DISSERTATION

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

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SOKA UNIVERSITY

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ABSTRACT

Seasonal changes in the abundance and biomass of copepod nauplii and other heterotrophic organisms with environmental factors were investigated for three years at Manazuru Port (embayment area) located in Sagami Bay, Japan. Monthly samplings were conducted from November 2012 to November 2015. The abundance of copepod nauplii showed peaks in spring, summer, and autumn and was significantly correlated with water temperature, pico- (<2 μm) and nano-sized (2–20 μm) chlorophyll *a* concentration, and the abundances of other heterotrophic organisms. *Acartia* spp. nauplii were most dominant in terms of biomass during the study period, and *Acartia steueri* dominated the biomass of *Acartia* copepodites and adults. The gut contents of *Acartia* spp. nauplii were observed through scanning electron microscopy using a newly developed fracturing device. Bacteria-like cells, cyanobacteria-like cells, frustules of diatoms, and cells with a flagella-like structure were observed as the gut contents and the prey:predator size ratios between the gut contents and *Acartia* spp. nauplii were revealed to be 0.3–6.5%. Laboratory experiments with haptophyte *Isochrysis galbana* as the optimum sized food were conducted on *Acartia steueri* nauplii using diffluent developmental stages, food concentrations, and water temperatures. The ingestion rates increased with food concentration at all stages, and two significant relational equations were obtained between carbon weight and two feeding parameters of the type III functional model. These equations were used to create an empirical model that can estimate *in situ* ingestion rates using food concentration. Ingestion rates also increased with increasing temperature, and the temperature quotient (Q_{10}) was determined to be 2.4. Food requirements of copepod nauplii estimated using the empirical model, an empirical model specifically constructed for cyclopoid nauplii, and the Q_{10} showed that copepod nauplii constituted 6.4% of the total food requirement of microzooplankton. Seasonal food requirements of copepod nauplii were 9.7% of the total food requirement in winter, 32% in spring, 2.1% in summer, and 4.4% in autumn. The estimated production rates of copepod nauplii were quite lower than those of micro protozoans and

phytoplankton. In addition, the assimilation efficiency of copepod nauplii was considerably lower than that of other microzooplankton communities. This result suggests that a lot of the carbon ingested by copepod nauplii was released as pellets or dissolved organic carbon from the microbial food web at the study site. According to the results, it is clarified that copepod nauplii seasonally play a role as an inefficient pathway transferring the primary and bacterial production of the microbial food web to higher trophic levels; that is, the material transfer efficiency of the temperate embayment water is poor when the relative role of copepod nauplii as a predator is dominant.

Chapter I

General introduction

1.1. Copepod nauplii in marine food webs

The qualitative and quantitative elucidation of material and energy transfer processes via predator-prey interactions in a food web is essential to understand the structure and function of a natural ecosystem, and also to evolve strategies to maintain, sustainably use, and restore the ecosystem. Generally, there are two food webs in a pelagic marine ecosystem: grazing and microbial food webs. The organic materials produced by nanophytoplankton (2–20 μm) and microphytoplankton (20–200 μm) are transferred to high trophic levels, such as that with fish, via grazing by mesozooplankton (0.2–20 mm) in the classical food web (Figure 1-1). In contrast, the organic materials produced from dissolved organic carbon by bacteria are transferred into mesozooplankton via ingestion of bacteria by heterotrophic nanoflagellates (HNF) (2–20 μm) and sequential ingestion of HNF by microzooplankton (20–200 μm) (Figure 1-1). In addition, picophytoplankton (0.2–2 μm) and nanophytoplankton are also consumed by HNF and microzooplankton, respectively. These two food webs are separated from each other at a small size range, but both are combined by the mesozooplankton. Copepods are well known as the main taxon representing the mesozooplankton (Lalli and Parsons 1993), and are regarded as the key organisms of marine food webs. On the contrary, the larvae of copepods just after hatching from eggs called “nauplii,” defined as larvae in the first developing stage (the period until the larvae have completed sixth molt), would be important in the microbial food web, as they are often classified into microzooplankton depending on their size.

As the body length of copepod nauplii is approximately one tenth that of the adults, they can ingest small organisms of the microbial food web: pico-sized plankton (0.2–2 μm), such as picophytoplankton, bacteria that are considered unusable for adults due to their small size (Turner and Tester 1992; Roff et al. 1995; Böttjer et al. 2010), and nano-sized plankton, including

nanophytoplankton and HNF (Uye and Kasahara 1983; Berggreen et al. 1988; Meyer et al. 2002; Böttjer et al. 2010). In addition, the carbon-specific ingestion rate of nauplii is considerably higher than that of adults; it often exceeds the naupliar body weight (White and Roman 1992; Kiørboe and Sabatini 1995; McKinnon and Duggan 2003; Saiz and Calbet 2007). Therefore, copepod nauplii are considered as one of the predators of the microbial food web, and some pioneering studies have reported the fragmentary aspects of material flow via copepod nauplii in temperate coastal waters. Copepod nauplii were the most abundant group among metazooplankton and showed higher phytoplankton ingestion than adults (White and Roman 1992; López et al. 2007a). Copepod nauplii were reported as the second largest group in the microzooplankton community in terms of grazing impact on phytoplankton standing stock in the coastal waters of Asturias in spring, which varied from 0.6–7.4% and occupied 22% of the average grazing impact of the microzooplankton community (Quevedo and Anadón 2000). Food requirements of copepod nauplii constituted 5–30 % of the total food requirement of microzooplankton in the Inland Sea of Japan (Godhantaraman and Uye 2001). On the other hand, micrometazoans, which are mainly composed of copepod nauplii and copepodites, showed low grazing impact (<2.5%) on the standing stock of heterotrophic and phototrophic nanoflagellates, dinoflagellates, diatoms, and ciliates, which seems insufficient to exert relevant control on phytoplankton and protozoan dynamics (Almeda et al. 2011). In order to quantitatively investigate the role of copepod nauplii as a predator and the material transferred via them, the *in situ* ingestion rate of copepod nauplii was estimated using several methods described in previous studies.

1.2. Estimation of *in situ* naupliar ingestion rates

Naupliar ingestion rates are particularly influenced by environmental variables such as prey quality (size, taxa, and mobility), food concentration, and water temperature (e.g., Paffenhöfer 1971; Meyer et al. 2002; Henriksen et al. 2007) and also by their own body carbon weight (Almeda et al. 2010; Helenius and Saiz 2017). Therefore, the effects of these factors on naupliar ingestion rates must be

considered to estimate the *in situ* ingestion rates of copepod nauplii; however, food concentration is not currently used for this estimation. Generally, the ingestion rates of zooplankton including copepod nauplii are estimated using respiration rates estimated from body carbon weight and temperature (Ikeda and Motoda 1978). Respiration rates are estimated using the constants 0.7 and 0.3 as expedient values for the assimilation and gross growth rate despite the fact that the both rates actually vary among different prey items and food concentrations in the field. In another method, ingestion rates are estimated from production rates, which are estimated using the body carbon weight and water temperature. The production rates are calculated using specific growth rates estimated from empirical models, which are commonly obtained from results of incubation experiments with sufficient food concentrations (Uye et al. 1996). Therefore, the specific growth rates can be regarded as the maximal value; that is, the estimated ingestion rates can describe only potentially maximum ingestion rates, which does not reflect the effect of food concentration. Numerical formulas or fixed numbers obtained from laboratory experiments regarding ingestion rates of copepod nauplii were often used for the estimation although field environments, in terms of factors such as food concentration, are not the same as experimental environments. The formulas or fixed numbers are determined using the food removal technique (Frost 1972; Böttjer et al. 2010) and gut fluorescence technique (Roff et al. 1995; López et al. 2007b; Vogt et al. 2013).

The quantitative investigation of the ecological role of copepod nauplii considering changes in the food environment has never been conducted, although the food environment changes in most marine waters including temperate embayment waters. For example, the range of chlorophyll *a* concentration is remarkably wide, ranging in several orders over the season in the Antarctic Sea (0.11–14.1 μg L^{-1}) (Walker et al. 2000), the sub-polar frontal area of the Japan/East Sea (0.25–1.73 mg m^{-3}) (Kim et al. 2007a), and the temperate shallow embayment area located on the coast of South Korea facing the East Sea (0.29–127 μg L^{-1}) (Kim et al. 2007b). Therefore, the challenge of developing an estimation method for naupliar ingestion rates reflecting the effect of the food environment is necessary and greatly contributes to the ecological role of copepod nauplii in these

waters. The variation in ingestion rate with changes in food concentration has been explained by functional response models (Holling 1959a, b, 1965), which can estimate ingestion rates using food concentration; thus, the models might be useful for the estimation reflecting the food environment. However, these models cannot be directly applied to field data because they are determined using a controlled laboratory environment involving a certain naupliar developmental stage, food item, and water temperature, unlike the conditions observed in the field. This problem might be resolved by modifying the models against the factors. As constants of the functional response model relate significantly with the body carbon weight (Almeda et al. 2010), an empirical model with explanatory variables of food concentration and body carbon weight can be constructed by substituting the relational expressions between the constants and body carbon weight into the functional model. By considering the optimal prey:predator size ratio, the empirical model constructed using a specific food item might be applied to other food items that have the same optimal ratio. For example, the optimal size ratio of *Acartia tonsa* nauplii was reported as 2–5%, and they showed relatively high clearance rates regardless of the prey quality within the range (Berggreen et al. 1988). The effect of water temperature on the ingestion rate is often supplemented as a temperature quotient (Q_{10}).

1.3. Site description

Sagami Bay, located on the southern coast of central Honshu is one of the representative temperate waters, and many researchers have investigated the physical, chemical, and biological environments intensively, over a long period in the bay (e.g. Ogura 1975; Horikoshi 1977; Nakata 1985; Kuwahara et al. 2000; Kanda et al. 2003, Fujiki et al. 2004; Ara et al. 2011). Sagami Bay is also well-known for the richness of its marine organisms, with great biodiversity (Tanaka 1953; Nakata 1985). For example, 25 orders, 220 families, and 946 species of fish have been reported in Sagami Bay (Nakata 1985). The total annual fishery capture has been maintained at ca. 30,000 tons wet weight $year^{-1}$ from the 1960s; therefore, this bay can be regarded as the most stable ground for

fishery production in Japan (Kobata 2003). Twenty rivers including two large rivers (Sakawa River and Sagami River) flow into the bay (Hirano 1969), which leads to the formation of low salinity water masses in nearshore areas. One of the areas influenced is the western, nearshore part of Sagami Bay includes Manazuru Port. Manazuru Port is a well-represented temperate site with clear food concentration change, and its environmental and biological variables have been methodically studied (Satoh et al., 2000; Toda et al., 2000; Nagao et al., 2001). A prior study of the primary production in the area reported an annual production of 2.9×10^4 mg C m^{-3} yr^{-1} (Satoh et al. 2000). Blooms occur from spring to summer over a relatively long period and the chlorophyll *a* concentration abruptly fluctuates by an approximate factor of 60 only for a few days. During the study, a summer phytoplankton bloom occurred after 3–4 days of persistent precipitation of 200 mm or more.

The calanoid copepod *Acartia steueri* Smirnov, 1936 is a dominant copepod in the inner bay of the temperate coastal waters of Asia, and is widely distributed in the embayment waters (e.g. the Russian coast in the Sea of Japan (Smirnov 1936; Kos 1958), the Korean coast feces on the Sea of Japan and the Yellow Sea (Yoo et al. 1991; Kang and Kang 2005), the Japanese coast faces on the Pacific Ocean and on the East China Sea (Ueda 1980; Uye 1980, 1981; Nishida 1985; Tanaka et al. 1987; Kurihara et al. 2004)). It also occurs in and dominates Manazuru Port (Onoue et al. 2006). Therefore, *A. steueri* was used as the representative species of temperate embayment waters in this study.

1.4. Objectives

The objective of the present study was to elucidate the ecological role of copepod nauplii as a predator in temperate embayment waters using *in situ* naupliar ingestion rates estimated by food concentration, body carbon weight, and water temperature. To achieve this objective, field investigation for three years and laboratory experiments regarding naupliar feeding ecology were carried out. In Chapter II, the seasonal changes in abiotic and biotic environmental factors and

abundance and biomass of heterotrophic plankton including copepod nauplii were measured as fundamental information to discuss the ecological role of copepod nauplii in the following chapters. In Chapter III, the gut contents of a dominant copepod naupliar group, which are revealed in Chapter II, were observed and identified with a scanning electron microscope to determine the *in situ* prey:predator size ratio. Feeding experiments of the dominant copepod nauplii were then conducted using a prey item selected according to the ratio. The effects of food concentration, carbon weight, and water temperature on the ingestion rate were evaluated to construct an empirical model and determine the Q_{10}. Finally, in Chapter IV, the food requirements of copepod nauplii were determined using the field data, constructed empirical model, and Q_{10}, and these food requirements were then compared with those of other microzooplankton to quantitatively elucidate the ecological role of copepod nauplii as a predator in the microbial food web in temperate embayment waters.

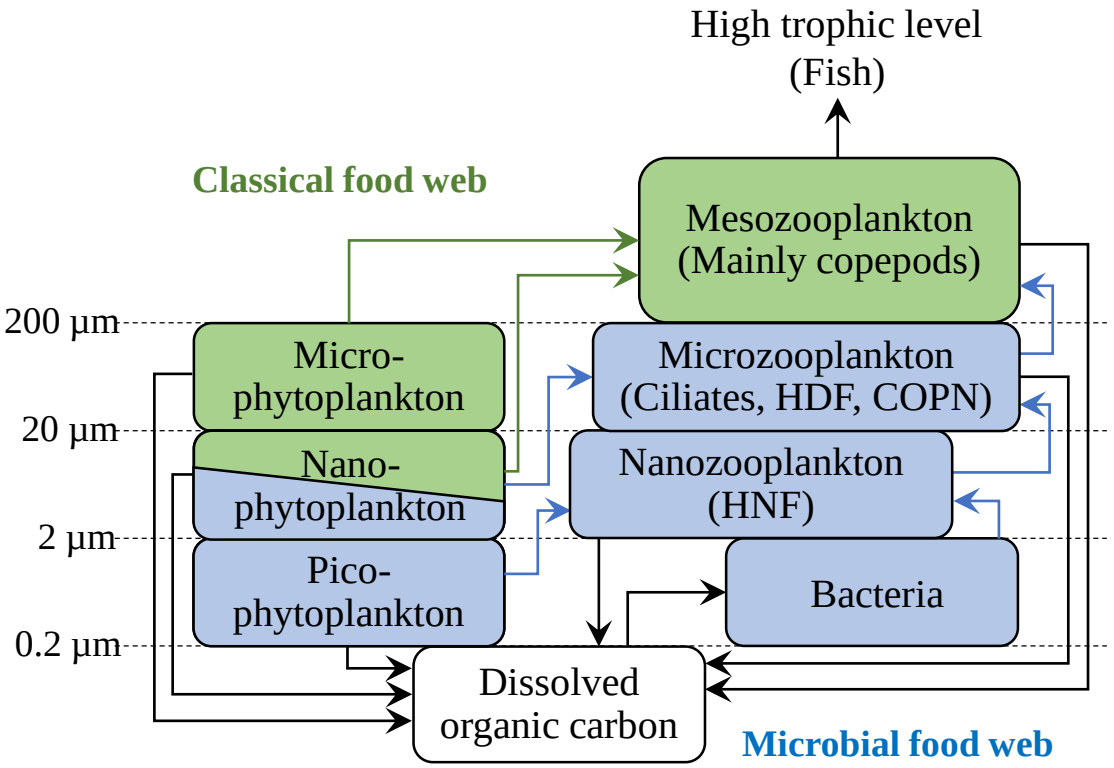


Figure 1-1. Diagram of two food webs, classical food web and microbial food web, in a marine ecosystem. HNF; heterotrophic nanoflagellates, HDF; heterotrophic dinoflagellates, COPN; copepod nauplii. Arrows and broken lines describe carbon flow and size classifications, respectively.

Chapter II

Seasonal changes of copepod nauplii in a temperate embayment water

2.1. Introduction

Copepod nauplii are distributed from polar to equatorial regions, and they appear from surface to deep-sea waters (e.g., Dagg et al. 1984; Dagg and Whitledge 1991; Lewis et al. 1996; Zhang and Wang 2000). Despite of the wide distribution, seasonal changes of abundance and biomass of copepod nauplii in natural field have not been studied well compared with those of adults. Embayment area is known as a natural nursey of fish larvae and relatively isolated from surrounding waters although there is an access connecting to neighbor open water (Mori 1995). In the embayment area, microbial food web is considered as key component in the ecosystem (Uye et al. 2000; Boissonneault-Cellineri et al. 2001; Safi et al. 2002; Lonsdale et al. 2006) like as other coastal waters in Japan (e.g. Ariake Sound (Nakamura and Hirata 2006), Sagami Bay (Ara and Hiromi 2009)). Since copepod nauplii are regarded as predators in microbial food web and considered as important food items of fish larvae (Lasker 1981; Graeb et al. 2004; Turner 2004), their ecological role in embayment area might be significant. The role of copepod nauplii as predators can be elucidated by comparing food requirement of copepod nauplii with those of the other heterotrophic microzooplankton. To estimate the food requirement of copepod nauplii, water temperature, naupliar biomass, food environment and daily ingestion rate are necessary. The variations in water temperature, naupliar biomass and food environment are able to be measured by field investigation. However, previous studies were carried out with samplings conducted only once or for one year, that is, no previous study has investigated the seasonal and year-on-year variation in copepod nauplii in embayment area. In addition, most previous studies have investigated variation in copepod nauplii without any classification of copepod nauplii (Uye 2000; Ara and Hiromi 2009) although identification of dominant naupliar group is quite helpful to determine representative species. Due to much less morphological characteristics of copepod nauplii compared with adults,

identification of copepod nauplii with species level is quite difficult but identification with order or family level is possible (Dahms et al. 2006).

