Ecological role of copepod nauplii in the microbial food web in temperate embayment waters

温帯内湾域の微生物食物連鎖におけるかいあし類ノープリウス幼生の生態学的役割

15D5702　名取　則明　　指導教員　戸田　龍樹

SYNOPSIS

かいあし類ノープリウス幼生は微生物食物連鎖における捕食者として知られ、その摂餌速度は主に炭素重量、水温、餌濃度の影響を受けて変動する。温帯内湾域では餌濃度は季節的に大きく変動するが、既存の現場摂餌速度の推定法では餌濃度の影響は考慮されていない。ノープリウス幼生の摂餌速度と餌濃度の関係は機能的応答モデル式によって説明されるが、モデル式は特定の発達段階および水温のもとで実験的に得られるため、それらの変動がモデル式に及ぼす影響を明らかにする必要がある。本研究では、炭素重量、餌濃度を説明変数に持つ摂餌速度を推定する経験式を新たに構築するとともに水温係数 Q_{10} を明らかにし、モデル式を現場観測へ応用することで温帯内湾域におけるノープリウス幼生の生態学的役割を定量的に明らかにすることを目的とした。相模湾内湾域にて 3 年間の現場観測を行った結果、水温、餌濃度、ノープリウス幼生の炭素重量は調査期間を通して大きく変動した。春季には *Acartia* spp.ノープリウス幼生が、夏季および秋季には Cyclopoida ノープリウス幼生が優占し、冬季には個体数は少なかった。成体のバイオマス組成においては *Acartia steueri* が最も優占した。新規に割断器を開発して *Acartia* spp.ノープリウス幼生へ応用し、消化管内容物の走査型電子顕微鏡観察をした結果、バクテリア様生物、シアノバクテリア様生物、珪藻類の外殻、鞭毛性細胞が検出され、餌-捕食者サイズ比が 0.3−6.5%の餌生物を主に捕食していることが明らかとなった。摂餌実験を *A. steueri* ノープリウス幼生 III−VI 期について行った結果、すべての発達段階において III 型の機能的応答モデル式が回帰された。モデル式の定数である最大摂餌速度および半飽和定数は炭素重量と有意な正の相関を示し、得られた関係式をモデル式に代入することで、餌濃度および炭素重量を説明変数にもつ経験式を構築した。摂餌速度は水温と正の相関を示し、Q_{10} は 2.4 となった。得られた経験式を現場観測データに応用した結果、ノープリウス幼生の年間餌要求量は冬季には 9.7%、春季には 32%、夏季には 2.1%、秋季には 4.4%を占めたことから、春季には微生物食物連鎖における主要な捕食者であることが明らかとなった。各プランクトン生物の生産速度を推定して比較した結果、ノープリウス幼生の同化効率は他のマイクロ動物プランクトン群集に比べて低く、メソかいあし類の生産にはほとんど結びついていないことが明らかとなった。これらのことから、温帯内湾域においてかいあし類ノープリウス幼生は、季節的に微生物食物連鎖の物質を高次栄養段階へ転送する経路として働く一方、その経路が相対的に優占するに従って、食物連鎖の効率が悪くなることが定量的に明らかとなった。

Keywords: Copepod nauplii, ecological role, microbial food web, empirical model, gut contents, *Acartia steueri*

INTRODUCTION

Copepod nauplii are the most abundant metazoans in marine planktonic communities and show a higher carbon intake relative to their carbon weight (McKinnon and Duggan, 2003). In addition, they can ingest pico-sized plankton such as bacteria, which are too small for adults to ingest efficiently (Roff et al., 1995; Natori et al., 2017). Due to these reasons, copepod nauplii are considered as a predator in the microbial food web. In order to clarify the ecological role of copepod nauplii as a predator quantitatively, estimation of the *in situ* naupliar ingestion rate is crucial.

