Digestion time and feeding of the pelagic chaetognath *Zonosagitta nagae* in the laboratory

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Abstract: Digestion time and feeding of the pelagic chaetognath *Zonosagitta nagae*, which occurs predominantly in warm waters around Japan, were investigated in the laboratory. Young individuals of *Z. nagae* ranging from 4.1–7.6 mm in body length were used for the experiments with adult females of the copepod *Acartia steueri* as prey. The mean digestion time of *Z. nagae* was 115 ± 3 minutes at 20°C. Temporal change in individual's feeding intensity was observed every 1.5 h for 24 h to determine the diel feeding pattern, feeding interval, feeding rates and daily ration. Almost all individuals showed no clear diel rhythm within 24-h at 20°C under 12L:12D cycle; time interval between feeding was 4.5–6 h (mode) for individuals that ingested multiple copepods. The number of copepods ingested by a chaetognath ranged from 0 to 5 and on average 2.6 copepods were consumed a day per individual. Specific daily ration in most individuals corresponded to ca. 50% of its dry weight while some individuals showed the extremes of non-feeding or voracious feeding ($>200\% \text{ d}^{-1}$), indicating that some of the young *Z. nagae* had an intermittent feeding mode, which would be adaptive to save energy expenditure at low prey densities and to reduce mortality by lowering the risk of predation.

Key words: Chaetognatha, digestion, feeding rate, feeding interval, daily ration

Introduction

Zonosagitta nagae (Alvarinho, 1967) is a dominant chaetognath species in the zooplankton community in warm waters around Japanese coastal areas (Marumo and Nagasawa 1973, Itoh et al. 2006, Miyamoto et al. 2012, Ohnishi et al. 2014). This species is particularly abundant in surface waters from spring to summer and predominantly preys on copepods and a variety of other organisms including fish larvae in the field (Nagasawa and Marumo 1972, 1976, Terazaki 2005). This indicates that *Z. nagae* can play an important role in the succession of planktonic communities. Nevertheless, its role in the food web has not been investigated in detail; particularly, accurate estimation on its daily ration (amount of food consumed per day) is lacking, which is crucial for understanding its ecological role

in planktonic food webs.

The feeding ecology of pelagic chaetognaths, including *Zonosagitta nagae*, have mainly been studied based on gut content analysis of field samples (Nagasawa and Marumo 1972, Feigenbaum 1991). To obtain daily ration from gut contents, one requires digestion time and diel feeding pattern data (Feigenbaum and Maris 1984), both of which are poorly known in *Z. nagae*. The only available information on digestion time for this species is reported as unpublished data by Terazaki (cited in Nagasawa and Marumo 1972), and it ranged widely from 120 to 300 min for copepod prey. The utility of this information is also limited by the lack of a description concerning the experimental conditions such as temperature and prey size. Diel changes in feeding intensity of *Z. nagae* is also uncertain. Active feeding by this species at night in the upper water column of Suruga Bay was reported by Nagasawa and Marumo (1972, 1976), while the feeding intensity became higher during the daytime in the population in the near bottom

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layer at the same sampling site (Nagasawa and Marumo 1976). These observations imply that feeding intensity in *Z. nagae* is controlled by ambient prey density, though the details have not yet been clarified. In this study, we determined the digestion time and temporal changes in feeding pattern of *Z. nagae* as well as its maximum daily ration in the laboratory. The results will improve our understanding of the ecological role of *Z. nagae*.

Materials and Methods

Specimens used in the experiments were collected on 14 July 2016 at a station in the north-western part of Sagami Bay (35°09.9'N 139°10.5'E) on board the R/V *Tachibana* of the Manazuru Marine Centre for Environmental Research and Education (MMCER), Yokohama National University. Chaetognaths were collected by towing a plankton net (80 cm mouth diameter, 0.3 mm mesh) slowly ($< 0.2 \text{ m s}^{-1}$) and obliquely from 50 m to the surface. Collected animals were kept in an insulating container filled with ambient surface seawater until use in the laboratory. Intact and healthy *Z. nagae* were selected and multiple individuals were placed into a PC bottle (diameter: 6 cm, height: 10 cm) containing 250 mL of $0.2 \mu\text{m}$ -filtered seawater (FSW) taken from the field. They were incubated at 20°C under 12L:12D conditions with prey (freshly caught adult females of *Acartia steueri* Smirnov, 1963 from Manazuru port) at a density of $>100 \text{ ind L}^{-1}$. After three days, surviving chaetognaths were selected and placed individually into a smaller PC bottle (diameter: 6 cm, height: 5 cm) filled with 125 mL FSW. Chaetognaths were transferred daily into a fresh medium with new food at a density of 20 copepods per 125 mL and maintained for 17 days in order to acclimate the chaetognaths to the experimental environment (20°C, 12L:12D). The density of prey copepods was 80 times higher than annual maximum of mesozooplankton abundance (2000 inds m^{-3}) in the surface waters (0–100 m) of the sampling site (Shimode et al. 2006).