In this chapter, seasonal variation in environmental factors, abundance and biomass of bacteria, HNF, and microzooplankton including naked ciliates, tintinnids, dinoflagellates and copepod nauplii were investigated in embayment area to evaluate whether the microbial food web is dominant and to determine the trophic position of copepod nauplii in the microbial food web.

2.2. Materials and methods

2.2.1. Field investigation and sampling

Monthly field investigations were conducted to three years from November 2012 to November 2015 at a fixed site, station A, located on Manazuru Port (Figure 2-1; maximum depth is 6 m). Manazuru Port is a typical temperate embayment site where environmental factors change abruptly as a result of irregular freshwater and tidewater inflows, as well as complex topography and seasonal changes (Satoh et al. 2000, Toda et al. 2000, Onoue et al. 2006, Tsuchiya et al. 2013). 15 L seawater was collected at a depth of 1 m using a 5-L Niskin bottle and its temperature was measured by a mercury thermometer. 5 L of the collected seawater was transferred into a 5-L shaded polycarbonate bottle after screening by a 180-μm nylon mesh to remove large plankton and detritus. This seawater sample was used for measurements of salinity, chlorophyll *a* concentration, bacterial abundance and biomass, and heterotrophic nanoflagellate (HNF) abundance and biomass. The other 10 L of the seawater was split and gently poured into two 5-L shaded polyethylene bottles without the mesh filtration. This non-filtered sample was used for microzooplankton abundance and biomass.

Net-samples for mesozooplankton abundance and biomass were collected by a vertical tow from bottom to surface using a plankton net (30-cm in mouth diameter, 180-μm in mesh opening size). After all samplings, these samples were immediately brought back to the laboratory and analyzed.

2.2.2. Measurements of physical and biological environmental factors

Chlorophyll *a* concentration was measured from four size fractions of <2 μm, <10 μm, <20 μm and <180 μm. <10 μm chlorophyll *a* concentration was measured from November 2012 to February 2014. The seawater samples for a size fraction of <180 μm was directly filtered on GF/F filters (Whatman). The samples for <2 μm, <10 μm and <20 μm were filtered on GF/F filters after pre-filtration by 2-μm pore size polycarbonate filter (Whatman), 10-μm pore size polycarbonate filter (Whatman) and 20-μm nylon mesh. To extract chlorophyll *a* pigments, these GF/F filters were immersed into 5 mL N, N-Dimethylformamide in 20-mL opaque glass vials and stored at -20°C until analysis (Suzuki and Ishimaru 1990). Chlorophyll *a* concentrations were determined by a fluorometer (Model 10-AU, Turner Design) according to Welschmeyer (1994). Biomass of phytoplankton was calculated by using a conversion factor of 40 μg C chlorophyll a^{-1} (Parsons et al. 1984; Montagnes et al. 1994).

Bacterial abundance was measured by microscopy. 15 mL sample was transferred into a 15-mL centrifuge tube and preserved by buffered formaldehyde (1% final concentration). Preserved sample was stored at -20°C until analysis. Bacteria were stained by SYBR Gold (Molecular Probes Inc.) (Shibata et al. 2006) and collected on 0.2-μm pore size black Nuclepore filter (Track-Etch Membrane Black, Whatman) by filtration of 1.6 mL stained sample with low vacuum pressure (<100 mmHg). Bacterial cells were counted using an epifluorescence microscope. At least 20 microscopic fields were examined on each filter, and more than 400 cells were counted. Bacterial carbon weight was calculated by a conversion factor of 34 fg C $cell^{-1}$ (Hamasaki et al. 1999).

HNF abundance was also measured by microscopy. Samples of 100 mL were transferred into 100-mL plastic bottle and preserved by glutaraldehyde (1% final concentration). Preserved samples were stored at 4°C until analysis. HNF were stained by primulin (Sherr et al. 1993) and collected on 0.8-μm pore size black Nuclepore filter (Track-Etch Membrane Black, Whatman®) by filtration of 10–30 mL stained sample with the low vacuum pressure. HNF were distinguished from autotrophic nanoflagellates by autofluorescence signals under the same epifluorescence

microscope as described in the previous section. At least 20 microscopic fields were examined on each filter, and more than 50 cells were counted. Using cell length measured by an eyepiece micrometer, cell volumes were calculated according to their geometry configurations. The carbon weight was calculated by cell volume and a conversion factor of 220 fg C μm^{-3} (Børsheim and Bratbak 1987).

For protozoans, duplicate 500 mL non-filtered samples were split and transferred into two 500-mL shaded plastic bottles, and then preserved by acidic Lugol's solution (5% final concentration). These samples were concentrated by settling to final volume of 60–100 mL. 10–30% of the concentrated sample was transferred into a Settling Chamber (HYDRO-BIOS) and settled for 24 hours (Ara and Hiromi 2009). Using an inverted microscope, all protozoans were counted and classified into three major groups—naked ciliates, tintinnids and heterotrophic dinoflagellates (Carey 1992; Chihara and Murano 1997). To calculate the carbon weight of naked ciliates or tintinnids, their cell volume (CV) or lorica volume (LV) were calculated according to their geometry configurations. The carbon weight of ciliated protozoans was calculated by conversion factor or formula of 0.19 pg C μm^{-3} for naked ciliates (Putt and Stoecker 1989) or pg C = 444.5 + 0.053LV for tintinnids (Verity and Langdon 1984), respectively. The cell volume of heterotrophic dinoflagellates was also calculated according to their geometry configurations, and their carbon weights were calculated using a conversion formula of pg C = $0.284CV^{0.900}$ (Menden-Deuer and Lessard 2000).

A ten little non-filtered sample was filtered on a 20-μm nylon mesh to collect copepod nauplii. These nauplii were concentrated and transferred into a 100-mL plastic bottle, and then they were preserved by buffered formaldehyde (5% final concentration). Using a dissecting microscope, copepod nauplii were classified into *Acartia* spp., other Calanoida, Cyclopoida, and Harpacticoida depending on their morphology (Dahms et al. 2006; Okada et al. 2009) and counted with measurements of their body lengths. Their individual carbon weight and biomass were calculated by the carbon-length equation of *Acartia steueri* nauplii (Figure 2-2) for *Acartia* spp. and a formula of

ng C = 1.51 × $10^{-5}BL^{2.94}$ (Uye et al. 1996) for the other copepod nauplii. To make the carbon-length equation (Figure 2-2), *A. steueri* nauplii were collected at Manazuru Port in July 2017 by using a plankton net (30-cm mouth diameter, 35-μm mesh size). Collected sample was fixed by 0.1% buffered formaldehyde, and then immediately conducted following handling. *A. steueri* nauplii were sorted and classified into several lengths, and body lengths of 20 individuals were measured for each classification. After measuring, nauplii were washed with 0.45 μm-FSW to remove attached particles. More than 400 individuals were sorted onto a combusted GF/F (Whatman) filter for each classification. Nauplii were then washed with slight distilled water and dried in an oven. Carbon weight was measured with a CHN corder (carbon, hydrogen, and nitrogen analyzer).

Copepod adults and copepodites were collected with 180-μm plankton net from November 2012 to November 2013. Net-samples were preserved by buffered formaldehyde seawater (5% final concentration). Adults and copepodites were counted and identified under the dissecting microscope. According to Chihara and Murano (1997), copepods were classified into genus levels for calanoids and cyclopoids, family levels for poecilostomatoids and order levels for harpacticoids whenever possible. To calculate the carbon weight of copepods, the length of appropriate body portions, *e.g.* prosome length for calanoids and body length for harpacticoids, were measured by an eyepiece micrometer. The carbon weights were calculated by conversion equations of μg C = 4.27 × $10^{-9}PL^{3.07}$ (Uye 1982) for copepods except harpacticoids and μg C = 8.51 × $10^{-10}BL^{3.26}$ × 0.47 (Hirota 1981) for harpacticoids, where PL and BL represent prosome length (μm) and body length (μm), respectively.

2.2.3 Statistical analysis

Interrelationships between abundances of heterotrophic organisms (bacteria, heterotrophic nanoflagellates, naked ciliates, tintinnids, and copepod nauplii) and physiological and biological environmental factors (temperature, salinity, and <2 μm, 2–20 μm and 20–180 μm chlorophyll *a* concentrations) were analyzed by Spearman rank correlation.

2.3. Results

2.3.1. Physical environmental factors and chlorophyll *a* concentrations

Water temperature varied within 10.1°C and 27.1°C during the study period, and it was lowest in December 2013 and highest in August 2013. Temperature showed common seasonal variation in temporal area in every year, and it was low in winter and high in summer (Figure 2-3).

Chlorophyll *a* concentration of microphytoplankton (20–180 μm) varied within 0.072 μg L^{-1} and 3.6 μg L^{-1} during the study period, and it was relatively high in November 2012, March–April 2014 and Jun 2015, while it was low in winter (Figure 2-3). The chlorophyll *a* concentration of nanophytoplankton (2–20 μm) often abundant in total chlorophyll *a* concentration, and it varied within 0.028 μg L^{-1} and 9.7 μg L^{-1} at December 2014 and February 2015. The chlorophyll *a* concentration of picophytoplankton (<2 μm) was relatively low throughout the study period comparing with the other two fractions without October 2014. Total chlorophyll *a* concentration was relatively high at spring and autumn blooms in 2013 and 2014. An additional chlorophyll *a* peak was observed in winter 2015 (Figure 2-3).

2.3.2. Plankton abundance and biomass

Bacterial abundance varied within 0.23×10^9 cells L^{-1} and 2.5×10^9 cells L^{-1} with $1.0\pm0.52\times10^9$ cells L^{-1} of the average. Bacterial abundance was low in winter and high in summer (Figure 2-4). Abundance of HNF varied within 0.06×10^6 cells L^{-1} and 1.1×10^6 cells L^{-1} with $0.31\pm0.20\times10^6$ cells L^{-1} of the average. HNF abundance shows seasonal variation similar with bacterial abundance, and it was low in winter and high in summer (Figure 2-4). Abundances of microzooplankton including copepod nauplii tended to be high in summer and low in winter (Figure 2-4). Abundance of each classification varied within 88 cells L^{-1} and 6583 cells L^{-1} (Average±SD: 2355±1671 cells L^{-1}) in naked ciliates, 0 cells L^{-1} and 1320 cells L^{-1} (Average±SD: 226±283 cells L^{-1}) in tintinnids, 0 cells L^{-1} and 3600 cells L^{-1} (Average±SD: 750±880 cells L^{-1}) in heterotrophic dinoflagellates, and 1.9 inds L^{-1} and 180 inds L^{-1} (Average±SD: 53±69 inds L^{-1}) in copepod nauplii (Figure 2-4).

Abundance of copepod nauplii showed three peaks in early spring, summer, and autumn (Figure 2-5). *Acartia* spp. nauplii were abundant in early spring in 2013 and 2015 while cyclopoid nauplii were most dominant from summer to winter, and other calanoid nauplii and harpacticoid nauplii tended to appear in autumn (Figure 2-5). In terms of seasonal change, *Acartia* spp. tended to appear in spring, and cyclopoid nauplii appeared in summer–autumn (Figure 2-6). Harpacticoid nauplii appeared in autumn, and other calanoid nauplii had not specific appearing season (Figure 2-6).

Microphytoplankton biomass varied from 0.22 μg C L^{-1} in Jun 2014 to 142 μg C L^{-1} in April 2014 (Figure 2-7). Nanophytoplankton biomass varied from 1.1 μg C L^{-1} in December 2014 to 387 μg C L^{-1} in February 2015. Picophytoplankton biomass varied from 1.7 μg C L^{-1} in June 2014 to 148 μg C L^{-1} in October 2014. Bacterial biomass varied from 7.9 μg C L^{-1} in January 2013 to 85 μg C L^{-1} in June 2013 and tended to be high in summer (Figure 2-7). HNF biomass varied from 0.52 μg C L^{-1} in November 2014 to 9.1 μg C L^{-1} in June 2013 (Figure 2-7). HNF biomass was much less than the other pico- and nano-sized plankton during the study period. Microzooplankton biomass varied from 0.36 μg C L^{-1} in January 2013 to 35 μg C L^{-1} in August 2014 (Figure 2-7). In the composition of microzooplankton biomass, naked ciliates were most dominant, and its composition occupied 2.1–95% with 71±26% of the average of the total microzooplankton biomass (Figure 2-7) while copepod nauplii were dominant in March 2013 (occupied 61%) and March 2015 (occupied 75%). In terms of biomass, *Acartia* spp. nauplii were more dominant in 2013 and 2014 (Figure 2-5). The composition of biomass of copepod adults and copepodites varied from 0.24 μg C L^{-1} to 14 μg C L^{-1} with the average of 3.6±4.1 μg C L^{-1} (Figure 2-8). Two species of *Acartia* spp. were identified as *A. steueri* and *Acartia japonica* but former was most dominant during the study period (Figure 2-8).

All of heterotrophic organisms showed positive coefficient against temperature (Table 2-1). Bacteria showed high negative coefficient against salinity although bacteria did not show significant coefficient against chlorophyll *a* concentrations. Tintinnids and copepod nauplii showed positive coefficient against <2 and 2–20 μm chlorophyll *a* concentrations.

2.4. Discussion

2.4.1. Physical environmental factors and chlorophyll *a* concentration

Seasonal variation of water temperature in the embayment area showed clear seasonality which is similar to those reported by previous studies in Sagami Bay (Onoue 2006; Ara and Hiromi 2009), and this variation was essentially repeated during all three years (Figure 2-1).

The average of total chlorophyll *a* concentration in this study (2.4 μg L^{-1}) was relatively high comparing with the neritic area of Sagami Bay (1.4 μg L^{-1}, Ara et al. 2011) but similar with the previous studies in Manazuru Port (2.1 μg L^{-1}, Onoue et al. 2006). However, the maximum chlorophyll *a* concentration of this study (10 μg L^{-1}) was much lower than 1995–1997 in Manazuru Port (>30 μg L^{-1}, Satoh et al. 2000; Toda et al. 2000). The bloom pattern of present study was different among each year. A distinct summer bloom like as Satoh et al. (2000) and Onoue et al. (2006), revealed by exceptionally high chlorophyll *a* concentration, was observed in 2013 and 2015 although the chlorophyll *a* concentration of August in 2015 was low. On the other hand, spring and autumn blooms which are generally observed in temperate coastal area (e.g. Ara and Hiromi 2009) were observed in 2014. Satoh et al. (2000) reported that the increasing relative contribution of micro phytoplankton to total chlorophyll *a* concentration in the summer phytoplankton bloom, and similar trend was observed in the present study except the winter bloom in 2015 which has very high nano phytoplankton chlorophyll *a* concentration. High concentration of micro-size chlorophyll *a* at bloom was also reported in previous studies conducted in the temperate coastal area of Sagami Bay (Ara and Hiromi 2009, Ara et al. 2011). The difference of bloom pattern may be happened related with the complex water mass structure of Sagami Bay affected by warm Kuroshio current, cool Oyashio current, rivers and Tokyo Bay (Iwata 1985).

2.4.2. Variation in abundance

Abundance of bacteria, HNF, and microzooplankton showed remarkable seasonal variation in the study site. Each seasonal variation of these three heterotrophic organisms was repeated throughout

the three-years sampling period, and the abundance was usually high in summer and low in winter. Such seasonal pattern was commonly observed in temperate coastal area of Sagami Bay (Ara and Hiromi 2009) and other temperate areas (e.g. Iwamoto et al. 1994; Fukami et al. 1996; Shinada et al. 2008; Kamiyama et al. 2009).

Due to the higher correlations between bacterial abundance and heterotrophic organisms than those between chlorophyll *a* concentration and heterotrophic organisms (Table 2-1), the microbial food web might be supported by the bacterial biomass in this study area. Previous studies have reported that significant positive correlation between bacterial abundance and chlorophyll *a* concentration because it is considered that the bacterial production is depending on dissolved organic carbon released from phytoplankton (Ara and Hiromi 2009, Okutsu et al. 2012). However, positive correlation between chlorophyll *a* concentration and heterotrophic organisms were shown at tintinnids and copepod nauplii in present study (Table 2-1). Since bacterial abundance was negatively correlated with salinity in this study (Table 2-1), it is suggested that the bacteria used dissolved organic carbon flowed from surround terrestrial area like as temperate estuary (Hitchcock and Mitrovic 2015).

2.4.3. Variation in biomass

Average bacterial biomass obtained in this study (34.5 μg C L^{-1}) was relatively high compared with neritic area of Sagami Bay (25.4 μg C L^{-1}, Ara and Hiromi 2009), Inland Sea of Japan (11.2–16.8 μg C L^{-1}, Imai 1984; Imai and Itoh 1984; Imai and Yamaguchi 1996), Osaka Bay (9.38 μg C L^{-1}, Imai and Yamaguchi 1997) and off Capes Esan and Usujiri, southern Hokkaido (range: 7.9–23.5 μg C L^{-1}, Shinada et al. 2003, 2005, 2008).

The average HNF biomass in the present study (2.65 μg C L^{-1}) is comparable to that found off Capes Esan and Usujiri (range: 0.8–8 μg C L^{-1}, Shinada et al., 2003, 2005, 2008), but is lower than the Inland Sea of Japan (5.5 μg C L^{-1}, Imai and Itoh 1984) and Hiroshima Bay (7.4 μg C L^{-1}, Imai and Yamaguchi 1996).

