

Grazing by the mysid *Neomysis mirabilis* during the warm season in a coastal lagoon in northeastern Japan

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ABSTRACT Mysids of the genus *Neomysis* are omnivorous, feeding on microalgae, zooplankton, and detritus, and are preyed upon by fish. *Neomysis mirabilis* is the most dominant mysid in the subarctic Notoro Lagoon, located on the coast of northeastern Hokkaido, Japan. The feeding habit of *N. mirabilis* was demonstrated as herbivorous during the icy season but has not yet been determined for the longer duration of the warm season. In this study, *N. mirabilis* were collected from the surface water of Notoro Lagoon in August to October 2023. To determine grazing by *N. mirabilis* on microalgae during the warm season we estimated the daily ration using gut pigment contents and the gut passage time in a gut evacuation experiment and then compared the data with the estimated carbon requirement. The average daily ration (carbon derived from the consumption of microalgae) ranged from 43.7 to 89.3 $\mu\text{g C ind}^{-1} \text{ day}^{-1}$ from August to October. The percent composition of the daily ration of microalgae to the metabolic requirement of *N. mirabilis* ranged from 24.7% to 54.7%. These results reveal that the feeding habit of *N. mirabilis* can be omnivorous during the warm season.

KEYWORDS: *Neomysis mirabilis*; Feeding habit; Gut pigment; Daily ration; Notoro Lagoon

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INTRODUCTION

Mysids of the genus *Neomysis* are omnivorous and feed on phytoplankton, zooplankton, detritus, and tiny benthic organisms, switching between filter-feeding and predation (Murano, 1963; Takahashi, 2004). In turn, mysids are fed upon by benthic invertebrates and fish (Murano, 1963; Chiba & Kawamura, 2011). Accordingly, *Neomysis* mysids play an important role in food webs in coastal waters. *Neomysis mirabilis* is a neritic species found in coastal waters of the subarctic northwestern Pacific (Ii, 1964; Mauchline, 1980). In Japan, this species also inhabits coastal lagoons and brackish waters (Yamada et al., 2007; Takahashi et al., 2015) and is the most dominant mysid in Notoro Lagoon, located in northeastern Hokkaido (Yusa & Goshima, 2011). Despite its potential importance in the coastal lagoons of northern Japan, information on the species' feeding ecology is limited to one study during the icy season (Takahashi et al., 2015). During winter, *N. mirabilis* in Saroma Lagoon fed primarily on epiphytes, such as pennate diatoms, found under the seasonal sea ice, which provided carbon amounts sufficient to meet their metabolic rate, demonstrating a primarily herbivorous habit in winter (Takahashi et al., 2015). To fully understand the feeding ecology of *N. mirabilis* in the subarctic region, we require information that includes the longer period of the warm season. Therefore, this study examined grazing by *N. mirabilis* on microalgae during the warm season by estimating the daily ration using gut pigment contents and comparing it with the estimated carbon requirement.

METHODOLOGY

Sampling

Neomysis mirabilis were collected from surface water using a 3- × 4-mm-mesh scoop net with a vinyl bottom, at a quay of the subarctic Notoro Lagoon in northeastern Hokkaido, Japan (Table 1), from

August to October in 2023. Immediately after collection the mysids were immersed in 7 mL of N,N-dimethylformamide (DMF) to extract gut pigments and then stored in a freezer at -60°C until they were analyzed.

Gut Evacuation

Gut evacuation experiment

To estimate the extent of grazing on microalgae from the gut pigment of zooplankton, an accurate estimate of the excretion rate is required (Willason & Cox, 1987). A gut evacuation experiment was conducted at the port of the lagoon, from 13:30 to 17:30, on 11 August 2023. After collecting and rinsing in filtered seawater, approximately 50 *N. mirabilis* were immediately transferred to 10-L polycarbonate bottles filled with surface seawater filtered through a glass fiber filter (GF/F, Cytiva) and then maintained in the seawater. Three mysids in the bottle were collected using a small hand net after different time points, at 0, 30, 60, 90, 120, 180 and 240 min, and immersed in 7.0 mL of DMF. The mysids were then transferred to a freezer and kept at -80°C until gut pigment analysis.