Determining *in situ* ingestion rates through time-consuming laboratory-based methods at every sampling date is highly labor-intensive and impractical. Thus, empirical models estimating the *in situ* ingestion rates based on factors obtained from basic field investigations are essential for conducting field investigations requiring frequent sampling. Since naupliar ingestion rates are particularly influenced by prey quality, food concentration, body carbon weight, and water temperature (e.g. Almeda et al. 2010; Helenius and Saiz 2017), the effects of these factors on naupliar ingestion rates must be considered to generate these models. However, models considering the effect of food concentration have never been established, even though food concentration greatly varies in most marine waters (e.g., Antarctic Sea, sub-polar frontal area, temperate coastal area, temperate embayment waters). The variation of ingestion rate with changes in food concentration is explained by functional response models (Holling 1965), but these models are obtained under constant naupliar developmental stage, food items, and water temperature. Therefore, modifying the functional response model based on body carbon weight, considering the optimum food items and the effects of water temperature is required.

Sagami Bay, located on the southern coast of central Honshu, is one of the representative temperate waters and can be regarded as the most stable ground for fishery production in Japan. The calanoid copepod *Acartia steueri* Smirnov, 1936 is a dominant copepod in the inner bay of the temperate coastal waters of Asia, and also occurs in and dominates the embayment waters of Sagami Bay where the food concentration varies greatly (Onoue et al. 2006).

In this study, a field investigation was carried out for three years, along with laboratory experiments. Seasonal changes in abundance and biomass of copepod nauplii were measured together with those of micro protozoans and environmental factors to examine the dominant copepod nauplii. Gut contents of *Acartia* spp. nauplii were observed and identified using a scanning electron microscope to determine the optimal prey alga for sequential ingestion experiments. The effects of body carbon weight, water temperature, and food concentration on the ingestion rates of *A. steueri* nauplii were examined through laboratory

experiments under various conditions of the abovementioned factors to construct an empirical model and to determine the temperature quotient (Q_{10}). The constructed empirical model and Q_{10} were applied to the dataset of the field investigation to estimate the *in situ* ingestion rates of *Acartia* spp. nauplii using food concentration and to compare the estimated ingestion rates with separately estimated ingestion rates using a previously established respiration method. Finally, the food requirements of copepod nauplii were estimated and compared with those of micro protozoans to quantitatively evaluate the ecological role of copepod nauplii as a predator in the microbial food web in temperate embayment waters.

MATERIALS AND METHODS

Study 1. Field investigations

Monthly field investigation was conducted from November 2012 to November 2015 at Station A located on Manazuru Port (35° 09' 49" N, 139° 10' 33" E) in Sagami Bay. Water temperature, salinity, size-fractionated (<2, 2–20, 20–180 μm) chlorophyll *a* concentration, and abundance and biomass of bacteria, heterotrophic nanoflagellates (HNF), micro protozoans (naked ciliates, tintinnids, heterotrophic dinoflagellates (HDF)), and copepods (nauplii, copepodites + adults) were measured.

Study 2. Estimation of ingestion rates using food concentration

2-1. Observations of gut contents

Nauplii of *Acartia* spp. were collected at Manazuru Port in March 2014. After sampling, collected nauplii were immediately preserved with 25% glutaraldehyde at a final concentration of 2% and stored at 4°C until the following sample preparation.

A fracturing device and its optimized protocol for the copepod nauplius were developed to observe their gut contents using a scanning electron microscope (Natori et al. 2017). The preserved nauplius was washed to remove attached debris, immersed in water, and frozen. The nauplius was fractured together with the ambient ice using the device. A pair of fractured nauplius was sublimated in a vacuum chamber. To make the nauplius conductive, an osmium coating was applied. Cross-sections of the nauplius were observed to inspect gut contents and to determine prey:predator size ratio under a low-vacuum scanning electron microscope and/or a field-emission scanning electron microscope. The elemental composition of the gut contents was analyzed to detect silicon using energy dispersive spectrometry with a low-vacuum scanning electron microscope.