The average biomass of microzooplankton obtained in this study (9.3 μg C L^{-1}) is relatively low compared to other bays and coastal waters in Japan (e.g. the Inland Sea of Japan (0.53–28.3 μg C L^{-1} Uye et al. 1996), Hiroshima Bay to Bungo Channel (0.39–4.24 C L^{-1} Godhantaraman and Uye 2001), Dokai Inlet (2.92–22.4 μg C L^{-1} Uye et al. 1998), Ise Bay (0.29–5.32 μg C L^{-1} Uye et al. 2000), and off Capes Esan and Usujiri (range: 0.6–40 μg C L^{-1} Shinada et al., 2003, 2005, 2008)). In terms of copepod nauplii, their biomass was relatively high compared with the other microzooplankton in spring. Therefore, the role of copepod nauplii as predators might be important in spring. *A. steueri* was greatly dominated in the biomass of copepodite and adult *Acartia* spp. in spring. Therefore, *A. steueri* nauplii could be regarded as representative copepod nauplii in this study area during spring.

The average biomass of copepod adults and copepodites obtained in the present study (3.56 mg C m^{-3}) are similar values obtained in neritic area of Sagami Bay (8.85 mg C m^{-3} Ara and Hiromi 2009), which are lower than those obtained for other high-productive waters in Japan, e.g. Osaka Bay (38.16 mg C m^{-3} Koga 1987), the Inland Sea of Japan (28.90 mg C m^{-3} Koga 1986; 12.0 mg C m^{-3} Uye et al. 1987) and Fukuyama Harbor (31.9 mg C m^{-3} Uye and Liang 1998).

In the present study, the plankton food web structure was assessed by the biomass structure of five components; phytoplankton, bacteria, HNF, microzooplankton and copepod secondary producers using the data of the first year (Figure 2-9). The average carbon biomass of the total plankton community was greater in summer and autumn (205 and 132 μg C L^{-1}) than in winter and spring (61 and 95 μg C L^{-1}) (Figure 2-9). The contribution of each component to the total plankton biomass was 50–65% by phytoplankton, 21–37% by bacteria, 1.4–3.9% by HNF, 3.3–11% by microzooplankton, 1.6–8.6% by copepod secondary producers (Figure 2-9). The biomass of small heterotrophs (i.e. bacteria, HNF and microzooplankton) was much higher than that of copepod secondary producers. In addition, phytoplankton <20 μm plus bacteria were dominant in the total plankton biomass, rather than phytoplankton >20 μm, throughout the study period. These facts indicate that the microbial food web would be the main route of carbon flow from primary and

bacterial production to higher trophic levels (adults and copepodites) in the study area, rather than the grazing food chain. Thus, carbon biomass dominated by pico- and nano-size phytoplankton and bacteria would be consumed principally by HNF and microzooplankton, and transferred to higher trophic levels (copepods) through the microbial food web (pico-/nanophytoplankton/bacteria–HNF/microzooplankton–copepods), which has similarly been observed in other coastal waters in Japan, (e.g., off Kii Channel, Pacific Ocean in early summer (Uye et al. 1999), the Oyashio region in summer (Shinada et al. 2001), off Usujiri in summer and autumn (Shinada et al. 2005) and off Cape Esan in summer–winter (Shinada et al. 2008)).

2.4.4. Seasonal changes of copepod nauplii

The abundance of copepod nauplii showed roughly similar variation with those of bacteria, HNF, and <20 μm chlorophyll *a* concentration (Figure 2-3, 3-4) and were positively correlated with water temperature, picophytoplankton, nanophytoplankton, bacteria, HNF, naked ciliates, tintinnids and HDF (Table 2-1). The similar correlations were observed in the coastal water (4.5 km off Enoshima Island) of Sagami Bay (Ara and Hiromi 2009). The significant relationship between copepod nauplii and <200 μm chlorophyll *a* was reported in the Inland Sea of Japan (Uye et al. 1996).

Since bacteria, HNF, picophytoplankton and nanophytoplankton were reported as food items of copepod nauplii by laboratory experiments in previous studies (e.g. Henriksen et al. 2007; López et al. 2007a; Böttjer et al. 2010; Helenius and Saiz 2017), it is suggested that the copepod nauplii were predators of those organisms in the study area. The abundance of copepod nauplii might be enhanced by the prey organisms as bottom up effect because peaks of predator and prey abundance are usually corresponding with positive correlation when bottom up effect is working (Ara and Hireomi 2009, Okutsu et al. 2012). In addition, the high positive correlation between water temperature and copepod nauplii can support the bottom up effect because feeding, growth and production rates generally increase with increasing of water temperature in suitable temperature range under proper food condition (Verity 1985; Sherr et al. 1988; Peters 1994; Uye et al. 1996;

Hansen et al. 1997). Top down effect of copepod nauplii would be low during the study period because peaks of predator abundance are usually opposite to that of prey abundance with negatively correlated between predator and prey abundances when top down effect works well (Tanaka and Taniguchi 1996, 1999; Tanaka et al. 1997; Jürgens et al. 2000).

As the conclusions of this chapter are followed: (1) the microbial food web was the main rote transferring materials in terms of the biomass structure in the study site (2) the relative biomass of copepod nauplii against microzooplankton biomass was high in spring (3) *Acartia* spp. nauplii and cyclopoid nauplii were the dominant groups during spring and from summer to autumn, respectively (4) picophytoplankton, nanophytoplankton, bacteria and HNF appeared as expected prey of copepod nauplii, and their biomass were low in winter and high in late spring, summer and early autumn.

The obtained dataset in this chapter can be used as fundamental information for the estimation of naupliar food requirement. To estimate the food requirement, estimation of *in situ* individual ingestion rates of copepod nauplii is necessary. In the next chapter, scanning electron microscope observation of gut contents of *Acartia* spp. nauplii and feeding experiments using *Acartia steueri* nauplii as predator were conducted.

Table 2-1. Coefficients of Spearman rank correlation between the variables and the abundance (cells L^{-1} or inds L^{-1}) of heterotrophic pico-, nano-, and micro-organisms. Significant correlation: *p<0.05; **p<0.01; ***p<0.001.

Heterotrophic organism	Temperature	Salinity	Chlorophyll *a*			Bacteria	Heterotrophic nanoflagellates	Naked ciliates	Tintinnids	Heterotrophic dinoflagellates	Copepod nauplii
			<2 μm	2-20 μm	20-180 μm						
Bacteria	0.548***	-0.525***	0.249	0.285	0.0662						
Heterotrophic nanoflagellates	0.364*	-0.277	0.088	0.296	-0.003	0.493**					
Naked ciliates	0.692***	-0.299	0.405	0.335	-0.104	0.490**	0.224				
Tintinnids	0.653***	-0.407*	0.347*	0.436**	0.134	0.572***	0.295	0.588***			
Heterotrophic dinoflagellates	0.721***	-0.478**	-0.000389	0.285	0.189	0.689***	0.443**	0.551***	0.613***		
Copepod nauplii	0.586***	-0.466**	0.360*	0.421*	0.171	0.570***	0.425**	0.479**	0.487**	0.647***	
Micro zooplankton	0.763***	-0.412*	0.312	0.354*	0.0147	0.602***	0.329*	0.585***	0.636***	0.587***	0.516**

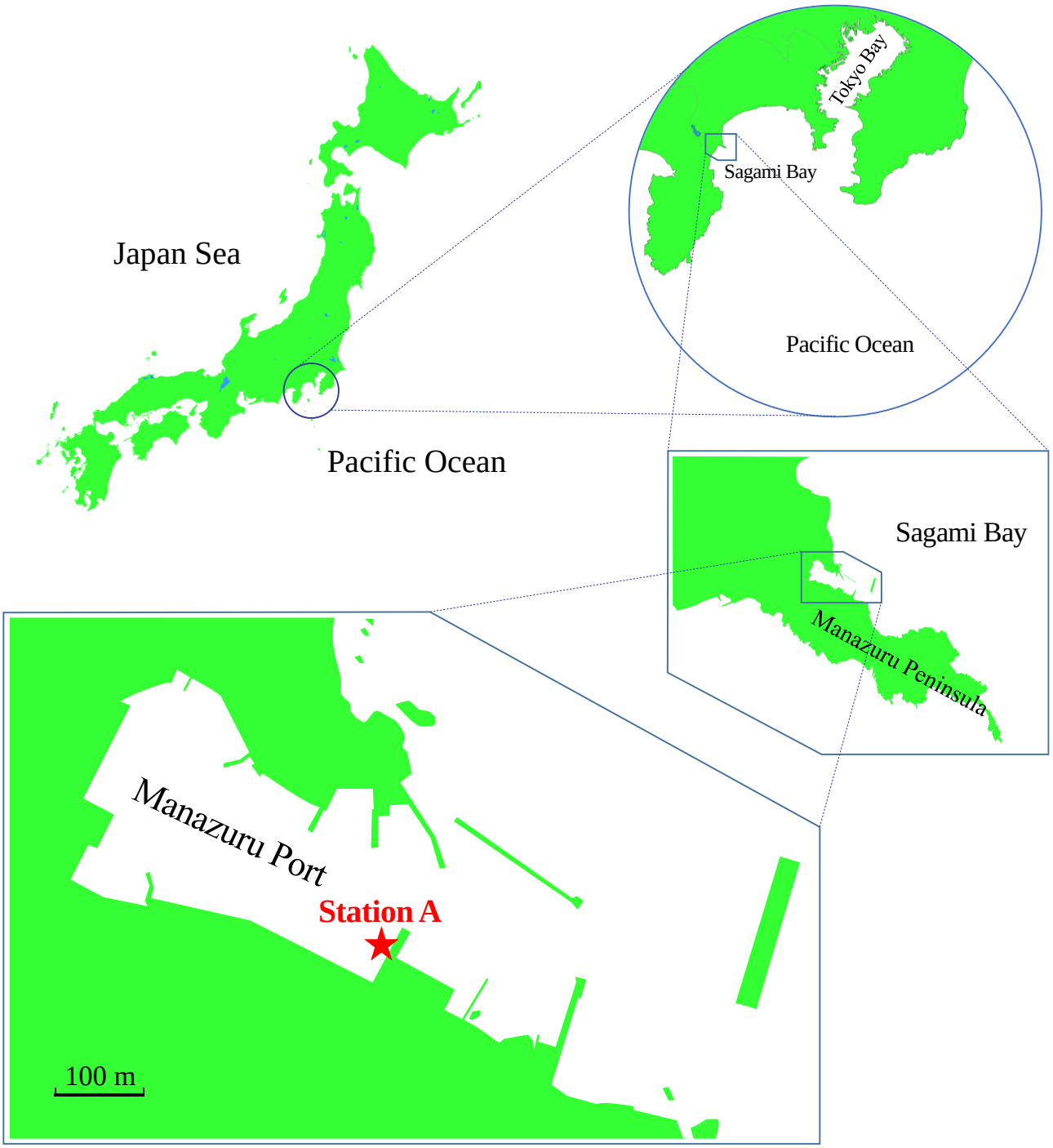


Figure 2-1. Location of station A (35° 09’ 49” N, 139° 10’ 33” E) in Manazuru Port.

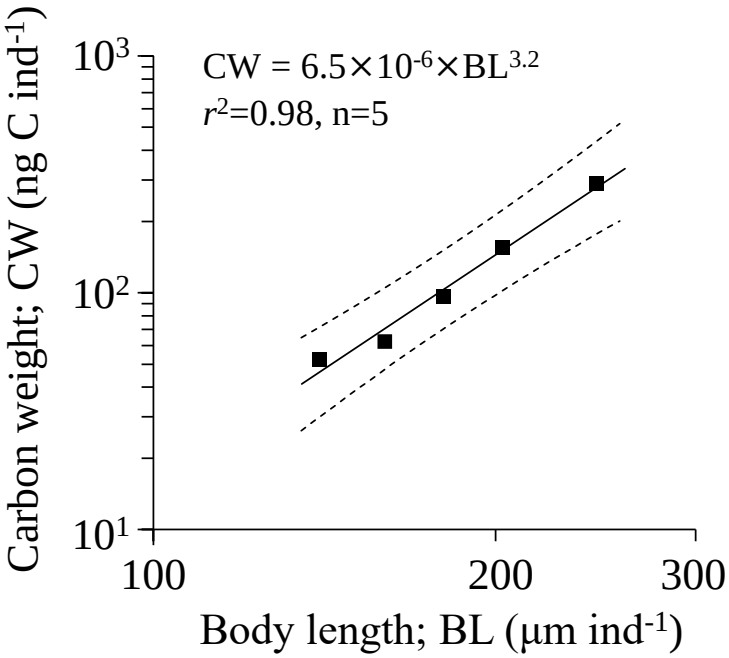


Figure 2-2. Relationship between body length and carbon weight of *Acartia steueri* nauplii. The continuous and broken lines correspond to regression line and 95% prediction interval, respectively.

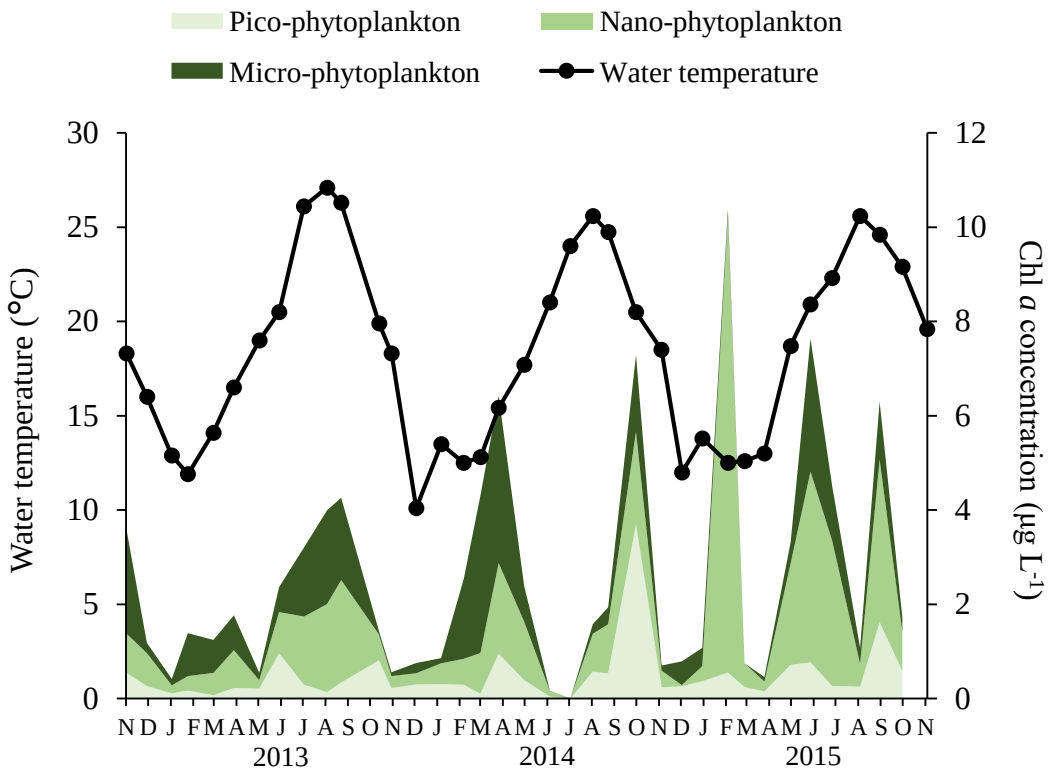


Figure 2-3. Seasonal variations of water temperature and chlorophyll *a* concentrations.

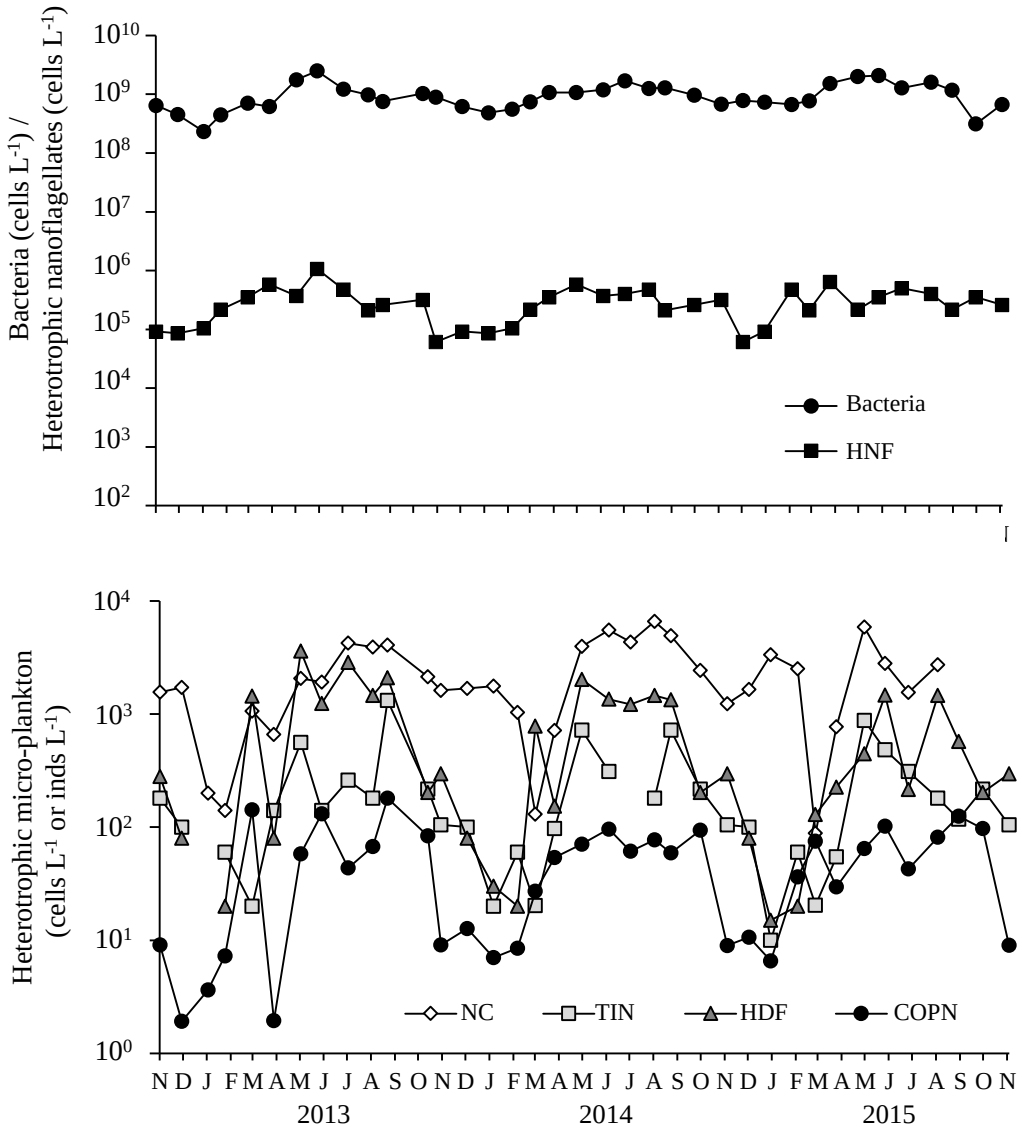


Figure 2-4. Seasonal variations of pico-, nano-, and micro-heterotrophic organisms (HNF: heterotrophic nanoflagellates; NC: naked ciliates; TIN: tintinnids; HDF: heterotrophic dinoflagellates; COPN: copepod nauplii) in Sagami Bay, from November 2012 to November 2015.