Gut pigment analysis

The gut pigment contents (chlorophyll *a* + pheopigment) of *N. mirabilis* was determined following the method of Holm-Hansen et al. (1965). Fluorescence values before and after adding a drop of HCl with 1 mol/L to DMF from which the gut pigment content was extracted were measured with a Trilogy Laboratory Fluorometer (Turner Designs). It was assumed that most chlorophyll is 100% degraded to pheopigment in the gut of *N. mirabilis*. Since the molecular weight of pheopigment is 66.3% that of chlorophyll *a* (Chl-*a*) (Dagg & Wyman, 1983), the measured amount of pheopigment was multiplied by 1.51. This study expressed the amount of gut pigment in terms of Chl-*a* content.

Gut evacuation rate and gut passage time

To estimate the daily ration using the amount of gut pigment, we determined the gut passage time based on the gut evacuation rate of *N. mirabilis*. We modified the method of Willason & Cox (1987) to determine the excretion rate and gastrointestinal transit time. In the present study, a decay curve was approximated using the Levenberg–Marquardt method, whereby the objective variable (*Y*) is the amount of gut pigment sampled over time, as the explanatory variable (*t*), in the gut evacuation experiment:

$$Y = a + b \times e^{-kt}$$

where *a* and *b* are constants, *k* is the gut evacuation rate, and $1/k$ is the gut passage time (*P*, h). Regression analysis, using the software KaleidaGraph version 5.0 (Synergy Software), was used to determine the significance ($p < 0.05$) of the decay curve.

Daily Ration

The daily ration of microalgae (*Dchl*, ng Chl-*a* ind.⁻¹ day⁻¹) consumed by *N. mirabilis* was estimated as follows:

$$D_{chl} = GP \times GT / P$$

where *GP* is the amount of gut pigment (ng Chl-*a* ind.⁻¹); *GT* is the feeding time (h), with the duration of feeding activity of *N. mirabilis* estimated as 24 h; and *P* is the gut passage time as determined in the gut evacuation experiment. To express the daily ration in terms of carbon content (*Dc*, ng C ind.⁻¹ day⁻¹), the ratio of the amount of carbon to Chl-*a* (C:Chl-*a*) of 47.63 (De Jonge, 1980) was used:

$$D_c = D_{chl} \times 47.63$$

Daily Metabolic Requirement

After analyzing the gut pigment content, the total body length (*TBL*, mm) of *N. mirabilis* was measured under a stereomicroscope using an ocular micrometer. The length data were used to estimate the dry weight (*DW*, mg) of the mysids, following the equation of Yamada (2008):

$$DW = 0.0104 \times TBL^{2.69}$$

Finally, the daily metabolic requirement (*RC*, $\mu\text{g C ind.}^{-1} \text{ day}^{-1}$) of *N. mirabilis* was calculated as:

$$RC = RO \times RQ \times 12/22.4 \times 24 / AE$$

where *RQ* is the respiration quotient, which in this study was 0.97 (Gnaiger, 1983); *AE* is the assimilation efficiency when microalgae are used as food, which in this study was 0.8 (Takahashi, 2004); and *RO* is the respiration rate ($\mu\text{L O}_2 \text{ ind.}^{-1} \text{ day}^{-1}$), calculated with the formula of Ikeda (1985) as:

$$\ln RQ = 0.5254 + 0.8354 \times \ln DW + 0.0601 \times T$$

where *DW* is the dry weight (mg) of the *N. mirabilis*, and *T* is the water temperature ($^{\circ}\text{C}$) at the sampling site.