Digestion time was measured during routine maintenance: whenever we observed the capture of prey by a chaetognath, that individual was immediately isolated into another 125 mL PC bottle filled with FSW without prey and its gut contents were visually checked every 5–10 minutes until evacuation. After evacuation, the body length of the chaetognath was measured under a dissecting microscope with a digital camera (Leica MZ16+DFC290). All observations were made under dim light at 20°C. Supplemental data obtained in October with different prey (*Oithona oculata* Farran, 1913) were also included in the analysis.

To ensure the healthy condition of the experimental animals, diel feeding rhythm and daily ration were measured with a total of 12 individuals of *Zonosagitta nagae* that survived 17 days of the pre-incubation, and the body length of which ranged from 4.1 to 7.6 mm. As these experiments were intended to measure the maximum dai-

ly ration, all individuals were starved for 24 h before the experiment; then 20 individuals of the copepod *Acartia steueri* were offered to each chaetognath at 8:30 h and the chaetognaths were visually checked for the presence/absence of gut contents every 1.5 hours to examine their feeding pattern, feeding interval and food consumption rhythm. Multiple prey items in the gut were distinguishable by eye, and the number of fecal pellets was also counted at the end of the incubation to confirm food-consumption. Diel feeding pattern of the population was expressed as mean food-containing ratio (ratio of chaetognaths with food in the gut) and mean feeding rate (number of copepods eaten by a chaetognath per hour). The experiment was carried out over a 24 hours period under the above-mentioned constant conditions, except for the brief moment when the chaetognaths were checked for gut contents under dim light. It appears that the experimental conditions were appropriate as all individuals used in the experiments survived for 3 to 25 days after the 24 h long observation.

Length-dry weight relationships were determined using live *Zonosagitta nagae* collected in June 2017, also using the same method. Body length of undamaged individuals were measured to the nearest 0.1 mm under a dissecting microscope (MZ16, Leica) with an eyepiece micrometer. The chaetognaths were then briefly washed with distilled water and placed on pre-weighed tin foil, dried for 24 h at 50°C and weighed to the nearest $0.1 \mu\text{g}$ with a microbalance (MSE6.6S, Sartorius). Dry weight of prey copepods was also measured: twenty to twenty-five freshly caught individuals were placed on precombusted glass fiber filters (Whatman GF/F) and rinsed, dried, and weighed in the same manner as above in order to determine the individual dry weight of prey.

Results

Digestion times measured for four individuals, plus supplemental data gathered in October, showed little variation at 20°C, with a mean of 115 ± 2.9 minutes (Table 1). Differences in values between the two prey species were not obvious.

The length-dry weight relationship was established based on 27 samples (Fig. 1) and is described by the following equation:

$$\text{BW} = 0.219 \text{BL}^{2.933} \quad (n=27, r=0.928)$$

where BW is body dry weight in μg and BL is body length in mm. Dry weights of the adult female *Acartia steueri* and *Oithona oculata* prey were $6.60 \pm 0.71 \mu\text{g ind}^{-1}$ ($n=4$) and $2.45 \pm 0.31 \mu\text{g ind}^{-1}$ ($n=3$), respectively.

The number of copepods ingested daily by each chaetognath ranged from 0 to 5 (on average 2.6). Almost all individuals showed no clear diel feeding rhythm (Fig. 2). Among the fed chaetognaths that fed, most individuals (85%) had a single copepod in their gut, but some individu-

Table 1. Digestion time of *Zonosagitta nage* observed in the laboratory at 20°C. All chaetognaths and copepods were collected from Sagami Bay.

Date	Observation time	Body length of <i>Z. nage</i> (mm)	Prey	Digestion time (min)
20 July 2016	14:05–15:55	5.6	<i>Acartia</i>	110
20 July 2016	16:45–18:45	4.4	<i>Acartia</i>	120
21 July 2016	21:30–23:00	5.4	<i>Acartia</i>	120
4 Oct 2017	16:00–17:50	5.6	<i>Oithona</i>	110
Mean±S.E.		5.3±0.3		115±2.9

Fig. 1. Allometric relationship between body length and dry weight of *Zonosagitta nage* collected in Sagami Bay in June 2017. Y axis is expressed with log scale.

als (15%) had 2–3 copepods in their gut. Feeding interval (i.e., time interval between feeding) was variable; the mode was within 4.5–6 h and the mean was 4.3 ± 3.2 h. Some individuals showed intensive feeding period with intervals shorter than 1.5 h (Fig. 3), while some did not feed at all within the 24 h period (Fig. 2).