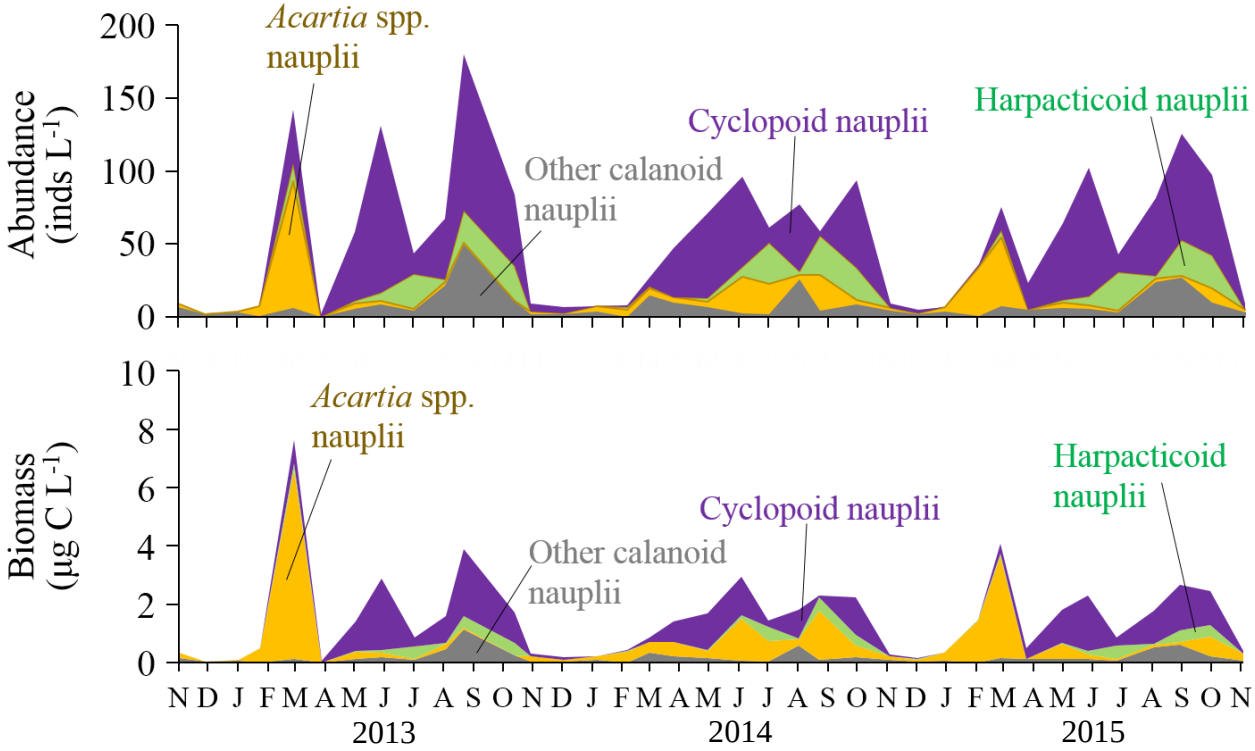
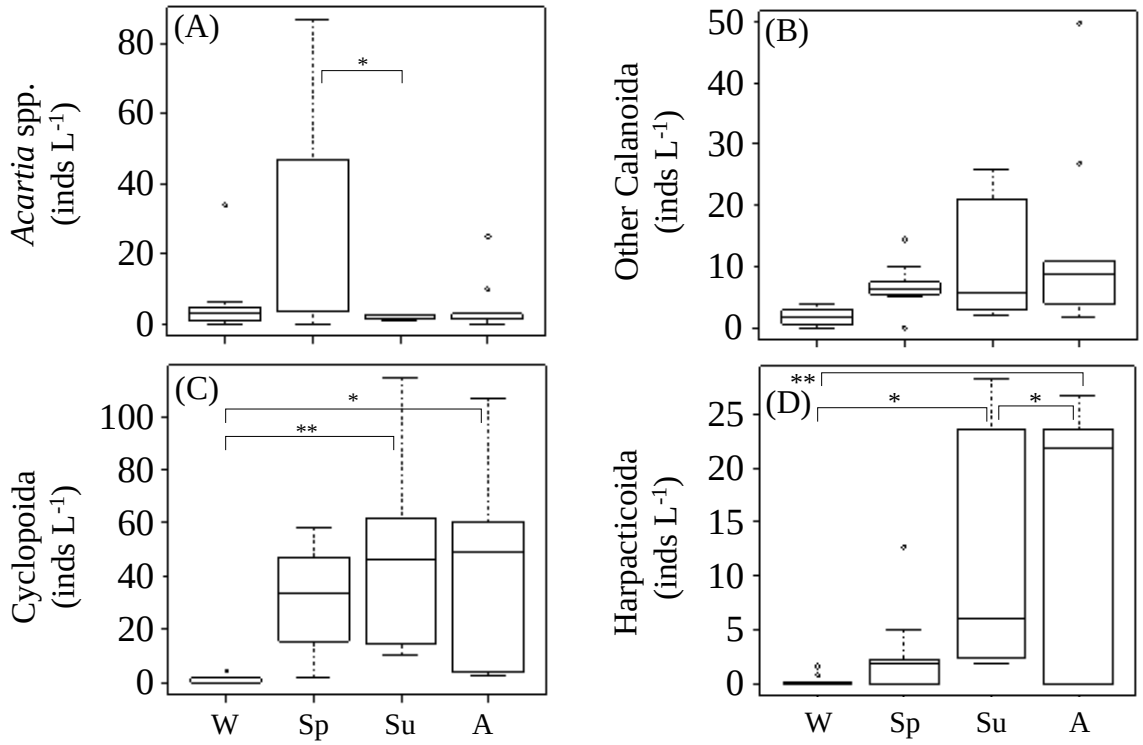


Figure 2-5. Seasonal variations in abundance and biomass of copepod nauplii.



W: Dec.-Feb., Sp: Mar.-May, Su: Jun-Aug., A: Sep. -Nov.

* p<0.05、 ** p<0.01

Figure 2-6. Abundance of copepod nauplii depending on each season. A; *Acartia* spp., B; other Calanoida, C; Cyclopoida, D; Harpacticoida.

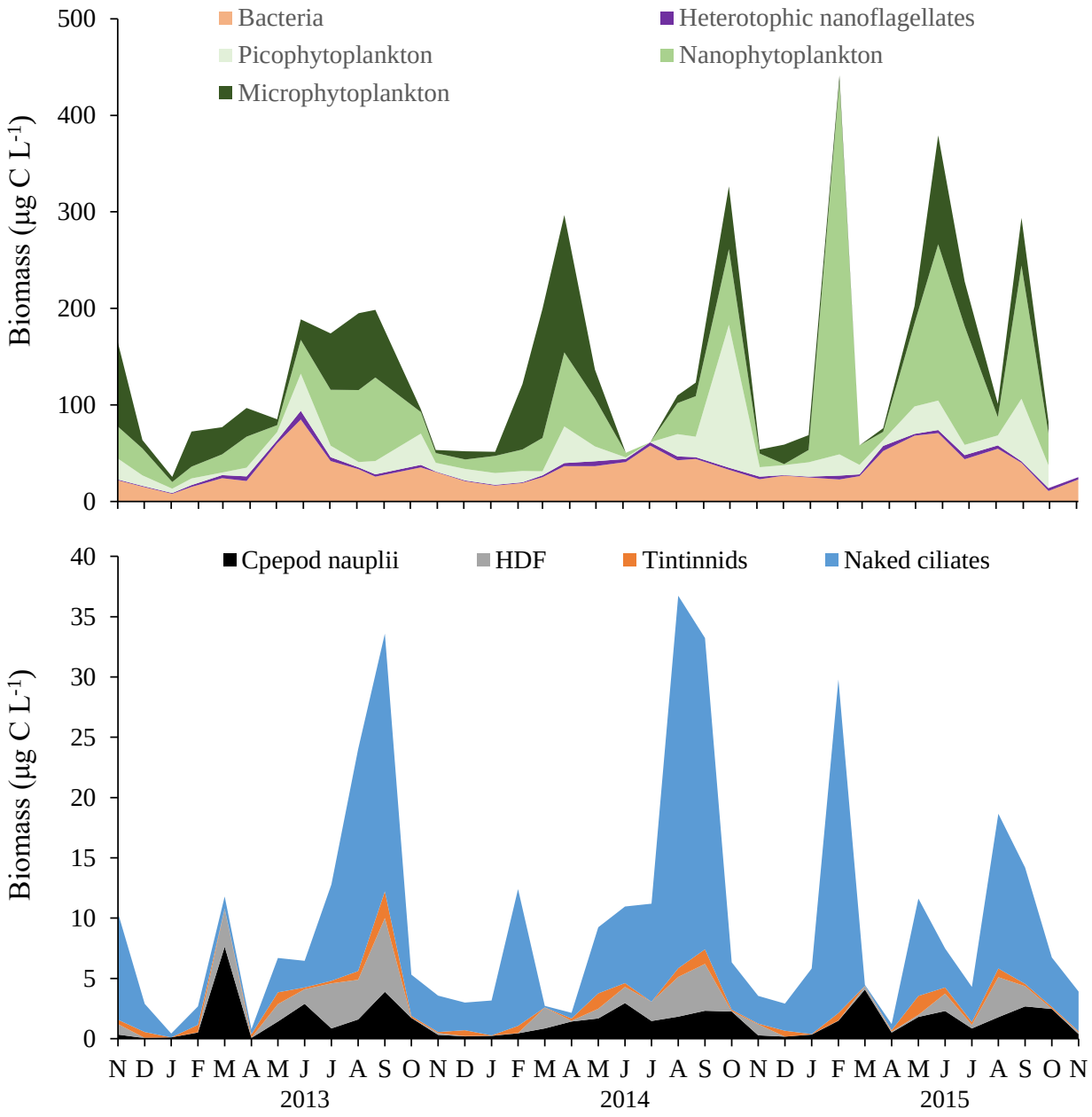


Figure 2-7. Seasonal variations of biomass, HDF; heterotrophic dinoflagellates.

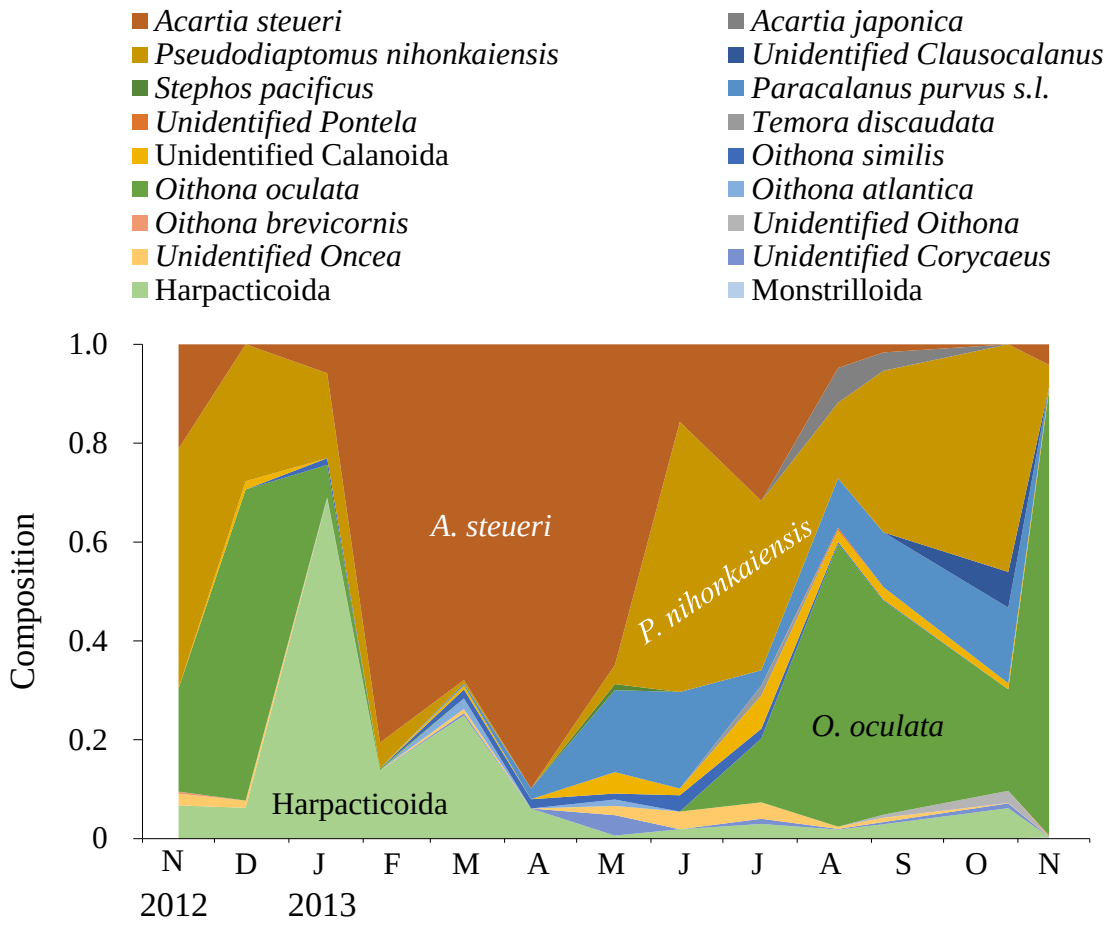


Figure 2-8. Biomass composition of copepod adults and copepodites during November 2012 to November 2013.

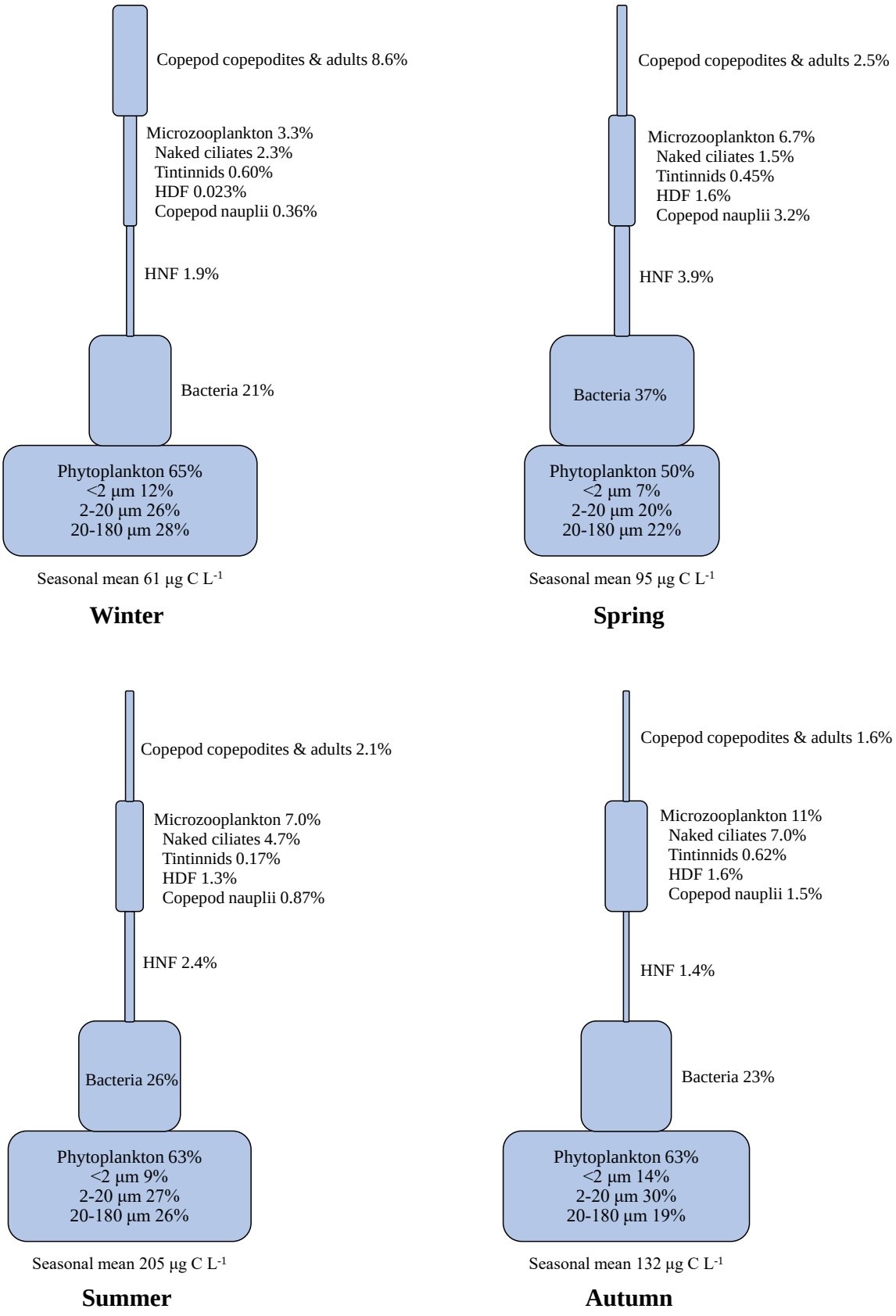


Figure 2-9. Biomass composition of plankton based on seasonal characteristics

Chapter III

Estimation of ingestion rates using food concentration

3.1. Introduction

To quantitatively verify the naupliar ecological role, understanding the factors which affect naupliar ingestion rates are essential. Prey quality, food concentration, individual carbon weight and water temperature are known as the factors which can affect naupliar ingestion rate (Paffenhöfer 1971; Meyer et al. 2002; Henriksen et al. 2007; Almeda et al. 2010; Helenius and Saiz 2017).