RESULT AND DISCUSSION

In the gut evacuation experiment, the gut pigment contents of *N. mirabilis* decreased over time, especially within the first hour after the start of the experiment (Figure 1). The regression analysis showed a significant decay curve. At this time, the gut evacuation rate (*k*) was 2.63, and the gut passage time ($1/k$) was 0.38 h. In another study, the gut passage time in the euphausiid *Euphausia pacifica* in filtered seawater was longer than that under a continuous feeding condition (Willason & Cox, 1987). Hence, *E. pacifica* seems to prolong its evacuation time under no-prey conditions. The gut passage time obtained in our experiment was likewise in filtered seawater. However, the gut passage time of *N. mirabilis* in the natural environment may be longer than that obtained in our experiment.

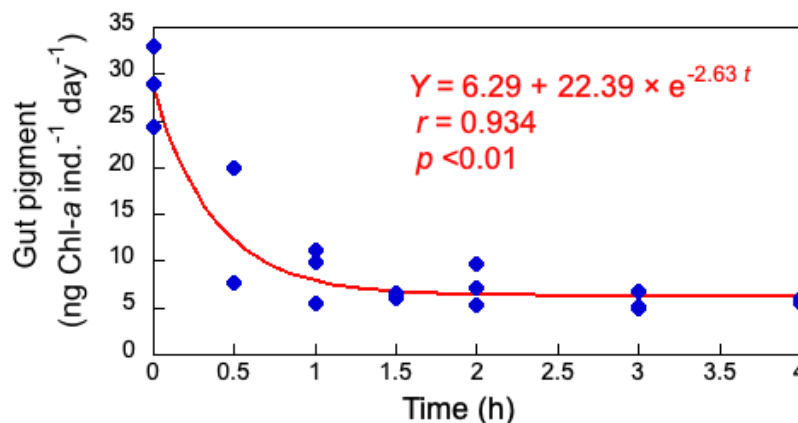


Figure 1. Results of the gut evacuation experiment conducted on *Neomysis mirabilis* in Notoro Lagoon, Hokkaido, Japan, on 11 August 2023, showing temporal variation in the gut pigment contents (ng Chl-*a* ind.⁻¹ day⁻¹). The solid line shows the fitted decay curve approximated using the Levenberg–Marquardt method.

To our knowledge, this study is the first to estimate the daily ratio of *N. mirabilis* during the warm season. In Notoro Lagoon, from August to October, the average daily ration of the mysids feeding on microalgae ranged from 43.7 to 89.3 $\mu\text{g C ind.}^{-1} \text{ day}^{-1}$ (Table 1), revealing a significant seasonal change ($p < 0.01$, Kruskal–Wallis test); the daily ration determined on 27 October was significantly lower than that for 16 September and 11 October (Figure 2). The estimated metabolic requirement of *N. mirabilis* ranged from 158.1 to 272.1 $\mu\text{g C ind.}^{-1} \text{ day}^{-1}$ from August to October, and the metabolic requirement determined on 27 October was relatively lower than that of earlier sampling dates (Table 1). Previous studies reported that water temperature affects the metabolism of marine zooplankton (Ikeda, 1974; 1985). Lower water temperatures, such as those recorded at the sampling site on 27 October, may affect individuals' tendency to graze on algae as well as the metabolic rate of this mysid species.