2-2. Ingestion rates of **Acartia steueri** *nauplii*

Laboratory feeding experiments were conducted on *A. steueri* nauplii under various conditions (Natori and Toda 2018). *A. steueri* nauplii used for feeding experiments were incubated from eggs produced by wild females. The females were collected at Manazuru Port in April and September 2014, and May and Jun 2017. In the laboratory, every 20 adult females were placed into 200-mL glass beakers along with 0.45 μm-filtered sea water (0.45 μm-FSW). The beakers were incubated at *in situ* temperature for 24 h under a 12 h light:12 h dark cycle. Batch-cultured haptophyte *Isochrysis galbana* which was considered as optimal prey for *Acartia* spp. nauplii in terms of size (see Results), was supplied as prey at satiating concentrations of > 1.0×10^4 cells mL^{-1}. Newly produced eggs were collected, and every 100 eggs were transferred into a 300-mL glass beaker with 0.45 μm-FSW and *I. galbana* (> 1.0×10^4 cells mL^{-1}). The eggs were incubated at 17 ± 1°C under a 12 h light:12 h dark cycle. Hatched nauplii were incubated until they had grown to desired developmental stages and were then used for the experiments.

Nauplii of 4 developmental stages (NIII–NVI) were used for experiments to determine the functional response as early developmental stages (NI–NII) do not ingest prey. The prey concentrations used in the experiments ranged from approximately 1.0×10^3 to 3.5×10^4 cells mL^{-1}, equivalent to 10 and 347 μg C L^{-1} using the conversion factor of 9.9 pg C $cell^{-1}$ which was previously measured using a carbon, hydrogen, and nitrogen analyzer (CHN corder). Nine 250-mL glass bottles—3 initial, 3 control, and 3 experimental bottles—were used for each experiment. All bottles were filled with a prey suspension of each concentration. Every 4 or 5 nauplii were added into these experimental bottles. At the start of the incubation, prey concentrations of initial bottles were measured. Three control and 3 experimental bottles were incubated for approximately 24 h under same conditions of incubation for the nauplii. After the incubation, concentrations of the control and experimental bottles were measured, respectively. According to Frost (1972), ingestion rates (cells ind^{-1} d^{-1}) were calculated using the measured concentrations. The cell-based ingestion rates were converted to carbon-based ingestion rates (μg C ind^{-1} d^{-1}) using the conversion factor. Carbon specific ingestion rates (d^{-1}) were calculated by dividing with the initial naupliar carbon weight. The carbon weights were calculated using the length-carbon content relationship of *A. steueri*, which was measured previously.

To examine the effect of temperature on the ingestion rates of *A. steueri* nauplii, NV were incubated under satiating food concentration (> 300 μg C L^{-1}) at 12, 17, 22, and 27°C. Nauplii and prey suspension were pre-acclimated for approximately 3 h to the experimental temperatures. After preconditioning, the bottles were incubated at each temperature for 24 h, and ingestion rates were calculated as described above. The effect of temperature on ingestion rate was determined by Q_{10} approximation:

$$Q_{10} = (M_2 / M_1)^{10/(T2-T1)} \quad (1)$$

where M_2 and M_1 are the rates of the studied process at temperatures T_2 and T_1 (°C), respectively.

RESULTS

Study 1. Field investigations

Temperature showed common seasonal variation in the temporal area in every year, and it was low in winter and high in summer. The nanophytoplankton (2–20 μm) were often dominant in the total chlorophyll *a* concentration except the

periods of phytoplankton blooms. Bacterial biomass varied seasonally and was high in summer (average 34.5 μg C L^{-1}).