Feedings of copepod nauplii on picoplankton such as cyanobacteria and bacteria; nanoplankton such as nanophytoplankton, diatoms and heterotrophic nanoflagellates; and microplankton like relatively small ciliates were reported from previous studies (Meyer et al. 2002; Ismar et al. 2008; Böttjer et al. 2010). Feeding rates of copepod on those prey items may be related to the size ratio between nauplii and prey. *Acartia tonsa* nauplii supplied 10 algal species (5 taxon) have shown high clearance rates when the predator:prey ratio is within 2–5%, and there is no relationship against prey taxonomy (Beggreen et al. 1988). Since no significant selective ingestion was reported on *Oithona* spp. nauplii supplied natural assemblages as food, copepod nauplii may opportunistically ingest preys with optimum size for them. However, little is known for *in situ* prey items actually ingested by copepod nauplii in natural environments because most knowledge was reported from artificially controlled laboratory experiments although DNA-based method was used to identify *in situ* prey of copepod nauplii (Craig et al. 2014). Since electron microscope (SEM) can observe small plankton such as pico and nanoplankton, SEM observation of gut contents of copepod nauplii might be suitable to determine the *in situ* food items of copepod nauplii.

Changing ingestion rate against change in food concentration is termed functional response and classified into type I, type II, or type III model by Holling (1959a, b, 1965). Type II and III functional models were described for copepod nauplii in previous literatures (Henriksen et al. 2007; López et al. 2007b; Almeda et al. 2010; Helenius and Saiz 2017). By using the model, naupliar

ingestion rate can be estimated based on food concentration. Almeda et al. (2010) reported that the constants—maximum carbon specific ingestion rate ($^{S}I_{max}$) and half saturation constant (K_m)—of the type III model showed significant relationships with naupliar carbon weight. This fact had led a simple idea that the functional response can be constructed with carbon weight. Almeda et al. (2010) also described that the effect of temperature can be considered by a supplement using temperate quotient (Q_{10}).

In this chapter, SEM observations of gut contents of *Acartia* nauplii were conducted to clarify the predator:prey size ratio and determine prey items for following feeding experiments. These experiments were conducted on several developing stages of *Acartia steueri* nauplii to clarify the effects of food concentration, carbon weight, and water temperature on naupliar ingestion rate, and then relationships between ingestion rate and the factors were investigated. Functional response of each developmental stage was determined by using wide food concentrations. Furthermore, Q_{10} was calculated on stage NV using several water temperatures under saturating food condition.

3.2. Materials and methods

3.2.1. Development of a novel fracturing device

A new fracturing device was designed (Figure 3-1) and developed in this study to observe gut contents of copepod nauplii (Natori et al. 2017). This device was modified from a device described by Ohta et al. (2011) for observing the internal structures of adult copepods. The empirical accuracy of Ohta's device is only approximately ± 0.1 mm, so this device cannot be used for microzooplankton samples such as copepod nauplii. The fracturing accuracy might be improved by reducing a volume of sample holding space as much as possible. Our device consisted of a brass base and two brass plates. The base had a space with a depth of 1.0 mm where the two plates were placed (Figure 3-1). These plates contained three slots that were 0.5 in mm width and 0.5 mm in maximum depth on the surface, respectively. A pair of slots became a sample holder, when the plates had been set into the base (Figure 3-1 I). Both plates were able to freely slide in the space of

the base (Figure 3-1 II). A boundary line between the plates was a fracturing line (Figure 3-1 broken line).

3.2.2. SEM observations of gut contents

In March 2014, field sampling was conducted at the Station A. Copepod nauplii were collected with a 35-μm mesh opening plankton net attached to a 1-L plastic bottle at the bottom. After sampling, the nauplii sample was immediately preserved in 25% glutaraldehyde (ES-grade, TAAB) at a final concentration of 2%. The sample was stored at 4°C in the dark until they could be processed. *Acartia* nauplii were sorted from the stored sample, and their body lengths were measured with an ocular micrometer mounted on a dissecting microscope. Average lengths were calculated as 170±36 μm (n = 75).

Thirteen individuals were used for observation of gut contents. Samples were washed to remove attached debris from the surface and then transferred into the device. As described by Natori et al. (2017), the samples were frozen and fractured by working of the device. The pairs of fractured samples were sublimated in a vacuum chamber attached on a freeze-drying system. The completely dried samples were picked up from the device and attached on carbon double-sided tape which was pasted on a carbon stub. Samples were then coated with osmium (VIII) to make the samples conductive. The cross-sections of samples were observed with a low-vacuum scanning electron microscope and/or a field-emission scanning electron microscope. Gut contents were observed, and its size were measured.

3.2.3. Incubation of *Acartia steueri* nauplii

As described in Natori and Toda (2018), *A. steueri* nauplii were reared from eggs which were obtained from an incubation of wiled females. The females were collected at Manazuru Port located on Sagami Bay, Japan, in April 2014, September 2014, May 2017 and Jun 2017. Copepod samples were collected by oblique tows from bottom to surface using a plankton net (30-cm mouth diameter, 180-μm mesh size) fitted with a cod-end (0.5-L). After the collection, seawater for incubations of

females and nauplii and for feeding experiments was obtained from the surface. The seawater was pre-screened with a 180-μm mesh size sieve to remove large zooplankton and suspended debris. In laboratory, hundreds of *A. steueri* females were sorted from the cod-end. Every 20 individuals were put into 200-mL glass beakers 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 (approximately 80 μmol photons m^{-2} sec^{-1}) after batch-cultured haptophyte *Isochrysis galbana*, which was selected as prey item for the feeding experiments based on the observation and measurement of gut contents by SEM (see Result), was added as prey at final concentration was over 1.0×10^4 cells mL^{-1}. To obtain a cohort of eggs, the females were removed by a 180-μm mesh size sieve after the incubation and all newly produced eggs were placed in a 3-L glass bowl. The eggs were then carefully concentrated into a 150-mL glass dish using a 35-μm mesh size concentrator. Every 100 eggs were sorted into a 300-mL glass beaker with 0.45 μm-FSW with *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 (approximately 80 μmol photons m^{-2} sec^{-1}) until hatched nauplii had grown up to desired developmental stages (Table 3-1). First feeding stage of copepod nauplii was reported as NII–NIII for some species including *Acartia* nauplii (e.g. *Acartia tonsa, Acartia grani, Calanus hyperboreus, Calanus helgolandicus, Calanus finmarchicus, Pseudocalanus newmani, Oithona davisae*) (e.g. Stoecker and Egloff 1987; Tsuda 1994; Calbet and Alcaraz 1997; Cook et al. 2007; Henriksen et al. 2007; Almeda et al. 2010; Jung-Madsen et al. 2013). NIII–NVI were used in the experiments. The culture medium was exchanged every day during the incubation period.

3.2.4. Feeding experiments with different food concentrations

The functional responses were determined for the different developmental stages of *A. steueri* nauplii (Table 3-1). Developmental stages were identified based on their morphological characteristics with a biological microscope according to Okada et al. (2009). The prey concentrations used for 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}, by using the conversion factor of 9.9 pg C $cell^{-1}$ which was measured with a CHN corder (Table 3-1). Each of prey suspension was coordinated in a 3-L polycarbonate jar by mixing a suitable volume of the stock culture of *I. galbana* to seawater sterilized by filtration through a 0.22-μm filter (0.22 μm-FSW). The cell concentration of the stock was pre-checked by a bacterial counting chamber. Feeding experiments were conducted by incubation of nauplii in bottles with prey item. Nine 250-mL glass bottles—3 initial, 3 control and 3 experimental bottles—were used for each experiment. All bottles were filled with the prey suspension. Every 4 or 5 nauplii were sorted from the cohort and added into the experimental bottles. At least 10 nauplii were also sorted from the cohort and preserved with 25% glutaraldehyde at final concentration of 0.1% to measure a naupliar body length. The body lengths were later measured with the biological microscope with an ocular micrometer. At the start of the incubation, 10 mL of the suspension of each initial bottle was preserved with 25% glutaraldehyde at final concentration of 0.5% to determine the initial concentration. To deduce any effect of naupliar excretion on algal growth, 1.25 mL of f/2 medium was added into 3 control and 3 experimental bottles. These bottles were sealed with polycarbonate caps and plastic paraffin films to prevent formation of air-bubbles and then incubated for approximately 24 h under same conditions of the incubations of eggs and nauplii. These bottles were carefully inverted every 4 h to ensure homogeneity of prey concentration during the incubation period. After the incubation, 10 mL of suspensions in the control and experimental bottles were preserved like as initial bottles. The prey concentration of each suspension was determined by a microscopic method. Two or 4 mL of the suspensions were filtrated on 0.45 μm-filters with low vacuum pressure (<100 mmHg). *I. galbana* cells were counted with an epifluorescence microscope using their autofluorescence signals. At least 20 microscopic fields were examined on each filter, and more than 400 cells were counted. According to Frost (1972), ingestion rates (cells ind^{-1} d^{-1}) and clearance rates (mL ind^{-1} d^{-1}) were calculated by using each prey concentration of the bottles when only the growth rates between grazing and control bottles were significantly different. 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. KyPlot software 5.0 was used for the statistical analyses, regressions, and curve-fittings. The curve-fittings were carried out by standard least-squares procedures.

3.2.5. Feeding experiments with different temperatures

To examine the influence of temperature on ingestion rates of *A. steueri* nauplii, NV nauplii were incubated under satiating food concentration at 12, 17, 22 and 27°C. Nauplii were preacclimated to the designed temperatures with satiating food concentration (3.5×10^4 cells mL^{-1}) for almost 3 hours. After this process, nauplii were washed with FSW at the designed temperature and used for incubation experiment as same method as described above. Bottles were incubated at each designed temperature (±0.1°C) in incubator with a 12 h:12 h light-dark condition for approximately 24 hours. Ingestion rates were calculated as previously described in the functional feeding responses. The effect of temperature on ingestion rate was estimated by Q_{10} approximation:

$$Q_{10} = (M_2/M_1)^{10/(T_2-T_1)} \quad \text{(Equation 3-1)},$$

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

3.3. Results

3.3.1. SEM observations

The cross sections of *Acartia* nauplii were obtained by using the device (Figure 3-2). As the results of the SEM observation of gut contents, fragments of diatoms were often present as gut contents (Figure 3-3 A). Chained cells were present in the foregut (Figure 3-3 B). The morphology of the cells was similar with filamentous cyanobacteria (Phlips and Zeman 1990; Miller et al. 2011). Bacterial like organisms were present in the foregut (Figure 3-3 C). Some cells with undulating tape-like structure were present in the midgut (Figure 3-3 D, E). The form of the undulating structure was similar with tentacle of *Noctiluca scintillans* although the size was greatly different

(Ohta et al. 2011). Undulating tape-like flagellums have been observed at a pathogenic dinoflagellate *Amyloodininm* cf. *ocellatum* (Landsberg et al. 1994). While the details of undulating tape-like structure were different with the previous findings, the cells observed here were regarded as some flagellated cells. These observed gut contents were smaller than 12 μm, and the prey:predator size ratios between observed gut contents and *Acartia* spp. nauplii were varied from 0.3% at bacteria to 6.5% at diatoms (Table 3-2). According to the prey:predator size ratio among the gut contents, haptophyte *Isochrysis galbana* was selected as prey algae of the feeding experiments (Table 3-3).

3.3.2. Feeding experiments

Carbon specific ingestion rates increased with increasing food concentrations in all feeding experiments (Figure 3-4). Conversely, clearance rates decreased with increasing food concentrations in all developments (Figure 3-4). For consistency, we fitted all data to a type III functional response model using the following functions:

$$^{S}I = {}^{S}I_{max} \times (C^2/(C^2+K_m^2)) \quad \text{(Equation 3-2)},$$

$$F = I_{max} \times (C/(C^2+K_t^2)) \quad \text{(Equation 3-3)},$$

where ^{S}I is the carbon specific ingestion rate (d^{-1}), C is the concentration of prey (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}), F is the clearance rate ($\times 10^{-3}$ L ind^{-1} d^{-1}), I_{max} is maximum ingestion rate (μg C ind^{-1} d^{-1}) and K_t is the food concentration as the threshold which shows maximum clearance rate. This functional response model fit well to the data in all developmental stages (p<0.05). The feeding parameters obtained for the different larval stages from the fitted functions are shown in Table 3-4. $^{S}I_{max}$ decreased through larval development from 22 d^{-1} in early nauplii (NIII–NIV) to 2.5 d^{-1} in late nauplii (NVI). On the other hand, K_m increased with development from 18 μg C L^{-1} in early nauplii to 78 μg C L^{-1} in late nauplii. I_{max} and K_t varied within 0.63–1.1 μg C ind^{-1} d^{-1} and 20–118 μg C L^{-1}. $^{S}I_{max}$ and K_m varied according to body weight as shown in Figure 3-5. Carbon specific ingestion rates in relation to body

weight fit well to a function (Figure 3-5 A), and the logarithmic form of the equations are given in Table 3-5. Half-saturation food concentration as related to body weight were also fit a function (Figure 3-5 B), and the logarithmic form of the equations were given in Table 3-5.

Feeding rates of *A. steueri* nauplii (NV) increased with increasing temperature between 12°C and 22°C (Figure 3-6) although feeding rate at 27°C was low. Ingestion rates increased from 4.2±1.7 μg C ind^{-1} d^{-1} at 12°C to 9.9±0.8 μg C ind^{-1} d^{-1} at 22°C (Q_{10} = 2.4). Feeding rate in relation to temperature were fitted to a function and the obtained equations are given in Table 3-5.

By substituting the equations obtained from the relationships to type III functional response model, an empirical model was provided as below:

$$^{S}I = 10^{-1.05\log CW+3.03}\times(C^2/(C^2+(10^{0.687\log CW+0.130})^2)) \text{ (Equation 3-4),}$$

this formula can be deformed to

$$^{S}I = 1.07\times10^{3}CW^{-1.05}\times(C^2/(C^2+(1.35CW^{0.687})^2)) \text{ (Equation 3-5),}$$

where ^{S}I, CW and C are carbon specific ingestion rate, individual carbon weight and food concentration, respectively. Estimated values calculated by using the empirical model was compared with measured values to verify the validity of the model (Figure 3-7 A). The empirical model well described carbon specific ingestion rate of *A. steueri* nauplii with high r^2 value.

3.4. Discussion

3.4.1. SEM observations of gut contents

The number of samples processed in the device in one fracturing is very low, with a maximum of three individuals (Natori et al. 2017). In addition, the observed prey tends to be identified only to a high taxonomic level because identification is dependent on morphological characteristics which are often broken by feeding and digestion processes. These limitations could be compensated for by the advantages of DNA-based methods, which can examine a large number of samples and identify a lot of potential prey to very low taxonomic levels. Conversely, the indirect information of DNA-based methods could be complemented by the robust evidence obtained from direct observation of

gut contents with the fracturing method. For example, DNA-based methods might identify a taxon that is unlikely to be prey due to contamination, presence of parasites or commensals, and prey of prey (*e.g.* bacteria consumed by heterotrophic nanoflagellates). The fracturing method could be used to assess whether the unlikely taxon was actually eaten by nauplii. Thus, by complementarily using fracturing alongside DNA-based methods, the fracturing method could improve our ability to examine the feeding of copepod nauplii.

Glutaraldehyde was used as a fixative solution in this study, which is often used for preserving bacteria and nanoflagellates in scanning electron microscopy. However, some flagellates and ciliated organisms are lysed by glutaraldehyde or formaldehyde (Sherr et al. 1993). Ingestion of *Tortanus dextrilobatus* nauplii (NIV–NVI) on phototrophic ciliate *Myrionecta* sp. as *in situ* food item was revealed by a DNA-based method (Craig et al. 2014). Therefore, a suitable fixing solution should be used against prey that can be expected to be eaten by copepod nauplii. The other aspect of the effect of fixative solution is problem of shrinkage. Meden-Deuer et al. (2001) reported that cell volumes of diatoms (8 species) and dinoflagellates (10 species) preserved with 2% Lugol's or 1% glutaraldehyde varied up to 50% shrinkage and almost 30% swelling although some species showed no change in cell volume. In addition, Choi and Stoecker (1989) reported that the cell volume of microflagellate *Paraphysomonas imperforata* preserved with 2% glutaraldehyde varied to approximately 50% shrinkage. Due to the facts, size distribution of the gut contents described here may have been potentially affected by fixative effects although the intensity of the effects may be different depending on taxa and species. In addition, the effects of fixative solution to gut contents may be different with those to ambient body.

Bacterivory on fluorescently labelled bacteria were different depending on species, and some species did not ingest the bacteria (Roff et al. 1995). *Oithona nana* and *Parvocalanus crassirostris* nauplius ingested bacteria in natural food suspension with no strong electivity but *Bestiolina similis* showed strong negative electivity against bacteria (Böttjer et al. 2007; Jungbluth et al. 2017). In terms of the prey:predator size ratio among the gut contents, the ratio of bacteria was lower than the

other gut contents (Table 3-2). Due to the facts, it is suggested that *Acartia* spp. nauplii may not efficiently ingest bacteria in the field.