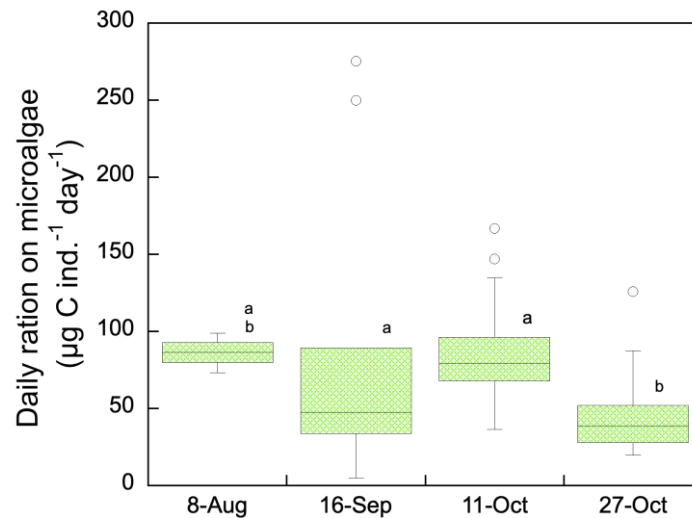


Figure 2. Average daily ration (carbon derived from the consumption of microalgae) ($\mu\text{g C ind.}^{-1} \text{ day}^{-1}$) for *Neomysis mirabilis* in Notoro Lagoon, Hokkaido, Japan, during the warm season, based on samples collected from August to October in 2023. The Kruskal–Wallis test showed a significant seasonal change in the daily ration ($p < 0.01$). Different superscript letters denote significant differences in the daily ration between sampling dates (Bonferroni multiple comparison test, $p < 0.05$).

Table 1. The estimated daily ration of microalgae based on the gut pigment contents, the daily metabolic requirement, and the percent composition of the daily ration to the metabolic requirement for *Neomysis mirabilis* in Notoro Lagoon, Hokkaido, Japan, during the warm season, from samples collected in 2023.

Date	Collected mysids (individuals)	Temperature (°C)	Ration on microalgae ($\mu\text{g C ind.}^{-1} \text{ day}^{-1}$)	Metabolic requirement ($\mu\text{g C ind.}^{-1} \text{ day}^{-1}$)	% Ration to metabolic requirement
8-Aug	3	23.5	86.4	272.1	31.8
16-Sep	9	22.6	89.3	263.7	33.9
11-Oct	24	16.4	86.4	158.1	54.7
27-Oct	31	12.8	43.7	176.6	24.7

The percent composition of the daily ration of microalgae to the metabolic requirement of *N. mirabilis* ranged from 24.7% to 54.7% from August to October (Table 1). The feeding habit of *N. mysids* is omnivorous (Takahashi, 2004), as their food sources include microalgae (such as diatoms), copepods, cladocerans, and detritus (Nomura, 1963). Based on morphological characteristics of the species, it is expected that *N. mirabilis* is also an omnivore. Therefore, *N. mirabilis* at our study site likely utilize prey other than microalgae to meet their metabolic requirements. In Saroma Lagoon, ~15 km east of Notoro Lagoon, *N. mirabilis* during winter were reported to feed on microalgae adhering

to the underside of sea ice, which met their metabolic requirement (Takahashi et al., 2015). Thus, the feeding habit of *N. mirabilis* may change from omnivorous during the warm season to primarily herbivorous during winter.

Our gut passage time estimates, however, may have been biased by laboratory conditions. Studies have shown that euphausiids exhibit longer gut passage times in filtered seawater compared to natural conditions with available prey (Willason & Cox, 1987). This suggests that our daily ration estimates may underestimate the actual feeding rates on microalgae in the field. Moreover, this study has limitations regarding sample size due to the difficulty in collecting *N. mirabilis*. The limited number of sampling dates and individuals may have reduced statistical power. Future year-round surveys with larger sample sizes would strengthen our understanding of this species' feeding ecology.

CONCLUSION

This study is the first to estimate the daily ration of *Neomysis mirabilis* during the warm season and compare it with the metabolic rate. The percent composition of microalgae in the daily ration of *N. mirabilis* ranged from 24.7% to 54.7%. Our findings indicate that *N. mirabilis* feeds on heterotrophic prey organisms, like zooplankton and detritus, as well as on microalgae, and that its feeding habit is omnivorous during the warm season. Since this study only examined microalgae as a food source, further studies that include observations and DNA metabarcoding of the gut contents, feeding experiments, and additional analysis of the gut pigments are needed to elucidate the feeding habits of *N. mirabilis*. Furthermore, long-term dietary studies of this species conducted over multiple years may allow for the assessment of global warming impacts on its feeding ecology.

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