In the results of abundance, all heterotrophic organisms showed a positive coefficient against temperature. Bacteria showed high negative coefficient against salinity although they did not show significant coefficients against chlorophyll *a* concentrations. *Acartia* spp. nauplii were abundant in early spring in 2013 and 2015 whereas cyclopoid nauplii were the most dominant from summer to winter. In terms of seasonal change, *Acartia* spp. tended to appear in the spring, and cyclopoid nauplii appeared in the summer–autumn (Fig. 1). Harpacticoid nauplii appeared in the autumn, and other calanoid nauplii showed not specific appearing season (Fig. 1).

In the composition of microzooplankton biomass, naked ciliates were the most dominant and occupied 2.1–95% with 71 ± 26% of the average of the total microzooplankton biomass whereas copepod nauplii were dominant in March 2013 (occupied 61%) and March 2015 (occupied 75%). In the results of adult and copepodite biomass, *A. steueri* and *Acartia japonica* were observed but *A. steueri* was the most dominant among *Acartia* spp. during the study period.

Study 2-1. The gut contents of* Acartia *spp. nauplii

The cross sections of *Acartia* spp. nauplii were obtained using the developed device, and the gut contents were inspected. Fragments of diatoms were often present as gut contents. Silicon was detected from a fecal pellet observed in a hindgut (Natori et al. 2017). Cyanobacteria-like and bacteria-like organism cells were present in the foregut. Nano-sized cells with flagella-like structures were present in the midgut.

These observed gut contents were smaller than 12 μm, and the prey:predator size ratios of observed gut contents and *Acartia* spp. nauplii varied from 0.3% for bacteria to 6.5% for diatoms.

Study 2-2. The ingestion rates of* A. steueri *nauplii

Carbon specific ingestion rates were increased with increasing food concentrations at all developmental stages. A type III functional response model was fitted to all data using the following functions:

$$^{S}I = {}^{S}I_{max} \times (C^2 / (C^2 + K_m^2)) \quad (2)$$

where ^{S}I is the carbon specific ingestion rate (d^{-1}), C is the prey concentration (μg C L^{-1}), $^{S}I_{max}$ is the maximum specific ingestion rate (d^{-1}), K_m is the half-saturation food concentration (μg C L^{-1}). This functional response model fit well to the data in all studied developmental stages ($p < 0.05$). $^{S}I_{max}$ (d^{-1}) decreased with increasing carbon weight from 22.2 d^{-1} in early nauplii (NIII-NIV) to 2.5 d^{-1} in late nauplii (NVI). On the other hand, K_m increased with increasing carbon weight from 17.8 μg C L^{-1} in early nauplii to 78.4 μg C L^{-1} in late nauplii. These two factors varied with individual carbon weight, and the logarithmic form of equations was obtained. By substituting the equations to the type III functional model, an empirical model was obtained as below:

$$^{S}I = 1.07 \times 10^{3} CW^{-1.05} \times (C^2/(C^2 + (1.35CW^{0.687})^2)) \quad (3)$$

where CW is the individual carbon weight (μgC ind^{-1}).

Feeding rates of *A. steueri* nauplii (NV) under food saturated condition increased with increasing temperature. The carbon specific ingestion rate increased from 8.3 ± 3.5 d^{-1} at 12°C to 20 ± 2 d^{-1} at 22°C. However, the carbon specific ingestion rate at 27°C was low. Q_{10} was calculated as 2.4 using the average ingestion rate at 12°C and 22°C. The empirical model described the carbon-specific ingestion rate of *A. steueri* nauplii well with a high r^2 value (0.92) (Fig. 2).