3.4.2. Carbon specific ingestion and clearance rates

The ingestion, carbon specific ingestion and clearance rates reported here for *A. steueri* nauplii approximately fall into the range of values found in the previous literature which were conducted to examine the feeding behavior of *Acartia* spp. nauplii (Table 3-6). The clearance rate which is expressed as the volume of water swept clear of food particles per unit of time was determined for *Acartia tonsa* nauplii NIII, and the clearance rates ranged within 11–49 mL ind^{-1} d^{-1} (Stoecker and Egloff 1987). In the present study, similar high clearance rates were found at *A. steueri* nauplii NIII (1.9–19 mL ind^{-1} d^{-1}), NIV (1.4–26 mL ind^{-1} d^{-1}) and NV (2.1–20 mL ind^{-1} d^{-1}). However, Smith et al. (2008) reported relatively low clearance rates which were below 1 mL ind^{-1} d^{-1} for *A. tonsa* nauplii NIV–VI. It is well known that clearance rate tends to be high at low food concentration and conversely be low at high food concentration (López et al. 2007b; Henriksen et al. 2007; Almeda et al. 2010; Helenius and Saiz 2017). Therefore, the difference of clearance rates can be explained by the rage of the food concentrations used in the study (Table 3-6). On the other hand, low clearance rates which ranged within 0.071–0.49 mL ind^{-1} d^{-1} were reported for *Acartia grani* nauplii NII–III, although a wide range of prey concentration was used (Henriksen et al. 2007). This low clearance rate may be explained by "edge effects." Böttjer et al. (2010) suggested that a volume of container which was used for experimental incubation may affect the ingestion rates of copepod nauplii, because a copepod tends to veer away from 1–2 cm of the walls of the container (O'Brien 1988; Köster et al. 2008). The volume of container used for *A. granii* nauplii NII-NIII by Henriksen et al. (2007) was 72 mL, and this volume was much smaller than the volume of bottle used in the present study (250 mL). The differences of ingestion and carbon specific ingestion rates between the study can be roughly explained by the food concentration and the volume of container like as clearance rate (Table 3-6). However, quite high carbon specific ingestion rates determined in the early developmental stages of *A. steueri* nauplii were not explained thoroughly by the above reasons.

Berggreen et al. (1988) determine optimal prey size for *A. tonsa* nauplii NII-VI, and the optimal relative prey size raged within 2–4%. The relative prey size of early *A. steueri* nauplii NIII–NIV to *I. galbana* ranged within 3.1–3.4%, and most previous study had used large (relative prey size; 9.3–39%) or small (relative prey size; 0.85–1.3%) size prey items for nauplii (Table 3-6). This fact suggests that *I. galbana* would be suitable prey for the early development of *A. steueri* nauplii in terms of size. The high ingestion, carbon specific ingestion and clearance rates found in present study could be explained by multiple effects of food concentration, container volume and prey size, but more examinations are necessary to inspect the effects.

3.4.3. Relationships between feeding rates and the factors

Previous study reported that copepod nauplius showed type II or III functional response model. In the case of *Calanus helgolandicus* nauplii NIV–V supplied *I. galbana* as prey, type III model was most fitted within the three types (López et al. 2007b). Type II model was reported for *Oithona davisae* nauplii NIII–IV supplied dinoflagellate *Heterocapsa* sp. (Henriksen et al. 2007) and for *A. granii* nauplii NII–III supplied *Heterocapsa* sp. or *Thalassiosira weissflogii* (Henriksen et al. 2007). Because functional response is changed by supplied prey items and its combination, *O. davisae* nauplii NI-VI supplied dinoflagellate *Oxyrrhis marina* shows type III model (Almeda et al. 2010). It is also reported that *Paracartia grani* nauplii NII changed their functional response between mono-prey and multi-prey conditions (Helenius and Saiz 2017).

According to the results, type III functional response model was fitted to all developmental stages of *A. steueri* nauplius (Figure 3-4). Type III functional response model is mainly different from the other two types at the low food concentration (Frost 1975). Since energy cost can be reduced by decreasing feeding effort at low food concentration (Price and Paffenhöher 1985), type III functional response can be regarded as more suitable to low food environment.

Although there is a disagreement about the actual effect of temperature in the field (reviewed in Saiz and Calbet 2007), temperature is known to accelerate the feeding rates of several copepod

species (Conover 1956; Anraku 1964). Almeda et al. (2010) reported positive relationships between water temperature and ingestion, carbon specific ingestion and clearance rates at satiating food conditions for *O. davisae* NII-III, and Q_{10} was determined as 2.5 for the carbon specific ingestion rates and as 3.0 for the clearance rates. Q_{10} determined for the carbon specific ingestion rate of *A. steueri* nauplii NV was 2.4 which was similar with the value of *O. davisae* nauplii. The carbon specific ingestion rates of *A. steueri* NV positively related with temperature up to 23°C but it greatly decreased in 27°C. *A. steueri* female showed that reducing reproduction rate at 27°C (Uye 1981). Therefore, 27°C may be regarded as the out of optimal range for *A. steueri* nauplii. The decreasing ingestion rate at high temperature might cause reducing their abundance during summer when *A. steueri* often disappeared (Onoue et al. 2006).

3.4.4. Empirical model of ingestion rate

Copepod feeding rates are mainly affected by body carbon weight, temperature, and food concentration (*e.g.* Mullin 1963; Ikeda 1977; Frost 1972). The effects of these factors on feeding parameters such as maximum carbon specific ingestion rates and half saturating food concentrations are foundational inputs for ecological models. Therefore, clarifying the factors which affect naupliar feeding can contribute to understanding the ecological role of copepod nauplii. Present results described significant relationships between carbon weight and maximum carbon specific ingestion rate, or half-saturated concentration (Figure 3-5). The empirical model constructed here could calculate carbon specific ingestion rates from carbon weights and food concentrations and be supplemented by Q_{10} to apply the model to different water temperatures (Figure 3-7 A); however, Q_{10} would be different among species. Thus, specific Q_{10} should be carefully tested when applying the present empirical model to other species.

To evaluate whether this empirical model 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), and the constructed model was then verified. Two equations of relationships between

carbon weight (W; ng C ind^{-1}) and feeding parameters, maximum ingestion rate (I_{max}; cells ind^{-1} day^{-1}) and half-saturation food concentration (K_m; cells mL^{-1}), were obtained from Almeda et al. (2010: Table 4) as

$$I_{max} = 8.9W^{0.77} \text{ (Equation 3-6)},$$

$$K_m = 259(1-e^{-0.029W}) \text{ (Equation 3-7)}.$$

These equations were substituted into a type III functional response model to construct the specific empirical model optimized for *O. davisae*:

$$I = 8.87W^{0.766}(C^2_{cell}/C^2_{cell}+(259(1-e^{-0.029W}))^2)) \text{ (Equation 3-8)},$$

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 constructed empirical model (Equation 3-8) using carbon weight (Almeda et al. 2010: Table 1) and food concentration (Almeda et al. 2010: Figure 1) were significantly related with measured rates, with a high r^2 value (0.99) and a P value less than 0.01 (Figure 3-7 B). This suggests that carbon weight and food concentration are the main explanatory factors of naupliar ingestion rate, and the empirical model proposed here can be applied for other species.

Table 3-1. Cell/body length and carbon weight of *Isochrysis galbana* and *Acartia steueri* nauplii used in the feeding experiments. Modified from Natori and Toda (2018).

Organisms		Length±SD (μm)	Carbon weight±SD (ng C ind^{-1})
Isochrysis galbana		4.5±0.28	(9.9±0.9) × 10^{-3}
Acartia steueri nauplii	NIII–NIV	133±14	42±12
	NIV–NV	146±9	56±11
	NV–NVI	195±15	141±36
	NVI	263±25	353±108

SD; standard deviation

Table 3-2. Prey:predator ratios between gut contents and *Acartia* nauplii.

Gut contents	Size (μm) Mean±SD	Range of ratio (%)	n
Cyanobacteria-like cells	5.6±1.7	1.7–5.7	16
Bacteria-like cells	0.86±0.25	0.31–0.73	23
Diatoms	6.8±2.2	2.3–6.5	15
Flagellate cells	8.1±2.0	4.6–6.2	5

Table 3-3. Prey:predator ratio between *Isochrysis galbana* (4.5 μm) and *Acartia steueri* nauplii.

Acartia steueri nauplii		*Isochrysis galbana*
Stage	Length (μm)	Ratio (%)
NIII	133	3.4
NIV	149	3.1
NV	195	2.3
NVI	263	1.7

Table 3-4. Feeding parameters at each developing stage of *Acartia steueri* nauplius. Modified from Natori and Toda (2018).

Feeding parameter	NIII–NIV	NIV–NV	NV–NVI	NVI
$^{S}I_{max}$ ±SE (d^{-1})	22±1	17±2	5.1±0.5	2.5±0.4
K_m ±SE (μg C L^{-1})	18±4	21±6	37±8	78±20
I_{max} ±SE (μg C ind^{-1} d^{-1})	0.63±0.24	0.94±0.28	0.60±0.18	1.1±0.3
K_t ±SE (μg C L^{-1})	20±5	25±7	30±9	118±31

$^{S}I_{max}$; maximum carbon specific ingestion rate, K_m; half-saturation food concentration, I_{max}; maximum ingestion rate, K_t; feeding threshold food concentration, SE; standard error

Table 3-5. Equations obtained from the experiments. Quitted from Natori and Toda (2018).

Relations	Equations
$^{S}I_{max}$ vs. Carbon weight	log $^{S}I_{max}$ = -1.1logCW+3.0
K_m vs. Carbon weight	log K_m = 0.69logCW+0.13
$^{S}I_{max}$ vs. Temperature	$^{S}I_{max}$ = 0.57T-2.9

$^{S}I_{max}$; maximum carbon specific ingestion rate (d^{-1}), K_m; half-saturation food concentration (μg C L^{-1}), CW; carbon weight (ng C ind^{-1}), T; temperature (°C)

able 3-6. Comparison of ingestion, carbon specific ingestion and clearance rates of *Acartia* nauplii. Quitted from Natori and Toda (2018).

Species	Stage	Body length (μm)	Temp. (°C)	Prey type	Relative prey size (%)	Food conc. (μg C L^{-1})	Ingestion rate (μg C ind^{-1} d^{-1})	Carbon specific ingestion rate (d^{-1})	Clearance rate (mL ind^{-1} d^{-1})	Density (ind mL^{-1})	Container volume (mL)	Ref.
Acartia tonsa	III	113[a]	20	Cultured ciliates (mixture; 12–65 μm)	11–57	0.15–6.5	0.0072–0.18	0.17–.0	11–49	0.04	250	[1]
	IV–VI	171–257[a]	22	*Isochrysis galbana* (4–5 μm)	3.9–1.8	648	0.11–0.48	0.77–3.4	0.35	0.28	72	[2]
				Aureococcus anophagefferens (2–4 μm)	2.1		0.29	2.08	0.44			
				Micromonas pusilla (1–3 μm)	1.3		0.19	1.32	0.33			
	-	-	-	Fluorescently labeled bacteria	0.85	-	0.1–1.2	1.9–16	1–8	0.17	18	[3]
	-	-	20	*Euplotes* sp.	-	75–600	0.3–1.1	0.40–16	-	0.10	200	[4]
Acartia grani	II–III	137	20	*Heterocapsa* sp. (ESD[b]=12.8)	9.3	5.1–535	0.0015–0.061	0.046–1.9	0.11–0.35	-	72	[5]
	II–III	134		*Thalassiosira weissflogii* (ESD[b]=14.4)	11	7.5–603	0.0015–0.030	0.046–0.91	0.071–0.49	-		
Acartia steueri	III–IV	133	17	*Isochrysis galbana* (4.5 μm)	3.4	8.1–370	0.086–0.88	2.1–25	1.9–19	0.02	250	[6]
	IV–V	146			3.1	11–374	0.085–0.94	1.6–21	1.4–26			
	V–VI	195			2.3	6.3–276	0.021–0.76	0.15–5.6	2.1–20			
	VI	262			1.7	22–360	0.0059–0.92	0.017–3.0	0.25–6.5			

a; body lengths are obtained from Leandro et al. (2006: Fig. 2, 22°C), b; ESD is the abbreviation of equivalent spherical diameter, blank cell; same as the just above cell, -; no data. Relative prey size is culculated by dividing average bodey length into average prey size. [1] Stoecker and Egloff (1987), [2] Smith et al. (2008), [3] Turner and Tester (1992), [4] Ismar et al. (2008), [5] Henriksen et al. (2007), [6] This study

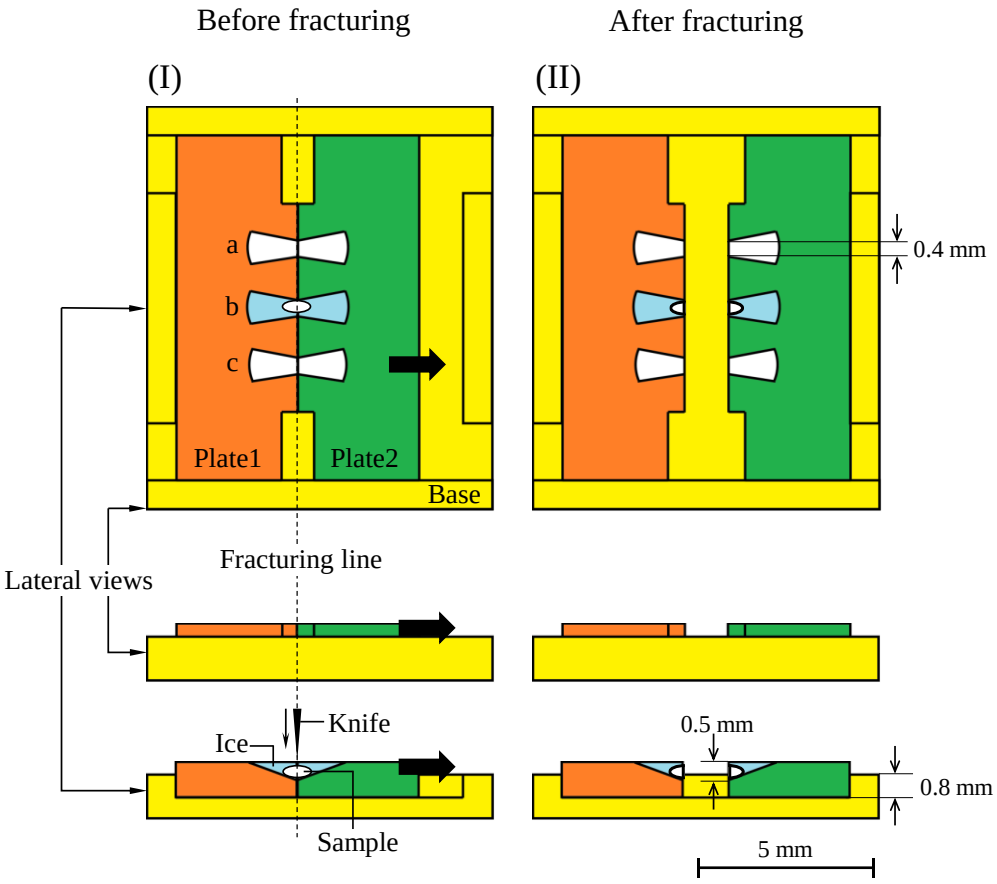


Figure 3-1. Device design and its fracturing mechanism. Schematics of the devise before fracturing (I) and after fracturing (II) modified from Natori et al. (2017).

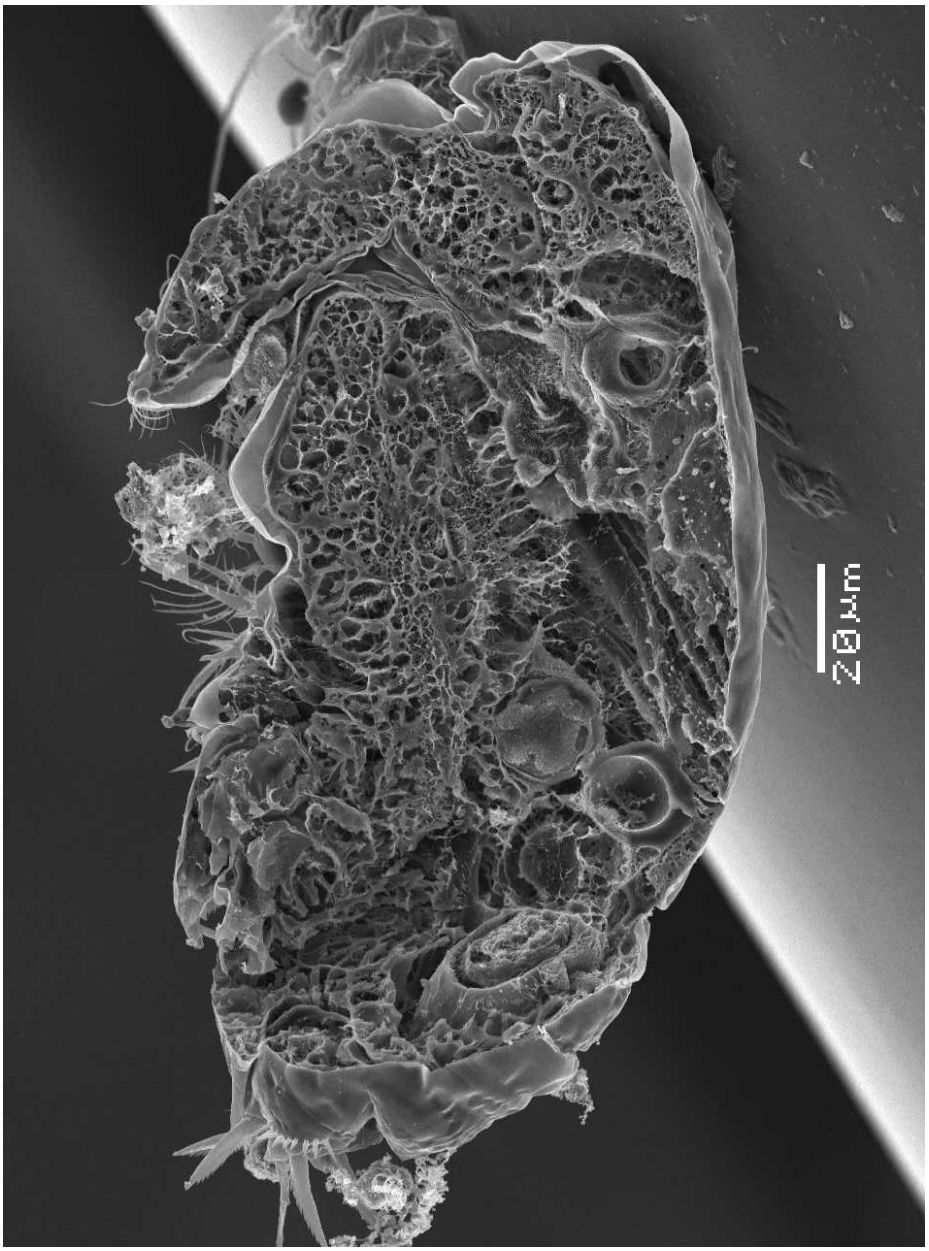


Figure 3-2. Scanning electron micrograph of internal structures of *Acartia steueri* nauplii.