The observed mean food-containing ratio was 14% and the difference in this value between light (15%) and dark (13%) conditions was not obvious (Fig. 4a). Mean feeding rate on *Acartia steueri* throughout the experiments was 0.11 ± 0.30 inds per chaetognath per hour and there was no statistical difference between light (0.13) and dark (0.09) periods (t-test, $p=0.42$, Fig. 4b). Although no significant difference was found between the first and the second half (10:00–20:30 vs. 22:00–8:30) of the observation period (t-test, $p=0.06$), a significant decreasing trend in feeding rate with incubation time was observed ($n=16$, $r=0.52$, $p<0.05$).

Daily ration for chaetognaths used in the experiments was calculated with the allometric relationship (Fig. 1) and dry weight of the prey. Generally, the daily ration seemed to correlate with chaetognath body size, while some individuals also showed high variability in feeding activity, ranging from intensive feeding to no feeding (Fig. 5a).

Fig. 2. Temporal change in individual feeding activity in *Zonosagitta nage* on the copepod *Acartia steueri* in the laboratory during a 24-hour observation period in the laboratory. Body length of experimental animal is shown in each panel. Grey shading indicates the dark period.

Overall, the specific daily rations for most individuals were about 50% of their body dry weight, though it varied from 0 to 200% of their body weight, particularly in smaller individuals where the body length was 4.3–5.0 mm (Fig. 5b).

Fig. 3. Frequency distribution in feeding interval in *Zonosagitta nagaе* that fed on multiple prey items during a 24-h observation period in the laboratory.

Discussion

The mean digestion time for *Zonosagitta nagaе* was 115 ± 3 minutes for small copepod prey at 20°C in our experiments. This value was within the range of the reported digestion time in other chaetognaths at the same temperature range (Table 2). Digestion time in *Z. nagaе* has been reported at 120–300 minutes for copepod prey (Terazaki, unpublished data cited in Nagasawa and Marumo 1972). However, it is difficult to compare the two studies as Terazaki's data lack information about water temperature, size of prey and size of chaetognaths, all of which are known to affect the digestion time (Feigenbaum and Maris 1984). This study is, therefore, the first report of digestion time in *Z. nagaе* with detailed information such as water temperature (20°C), body length of chaetognaths (4.4–5.6 mm) and prey species (*Acartia steueri* and *Oithona oculata*). Our results can be used as a benchmark when estimating food intake by *Z. nagaе* in the field, since the small copepods used in this study are among the main prey of *Z. nagaе* smaller than 10 mm (Nagasawa and Marumo 1976). As *Z. nagaе* larger than 10 mm also ingest larger copepods such as *Calanus* spp., *Candacia* spp. and *Pareuchaeta* spp. in the field (Nagasawa and Marumo 1976), further studies concerning digestion time with larger prey are required.

Zonosagitta nagaе did not show evidence of a diel feeding rhythm in our experiments and preyed on copepods regardless of light condition, even though it is generally known that planktonic chaetognaths, including *Z. nagaе*, feed more actively at night than in daytime in the field (Nagasawa and Marumo 1972, 1976, Feigenbaum and Maris 1984). Several explanations can be offered for this discrepancy. First is the effect of starvation. Although there was no statistical difference in the mean feeding rates in the first half and the second half of the experimen-

Fig. 4. Diel feeding pattern of *Zonosagitta nagaе* on the copepod *Acartia steueri* during a 24-h observation period in the laboratory. a) mean food-containing ratio and b) mean feeding rate. Vertical bars indicate ± 1 S.E. Grey shading indicates the dark period.

tal period, feeding rate gradually decreased with time during the observations. The young specimens (4.1–7.6 mm) used in this study tend to have small energy reserves and high metabolic requirements; therefore, the 24-h starvation period during the pre-incubation may have affected their feeding patterns as has been reported in other chaetognath species (Kuhlmann 1977). Moreover, constant prey concentrations may affect the diel feeding pattern. Nagasawa and Marumo (1976) reported that *Z. nagaе* in a near bottom habitat fed more actively on copepods during the day than at night, while the feeding activity in the population in the upper water column was higher at night (Nagasawa and Marumo 1972, 1976). This may be attributed to a daytime increase in prey density in the near bottom habitat due to diurnal descent in the vertically migratory prey. Based on these observations, Nagasawa and Marumo (1976) concluded that the daily cycle in feeding activity in *Z. nagaе* varied depending on prey density rather than on the ambient light conditions. The results of this study agree

with their conclusion and indicate that the presence of ambient light was not a critical factor inhibiting the feeding behaviour of *Z. nage* in its young stages.