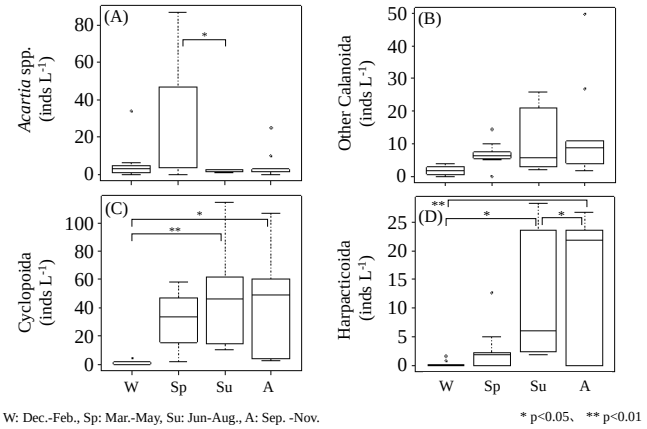

Figure 1. Abundance of copepod nauplii depending on each season. A; *Acartia* spp., B; other Calanoida, C; Cyclopoida, D; Harpacticoida.

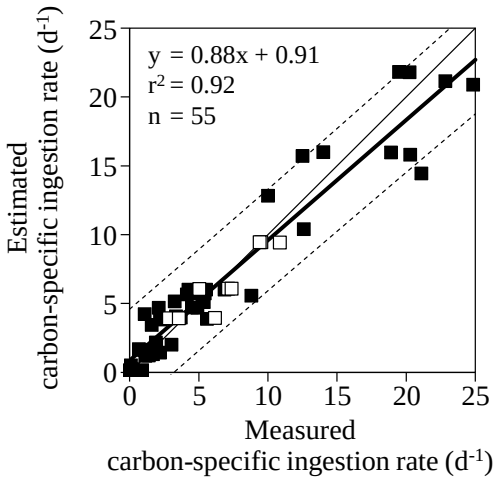

Figure 2. Relationship between measured and estimated carbon specific ingestion rate. The open dots correspond to the results of temperature experiment supplemented by using Q_{10} = 2.4. The continuous and broken lines correspond to regression line and 95% prediction interval, respectively (Natori and Toda 2018).

DISCUSSION

The average bacterial biomass was relatively higher than that of the neritic area of Sagami Bay (25.4 μg C L^{-1}, Ara and Hiromi 2009). Since bacterial abundance was not correlated with chlorophyll *a* concentration, even though the previous studies have reported significant positive correlation between bacterial abundance and chlorophyll *a* concentration (Ara and

Hiromi 2009; Okutsu et al. 2012), it is suggested that the bacteria used dissolved organic carbon flowing from the surrounding terrestrial area (Hitchcock and Mitrovic 2015) in this study site. The fact that the biomass of < 20 μm phytoplankton plus bacteria was dominant in the total plankton biomass throughout the study period indicates that the microbial food web was the main route of carbon flow from primary and bacterial production to higher trophic levels in the study site.

To determine which factors (water temperature, food concentration, carbon weight) are the most affectable to the estimation of *in situ* ingestion rates, ingestion rates of *Acartia* spp. nauplii were estimated using the empirical model (3) and were compared with ingestion rates estimated using the previous respiration method (Ikeda and Motoda 1978). Standardized partial regression coefficients of each factor (Table 1) show that the ingestion rates estimated by the empirical model varied more likely with the change in food concentration rather than with carbon weight although the estimated ingestion rates by the respiration method varied with the change in temperature and carbon weight. These results indicated that food concentration is the most considerable factor for the estimation of naupliar ingestion rates in the study site. To evaluate whether the empirical model described here can be applied for another species, an empirical model was constructed for *Oithona davisae* nauplii NI–NVI using the data obtained from Almeda et al. (2010):

$$I = 8.87W^{0.766}(C^2_{cell}/C^2_{cell} + (259(1-e^{-0.029W}))^2)) \quad (4)$$

where I and C_{cell} are the ingestion rate (cells $ind^{-1}day^{-1}$) and food concentration (cells mL^{-1}), respectively. The estimated ingestion rates by the specifically constructed empirical model (4) using carbon weight (Almeda et al. 2010: Table 1) and food concentration (Almeda et al. 2010: Fig. 1) were significantly related with the measured rates, with a high r^2 value (0.99) and a P value less than 0.01. This result suggests that the empirical model proposed here can be applied for other species using specific data sets.