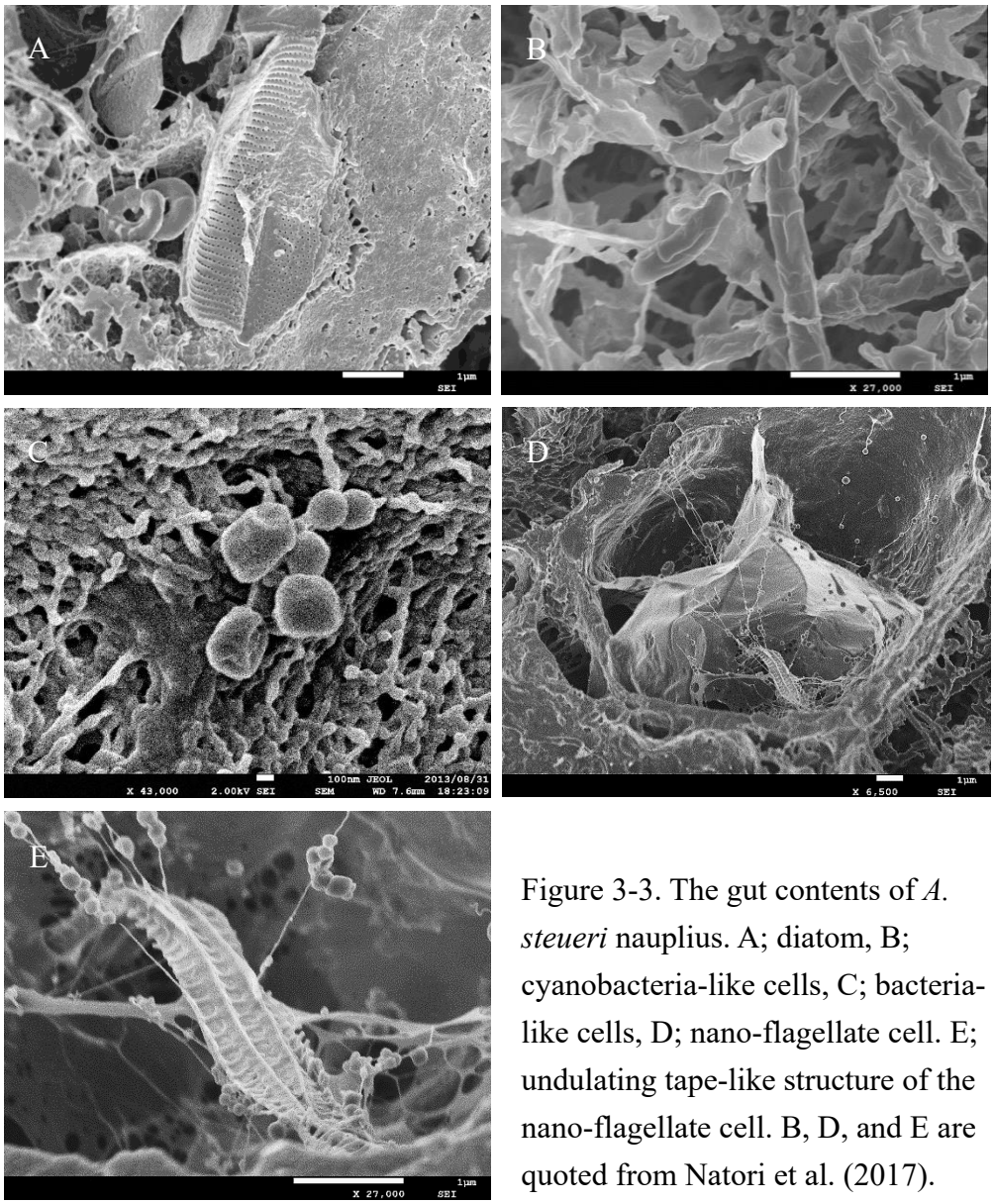


Figure 3-3. The gut contents of *A. steueri* nauplius. A; diatom, B; cyanobacteria-like cells, C; bacteria-like cells, D; nano-flagellate cell. E; undulating tape-like structure of the nano-flagellate cell. B, D, and E are quoted from Natori et al. (2017).

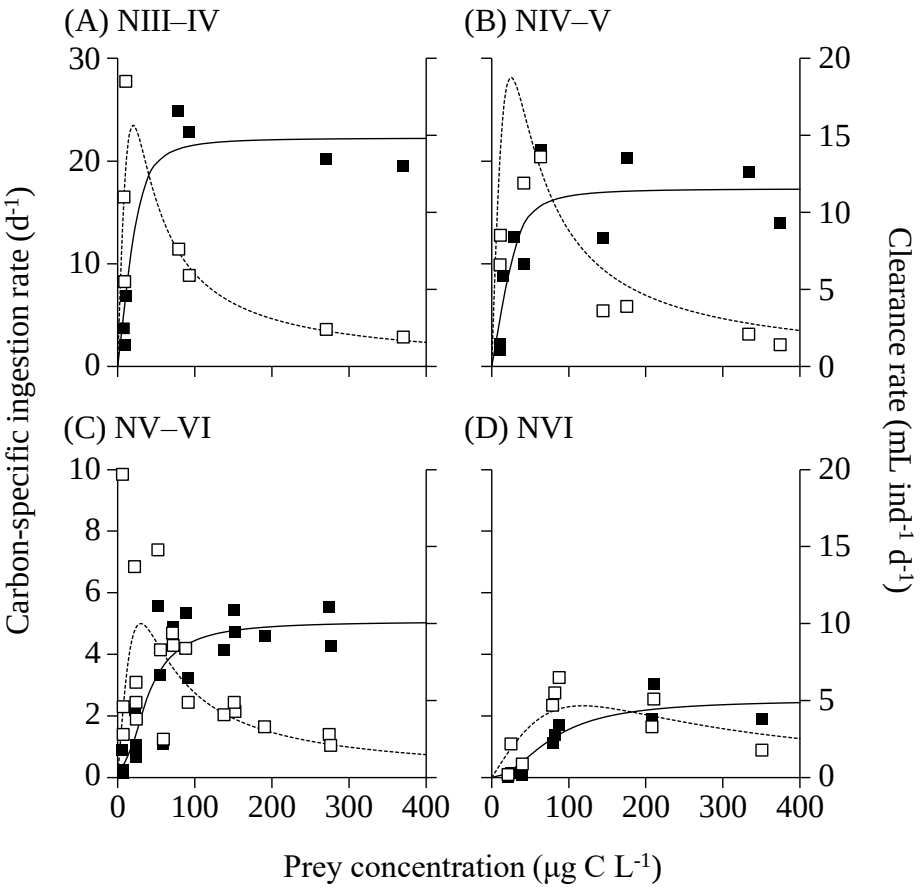


Figure 3-4. Relationship between food concentration and carbon specific ingestion or clearance rate for different developmental stages of *Acartia steueri* nauplii: (A) NIII–IV, (B) NIV–V, (C) NV–VI and (D) NVI. Closed and open dots describe carbon specific ingestion and clearance rate, respectively. The continuous and broken lines correspond to the models fitted on the data. The estimated equations were obtained:

(A) $^{S}I = 22\times(C^2/(C^2+18^2))$

$F = 0.63\times(C/(C^2+20^2))$

(B) $^{S}I = 17\times(C^2/(C^2+21^2))$

$F = 0.94\times(C/(C^2+25^2))$

(C) $^{S}I = 5.1\times(C^2/(C^2+37^2))$

$F = 0.60\times(C/(C^2+30^2))$

(D) $^{S}I = 2.5\times(C^2/(C^2+78^2))$

$F = 1.1\times(C/(C^2+118^2))$

where ^{S}I is the carbon specific ingestion rate (d^{-1}), C is the concentration of prey (µg C L^{-1}), F is the clearance rate ($\times10^{-3}$ L ind^{-1} d^{-1}). Modified from Natori and Toda. (2018).

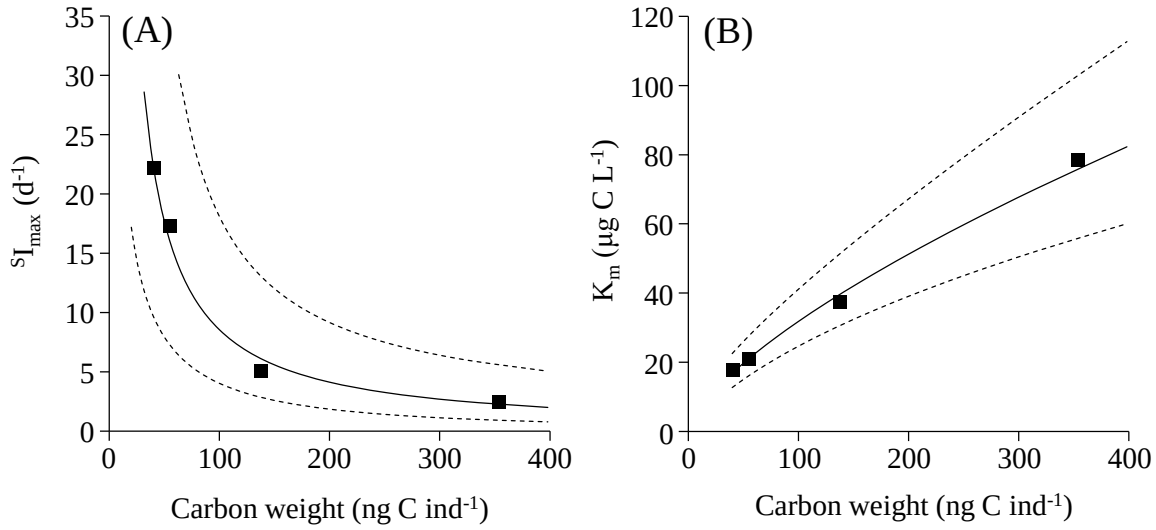


Figure 3-5. Relationships between feeding parameters and carbon weight. $^{S}I_{max}$ is maximum carbon specific ingestion rate (A), and K_m is half-saturation food concentration (B). The continuous lines correspond to regression curves fitted on the data. The broken lines correspond to 95% prediction interval. The regression equations were obtained:
(A) $^{S}I_{max} = 10^{3.0} \times CW^{-1.1}$
(B) $K_m = 10^{-0.13} \times CW^{0.69}$
where CW is the carbon weight. Modified from Natori and Toda (2018).

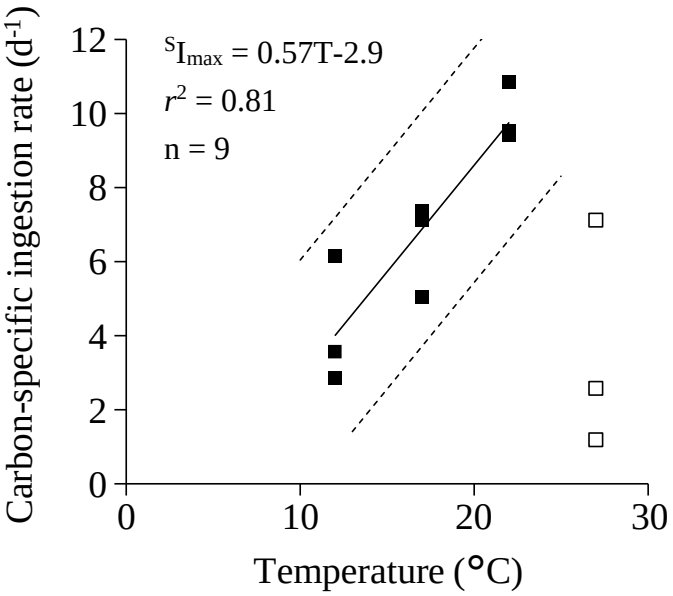


Figure 3-6. Relationship between temperature and carbon specific ingestion rate of NV. The continuous line and broken lines correspond to regression line and 95% prediction interval, respectively. The open dots were removed from analysis. Modified from Natori and Toda. (2018).

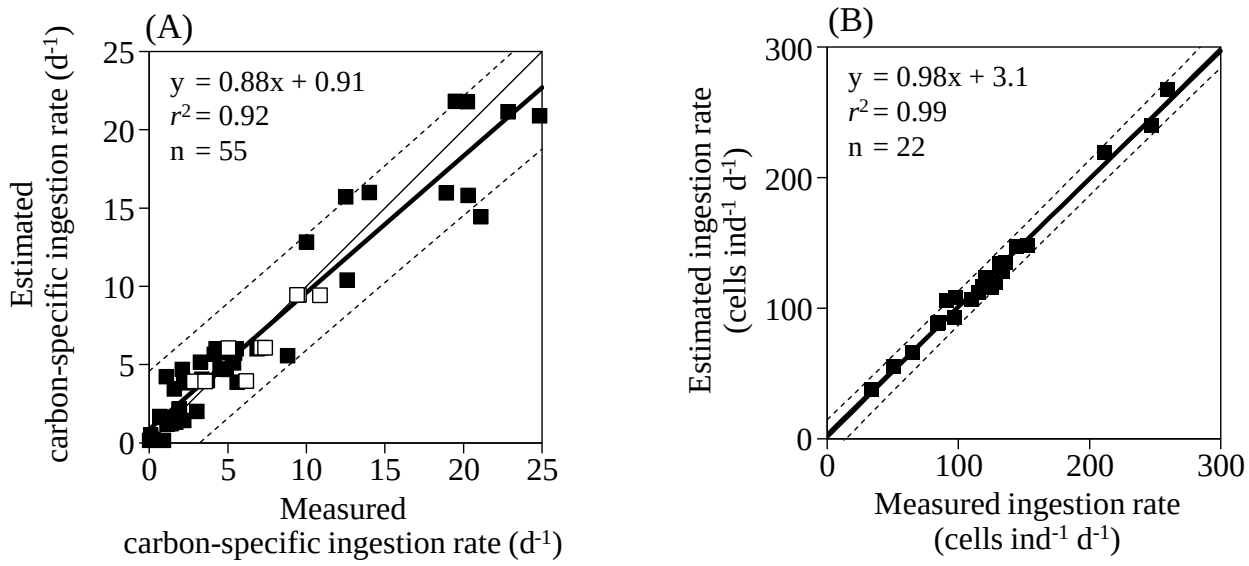


Figure 3-7. Relationships between measured and estimated ingestion rates. (A) Carbon specific ingestion rates of this study. The open dots correspond to the results of temperature experiment supplemented by using $Q_{10} = 2.4$. (B) Ingestion rates calculated by using the date set of Almeda et al. (2010). The continuous and broken lines correspond to regression line and 95% prediction interval, respectively. Modified from Natori and Toda. (2018).

Chapter IV

General discussion

This study newly proposed an estimation method for *in situ* naupliar ingestion rate reflecting the effects of food concentration and demonstrated its application to a field investigation in the temperate embayment waters of Sagami Bay to investigate the ecological role of copepod nauplii as a predator. The seasonal variations in environmental and biological variables including copepod nauplii were described in Chapter II; the determination of the prey:predator ratio of *Acartia* spp. nauplii and the effects of food concentration, individual carbon weight and water temperature on the carbon specific ingestion rate of *A. steueri* nauplii as described in Chapter III.

4.1. Factors affecting ingestion rates of copepod nauplii

To determine the factors (water temperature, food concentration, carbon weight) most affecting the estimation of *in situ* ingestion rates, the ingestion rates of *Acartia* spp. nauplii were estimated using the empirical model (Equation 3-5) described in Chapter III with water temperature, biomass, and food concentration as investigated in Chapter II and were compared with the ingestion rates estimated using the previously reported respiration method (Ikeda and Motoda 1978). According to the results presented in Chapter III, picophytoplankton, 2–10 μm phytoplankton, and HNF can be regarded as *in situ* food items. Therefore, *in situ* ingestion rates were estimated in the month during which < 10 μm chlorophyll *a* concentration was measured. The effect of water temperature was supplemented using the Q_{10} that was estimated in Chapter III when the *in situ* water temperature was lower than 22°C. When the temperature was higher than 22°C, a conversion factor of 0.29 calculated by dividing the ingestion rate at 27°C estimated by the regression line described in Figure 3-6 with the average ingestion rate actually measured at 27°C, was used to supplement the effect of temperature. As a result, the estimated ingestion rates by the respiration method varied with the change in carbon weight (Figure 4-1). On the other hand, the ingestion rates estimated by

the empirical model varied with the change in food concentration rather than by carbon weight. The standardized partial regression coefficient of each factor was calculated to compare the contribution of factors to the estimated ingestion rates (Table 4-1). Food concentration was most explanatory factor of the estimated ingestion rates in terms of the empirical model, whereas temperature was not significant. On the other hand, the temperature and carbon weight contribute equally to the estimation in terms of the respiration method. The non-significance of temperature suggested that the effect of change of food concentration on naupliar ingestion rates was quite larger than that of temperature during the study period. These results show that the empirical model would be useful to estimate naupliar ingestion rates in regions where its food concentration varies largely.