Since we observed individual feeding events at intervals shorter than the digestion time, the results allowed analysis

Fig. 5. Relationship between body weight and a) daily ration and b) specific daily ration in *Zonosagitta nage* with the copepod *Acartia steueri* as prey, calculated based on the data from a 24-hour incubation in the laboratory.

of the feeding interval which has never been reported in pelagic chaetognaths. The mode of the feeding interval in chaetognaths that had ingested multiple copepods during the experiment was found to be 4.5–6 h, although another peak was found at 0–1.5 h, implying that foraging behaviour in *Zonosagitta nage* may be enhanced as a hunger response after several hours of digestion and assimilation of ingested prey.

Daily ration of *Zonosagitta nage* generally increased with body size, except for some individuals that showed the extremes of non-feeding or voracious feeding. Overall, the specific daily ration of *Z. nage* corresponded to ca. 50% of its dry weight and the voracious feeding individuals ingested >200% of their dry weight, while two out of 12 individuals did not feed at all in 24 h. In the field population, the mean specific daily ration of *Z. nage* of 8–23 mm in body length was estimated at 37.6% of its dry weight by Nagasawa and Marumo (1972), while Feigenbaum and Maris (1984) claimed a possibility of overestimation and revised it to 18.8%. In either case, the daily ration shown in this study is clearly higher than the estimates for field populations. This may be caused by the high prey concentration in the experimental bottle in addition to the presence of a 24 h starvation period prior to the experiments, indicating that the daily ration shown in this study is a maximum for this species in this size range. Furthermore, the small animal size in our experiments (4.1–7.6 mm) is likely another cause, since young chaetognaths generally have higher specific daily rations than larger individuals (Feigenbaum 1979, Reeve 1980). Extremely high specific feeding rates (>200%) found in some individuals also indicate elevated feeding activities of young individuals. At the same time the presence of non-feeding individuals, even after 24 h starvation, indicates that small sized individuals of this species are able to survive in low food environments at least for a few days. The pattern found in the specific feeding rates of *Z. nage* implies that this species has two feeding modes; continuous and intermittent feeding. A continuously feeding individual consumes 50% of its body dry weight a day, while an intermittently feeding individual might feed intensively in-between extended non-feeding periods. Although we were not able to determine the cause for the intermittent feeding, this feeding

Table 2. Comparison of digestion time of chaetognaths with copepod prey at 20–25°C (Modified from the Table in Feigenbaum 1991).

Species	Temperature (°C)	Prey	Digestion time (min)	References
<i>Aidanosagitta crassa</i>	22–24	<i>Acartia clausi</i>	102	Nagasawa (1985)
	22–24	<i>Tigriops japonicus</i>	168	
<i>Ferosagitta hispida</i>	25	Copepods	53	Canico and Grant (1985)
	21	Copepods (2inds)	137	
<i>Flaccigitta enflata</i>	23	Copepods	190	Spyzer (1978)
<i>Parasagitta tenuis</i>	21, 25	<i>Acartia tonsa</i>	69	Canico and Grant (1985)
<i>Zonosagitta nage</i>	20	<i>Acartia steueri</i>	116	This study
	20	<i>Oithona oculata</i>	110	

mode is potentially adaptive to save energy expenditure when prey density in the environment is low. An intermittent feeding mode would also help lower the mortality of young chaetognaths as less active foraging behaviour decreases the encounter rate with potential predators, including other chaetognaths. Cannibalism is known to occur commonly in chaetognaths, including *Z. nagae* (Nagasawa and Marumo 1972, Pearre 1982).

Chaetognaths are ubiquitous in the marine environment and are among the most abundant carnivorous plankton, often exceeding 20–30% of the copepod biomass (Reeve 1970); hence they are important secondary consumers in planktonic food webs. Nevertheless, information on their physiological parameters, which is vital for ecosystem models, is limited partly because of the difficulty in obtaining the data using chaetognaths in captivity. This study shows that *Zonosagitta nagae* can be maintained in the laboratory for more than two weeks and thus further study about its feeding and growth characteristics in the laboratory will improve our understanding of pelagic chaetognaths.

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