Food requirements of copepod nauplii were estimated using the empirical models (3 and 4) and Q_{10} and were compared with those of micro protozoans estimated using equations described in previous studies, for evaluating the naupliar ecological role as a predator. The naupliar food requirement varied from 0.006 μg C L^{-1} d^{-1} in January 2013 to 31 μg C L^{-1} d^{-1} in April 2014 and was high in spring and autumn. The food requirement of naked ciliates was highest in the community and constituted 78% of the total microzooplankton food requirement in the annual mean. Copepod nauplii showed the third highest food requirement in the community, which constituted 6.4% of the total food requirement. In terms of seasonal food requirements, copepod nauplii accounted for 9.7% of the total food requirement in winter (December–February), 32% in spring (March–May), 2.1% in summer (June–August), and 4.4% in autumn (September–November). According to the results, copepod nauplii can be regarded as one of the major predators in the microbial food web during spring. Production rates of five components (phytoplankton, HNF, micro protozoans, copepod nauplii, copepod secondary producers) were estimated to assess the trophodynamics described by carbon flow. The result indicated that the assimilation efficiency of copepod nauplii was considerably lower than those of other microzooplankton communities. In addition, the naupliar production was quite lower than those of phytoplankton and micro protozoans. These results suggest that a lot of the carbon ingested by copepod nauplii was released as pellets (Pasternak et al. 2000) or dissolved organic carbon from the microbial food web at the study site.

Table 1. Standardized partial regression coefficient (SPRC) of each factor.

Factor	SPRC	Standard deviation	*t* value	*p* value
Empirical model				
Temperature	-0.44	0.32	-1.4	0.19
Food concentration	0.93	0.32	2.9	0.012*
Carbon weight	0.61	0.17	3.6	0.0034**
Respiration method				
Temperature	0.74	0.056	13	6.6×10^{-9}***
Carbon weight	0.73	0.056	13	8.2×10^{-9}***

SUMMARY

This study newly proposes an estimation method for ingestion rate reflecting the effects of food concentration and demonstrates its application to field investigations of temperate embayment waters in Sagami Bay. The results indicated that the microbial food web was the main route of carbon flow in the study site. *Acartia* spp. nauplii and cyclopoid nauplii were the dominant groups in the study site. The gut contents of copepod nauplii were first observed in the present study and the prey:predator size ratio of *Acartia* spp. nauplii were revealed. An empirical model that can estimate naupliar ingestion rate reflecting the effect of food concentration was obtained, and the applicability of the model to other species was described statistically. Copepod nauplii seasonally play the role as an inefficient pathway transferring primary and bacterial production of the microbial food web to higher trophic levels, that is, the material transfer efficiency of the temperate embayment water will be poor when the relative role of copepod nauplii as a predator has been dominant.

LITERATURES CITED

Almeda et al. (2010) J. Exp. Mar. Biol. Ecol. 387: 24–35. Frost (1972) Limnol. Oceanogr. 17: 805–815. Godhantaraman and Uye (2001). Plankton Biol. Ecol. 48: 19–27. Helenius and Saiz (2017). PLoS ONE 12: e0172902. Holling (1965) Memoirs of the Entomol. Soc. of Canada 45: 5–60. Ikeda and Motoda (1978) Fish. Bull. 76: 357–367. Jungbluth et al. (2017) Mar. Ecol. Prog. Ser. 572: 57–76. McKinnon and Duggan (2003) Mar. Biol. 143: 897–907. Natori et al. (2017) Limnol. Oceanogr.: Methods. 15: 567–571. Natori and Toda (2018) Mar. Biol. 165: 1–11. Onoue et al. (2006) Coast. Mar. Sci. 30: 353–359. Pasternak et al. (2000) Sarsia 85: 439–452. Roff et al. (1995) Aquat. Microb. Ecol. 9: 165–175. White and Roman (1992) Mar. Ecol. Prog. Ser. 86: 251–261.