growth of an intertidal scavenger. *Marine Ecology Progress Series* 148(1): 109-114. 1997.
21. Morton B. Observations on the feeding behavior of *Nassarius clarus* (Gastropoda: Nassariidae) in Shark Bay, Western Australia. *Molluscan Research* 23(3): 239-249. 2003.
22. Morton B, Chan K. Hunger rapidly overrides the risk of predation in the subtidal scavenger *Nassarius siquijorensis* (Gastropoda: *Nassariidae*): an energy budget and a comparison with the intertidal *Nassarius festivus* in Hong Kong. *Journal of Experimental Marine Biology and Ecology* 240(2): 213-228. 1999.
23. Morton B, Chan k, Britton JC. Hunger overcomes fear in *Nassarius festivus*, a scavenging gastropod on Hong Kong shores. *Journal of Molluscan Studies* 61(1): 55-63. 1995.
24. Schulz DJ, Huang ZY, Robinson GE. Effects of colony food shortage on behavioral development in honey bees. *Behavioral Ecology and Sociobiology* 42(5): 295-303. 1998.
25. Stenzler D, Atema J. Alarm response of the marine mud snail, *Nassarius obsoletus*: specificity and behavioral priority. *Journal of Chemical Ecology* 3(2): 159-171. 1977.
26. Stockhoff BA. Starvation resistance of gypsy moth, *Lymantria dispar* (Lepidoptera: *Lymantriidae*): tradeoffs among growth, body size, and survival. *Oecologia* 88(3): 422-429. 1991.
27. Wonham MJ, O'Connor M, Harley CD. Positive effects of a dominant invader on introduced and native mudflat species. *Marine Ecology Progress Series* 289: 109-116. 2005.
28. Zar JH. Multisample Hypotheses: The Analysis of Variance. In: *Biostatistical analysis,* 4th Edition, edited by Zar JH: Dorling Kindersley, pp.137-158. 2006.
29. Zhao Q, Cheung SG, Shin PKS, Chiu JMY. Effects of starvation on the physiology and foraging behavior of two subtidal nassariid scavengers. *Journal of Experimental Marine Biology and Ecology* 409(1): 53-61. 2011.