The survival strategy of copepod nauplii can be discussed using the results of the investigated food environment in Chapter II and that of the functional response model obtained in Chapter III. The higher maximum carbon specific ingestion rate of *A. steueri* nauplii during earlier development stages suggests that they need high carbon uptake to grow rapidly. The half saturation constant, K_m, describes the concentration that provides the half-maximum ingestion rate; that is, the ingestion rate is restricted by over 50% at low K_m. Thus, lower K_m is suitable for low-food environments. Conversely, high K_m results in high feeding capacity in abundant food environments. The results of this study showed an increase in K_m with increase in the carbon weight, indicating that *A. steueri* nauplii might be better suited for survival in low-food environments during the early stages and may be more suited for growth in high-food environments during the later stages of development. Such adaptations of relative suitability to low food environments during early developmental stages can be advantageous for the survival of hatched nauplii in temperate embayment waters, where the environment with respect to food varies significantly over short and long periods (Onoue et al. 2006; Ara and Hiromi 2009; Chapter II).

4.2. Relative role of copepod nauplii as a predator in microbial food web

To evaluate the ecological role of copepod nauplii in microbial food webs, their food requirements

were estimated using the empirical model and were compared with those of other microzooplankton—naked ciliates, tintinnids, and heterotrophic dinoflagellates—estimated using the equations obtained from previous studies. The food requirement of *Acartia* sp. nauplii was estimated using the following equation:

$$FR_{NAUP} = {}^{S}I \times B_{Acartia} \quad \text{(Equation 4-1)}$$

where, ${}^{S}I$ is the carbon specific ingestion rate (d^{-1}) estimated using the empirical model (Equation 3-5) obtained in Chapter III. As mentioned above, picophytoplankton, 2–10 μm phytoplankton, and HNF were regarded as *in situ* food items. However, < 10 μm chlorophyll *a* concentration was measured from November 2012 to February 2014. Therefore, the ratio of < 10 μm chlorophyll *a* concentration against < 20 μm chlorophyll *a* concentration was calculated in each season, and the < 10 μm chlorophyll *a* concentration estimated from < 20 μm chlorophyll *a* concentration by the ratios was used for the three-year sampling period. $B_{Acartia}$ is the biomass of *Acartia* spp. nauplii (μg C L^{-1}) investigated in Chapter II. The food requirements of other copepod nauplii were estimated using the investigated biomass and Equation 3-5 for Calanoida and Harpacticoida, and Equation 3-8 for Cyclopoida. To compare the food requirements of copepod nauplii and those of other microzooplankton, the food requirements of other microzooplankton were estimated using the equations (Table 4-2) described by Ara and Hiromi (2009). The results revealed that the food requirement of copepod nauplii varied from 0.006 μg C L^{-1} d^{-1} in December 2014 to 26 μg C L^{-1} d^{-1} in April 2014 (Figure 4-2). The month that showed the peak for food requirement was different based on the year, but the food requirement tended to be high during spring and autumn in the study period (Figure 4-2). Although the food requirement of naked ciliates was the highest in the community, accounting for 78% of the annual mean food requirement (Figure 4-3), copepod nauplii showed the food requirement accounting for 6.4% of the annual mean food requirement. The food requirement of tintinnids and heterotrophic dinoflagellates accounted for 4.1% and 11%, respectively. In terms of seasonal food requirements, copepod nauplii accounted for 9.7% during winter (December-February), 32% during spring (March-May), 2.1% during summer (June-

August), and 4.4% during autumn (September-November) (Figure 4-4). According to these results, copepod nauplii can be regarded as one of major predators in the microbial food web during spring. In Chesapeake Bay, the daily food requirement of *A. tonsa* nauplii varied from 0.6 to 47 μg C L^{-1} d^{-1}, and accounted for 7.4–52% of the total depth-integrated food requirement of heterotrophic organisms including copepodites and adults (White and Roman 1992), which is similar to that in the present study.

The feeding impact of microzooplankton on < 20 μm phytoplankton and HNF was compared with the biomass of those food items (Figure 4-5). The feeding impact varied from 0.21% in January 2013 to 487% in June 2014, and the removal percentage exceeded 100% in every summer. This result suggests that the summer prey environment was inadequate for copepod nauplii and that the competition with micro protozoans was keen.

As the efficiency of the microbial food web is lower than that of the classical food web, it has been suggested that the inflow of dissolved organic carbon increases with an increase in precipitation and river runoff by climate change, favoring a bacteria-based food web and reducing pelagic productivity at higher trophic levels (Bergland et al. 2007). In addition, intensification of wind and rainfall in the past typhoons might be influenced by global warming (Elsner et al. 2008, Knutson et al. 2010, Yamada et al. 2010). The trophic role of copepod nauplii as a predator will be more important in regions where the domination of microbial food web by the intensification of terrestrial runoff is expected. Estimation of ingestion rate using food concentration as described here can contribute to evaluating the effect of these episodic events on the ecological role of copepod nauplii. This study has shown the quantitative contribution of copepod nauplii to the food requirement of microzooplankton in embayment waters based on the change in food concentration. However, further studies are needed to adjust and feedback the results of the laboratory incubation experiments to the natural field. First, the influence of multi prey environment on ingestion rates should be evaluated to consider an *in situ* complex prey environment. Although naupliar ingestion rate can be high within the optimal prey:predator size ratio (Berggreen et al. 1988), values of

constants of the functional response model obtained under multi prey conditions will be different from those values obtained under single prey condition (Helenius and Saiz 2017). Second, the empirical model constructed here should be applied to other regions, especially the region where the prey environment does not vary significantly, such as tropical waters, to evaluate how the variation in food concentration will be considerable in the estimation of naupliar ingestion rates.

4.3. Trophodynamics in the studied embayment area

To determine the ecological role of copepod nauplii, the trophodynamics are assessed by carbon flow through six components (phytoplankton, bacteria, HNF, micro protozoans, copepod nauplii, copepod secondary producers) (Figures 4-6, 4-7 A, B). Primary production was estimated by the relational equation of PP/Chl *a* ratio calculated from silicate concentration (see Appendix 1-1), which was reported in Manazuru Port by Tsuchiya (2014). Production of heterotrophic organisms was estimated using the equations described by Ara and Hiromi (2009). The primary production was 8.7–19 mg C L^{-1} d^{-1}, with an annual production of 52 mg C L^{-1} d^{-1}. The production of each component was estimated to be 0.18–2.0 mg C L^{-1} d^{-1} (3.4 mg C L^{-1} y^{-1} as an annual production) for HNF, 0.16–2.6 mg C L^{-1} d^{-1} (5.2 mg C L^{-1} y^{-1}) for micro protozoans, 0.013–0.029 mg C L^{-1} d^{-1} (0.083 mg C L^{-1} y^{-1}) for copepod nauplii, and 0.046–0.143 mg C L^{-1} d^{-1} (0.30 mg C L^{-1} y^{-1}) for copepod secondary producers (Figures 4-6, 4-7 A, B). The food requirement of each component was estimated to be 0.62–7.8 mg C L^{-1} d^{-1} (14 mg C L^{-1} y^{-1}) for HNF, 0.39–9.2 mg C L^{-1} d^{-1} (18 mg C L^{-1} y^{-1}) for micro protozoans, 0.0079–0.21 mg C L^{-1} d^{-1} (0.61 mg C L^{-1} y^{-1}) for copepod nauplii, and 0.19–0.57 mg C L^{-1} d^{-1} (1.2 mg C L^{-1} y^{-1}) for copepod secondary producers (Figures 4-6, 4-7 A, B).

The estimated production rate and food requirement of heterotrophic organisms were relatively low in winter and higher in summer and autumn. Copepod nauplii would be important predators in the microbial food web as discussed above but it is suggested that their contribution to the material flow to copepod secondary producers would be negligible because their production rate

was quite lower than those of phytoplankton and micro protozoans (Figures 4-6, 4-7 A, B). In addition, the assimilation efficiency of copepod nauplii was calculated to be 14% (annual), 16% (winter), 9% (spring), 14% (summer), and 12% (autumn) by dividing the food requirement into production rate (Figures 4-6, 4-7 A, B). On the other hand, the assimilation efficiency of micro protozoans was calculated to be 29% (annual), 40% (winter), 33% (spring), 28% (summer), and 29% (autumn). The low assimilation efficiency of copepod nauplii suggests that the carbon transferring efficiency of the microbial food web would be poor when the contribution of copepod nauplii as a predator is significant.

The range of feeding impact of microzooplankton on primary production or standing stock was larger in Sagami Bay compared with the other temperate coastal waters (Table 4-3), which may be caused by the large difference in microzooplankton biomass between summer and winter in Sagami Bay (Figure 2-9) (Ara and Hiromi 2009). Among Sagami Bay, the average feeding impact of microzooplankton was higher in the embayment area (this study) than in the neritic area (Ara and Hiromi 2009) (Table 4-3). The microbial food web in the embayment ecosystem might be more predominant as a pathway of material transfer compared to the microbial food web in the neritic ecosystem. The range of percentages of naupliar food requirement against the total food requirement in present study was similar to that in the Cantabrian coast (Quevedo and Anadón 2000) and Inland Sea of Japan (Godhantaraman and Uye 2001) (Table 4-3). Godhantaraman and Uye (2001) investigated the abundance, biomass, and production rate of microzooplankton including copepod nauplii from inshore to offshore at 11 stations (4 inshore and 7 offshore) in June and at 8 stations (4 inshore and 4 offshore) in February–March. The stations were several kilometers away from each other. A marked decline of naupliar abundance, biomass, and production rate from inshore to offshore was observed in June, whereas the geographical variations were less pronounced among inshore stations. Horizontal distribution of copepod nauplii in relatively small areas such as a port, an inlet, etc. may not be large compared to the difference between inshore and offshore.

The most part of carbon lost via copepod nauplii might be released as fecal pellets which inputs the carbon of pico- and nano-sized plankton to the benthic ecosystem by packaging them as a micro-sized suspension (Pasternak et al. 2000) or as dissolved organic carbon by sloppy feeding which has been observed in adult copepods (e.g. Strom et al. 1997; Møller 2005; Møller 2007). Møller (2007) investigated the relationship between the dissolved organic carbon produced by sloppy feeding and prey:predator size ratio in three species of marine copepod copepodites and/or adults (*Acartia tonsa*, *Centropages typicus* and *Temora longicornis*). Dissolved organic carbon production by sloppy feeding was observed when relatively large size phytoplankton (*Heterocapsa rotundata*; 15 μm in equivalent spherical diameter (ESD), *Ditylum brightwelli*; 39 μm in ESD) were supplied. On the other hand, sloppy feeding was not detected when relatively small size phytoplankton (*Rhodomonas salina*; 7 μm in ESD) were supplied. The dissolved organic carbon production was significantly related with the prey:predator ratio regardless of the copepod species. Of the total removed phytoplankton carbon, 15–36% was lost as dissolved organic carbon when the prey:predator size ratio was 4–6%. Sloppy feeding on large size prey has been suggested in *Paracartia grani* nauplii (Helenius and Saiz 2017). Therefore, sloppy feeding of copepod nauplii might be considerable as the carbon source of microbial food web when the high abundance, biomass, and ingestion rate of copepod nauplii appear in blooms with relatively high composition of large sized phytoplankton (Figure 2-3; Satoh et al. 2000; Ara and Hiromi 2009; Ara et al. 2011).

4.4. Conclusion

The present study proposes a novel estimation method of ingestion rate reflecting the effects of food concentration and demonstrates its application to a field investigation in temperate embayment waters in Sagami Bay. The results revealed that the empirical model of ingestion rate including the explanatory variables of food concentration and body carbon weight was constructed, and that the model quantitatively described the effect of food concentration on the *in situ* ingestion rate of copepod nauplii. In addition, application of electron microscopy to observe the naupliar gut content

was enabled using a newly designed fracturing device in the analysis process. The following conclusions were drawn: (1) the microbial food web was the main route of carbon flow in the study site; (2) *Acartia* spp. nauplii and cyclopoid nauplii were the dominant groups during spring and from summer to autumn in the study period; (3) the gut contents of copepod nauplii were firstly observed in the present study and prey:predator size ratio of *Acartia* spp. nauplii were revealed; (4) 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; (5) copepod nauplii seasonally play a role as one of main pathways transferring the primary and bacterial production of the microbial food web to higher trophic levels with low assimilation efficiency, that is, the material transfer efficiency of the temperate embayment water might be poor when the relative role of copepod nauplii as a predator has been dominant.

Table 4-1. 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	$6.6 \times 10\text{-}9$	***
Carbon weight	0.73	0.056	13	$8.2 \times 10\text{-}9$	***

Table 4-2. Relational equations used for calculation of ingestion rates of heterotrophic organisms (Hansen et al. 1997).

Heterotrophic organisms	Maximum specific ingestion rate (d^{-1})
Heterotrophic nanoflagellates	$I=21.89CV^{-0.16}$
Naked ciliates	$I=30.22CV^{-0.20}$
Tintinnids	$I=30.22LV^{-0.20}$
Heterotrophic dinoflagellates	$I=33.91CV^{-0.27}$

CV; cell volume, LV; lorica volume

Table 4-3. Comparison of food requirement, assimilation efficiency and feeding impact in temperate coastal waters.

Study	Community	Period	Parentage of naupliar food requirement to that of the total (%)	Assimilation efficiency		Feeding impact (%)	
				Micro protozoans	Copepod nauplii	Community	Copepod nauplii
Chesapeake Bay (White and Roman 1992)	Heterotrophic organisms	March, May August, October	7.4–52	-	-	-	7.5–28 on production
Cantabrian coast (Quevedo and Anadón 2000)	Microzooplankton	May	8.8–48	-	-	-	0.6–7.4 on standing stock
Inland Sea of Japan (Godhantaraman and Uye 2001)	Microzooplankton	June February−March	5–30	-	-	4.1−37 on standing stock	-
Chesapeake Bay (Sun et al. 2007)	Microzooplankton	Early spring	-	-	-	49–171 on standing stock	-
Bay of Biscay (López et al. 2007a)	<200 μm copepods	Annual	-	-	-	0.3–12 on standing stock	0.2–7.1 on standing stock
Yellow Sea (Yang et al. 2007)	Microzooplankton	Annual	-	-	-	60–80 om production	-
Suwannee River estuary (Quinlan et al. 2009)	Microzooplankton	Summer–winter	-	-	-	42–74 on standing stock	-
Sagami Bay (neritic area) (Ara and Hiromi 2009)	Microzooplankton	Annual	-	31–42 (including copepod nauplii)	-	1.8–439 (mean: 49) on production	-
Sagami Bay (embayment area) This study	Microzooplankton	Annual	2.1–32	28–40	9–16	0.21–487 (mean: 79) on standing stock	-

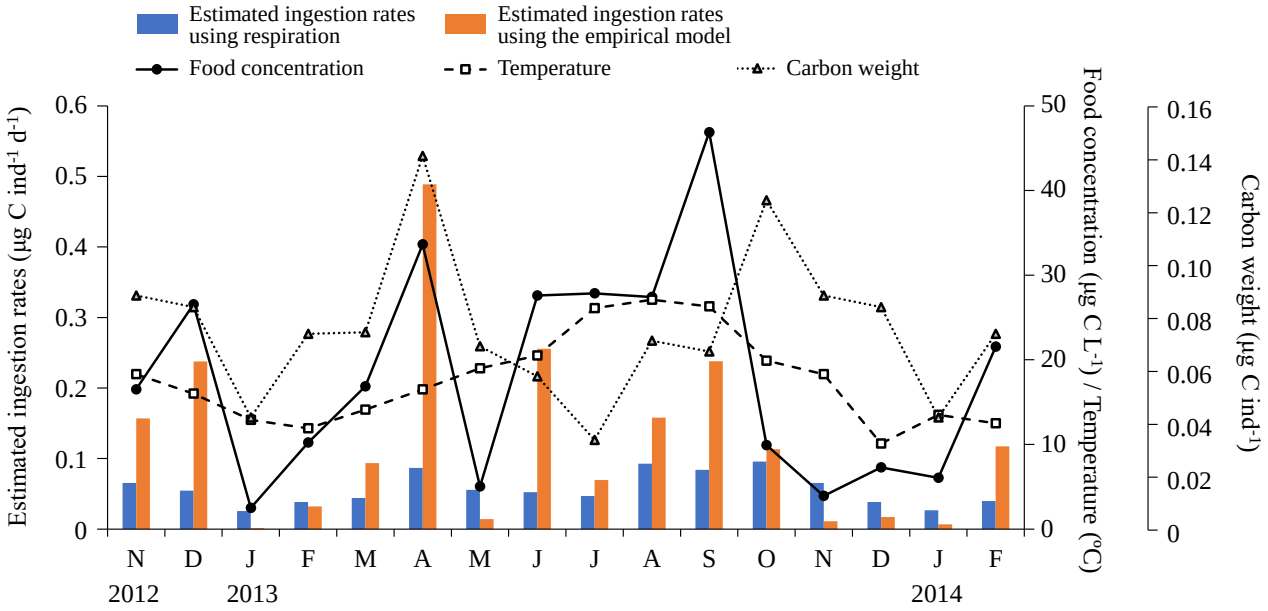


Figure 4-1. Comparison of estimated ingestion rates of *Acartia* spp. nauplii by using respiration rate method (Ikeda and Motoda 1978) and the empirical model obtained in this study.

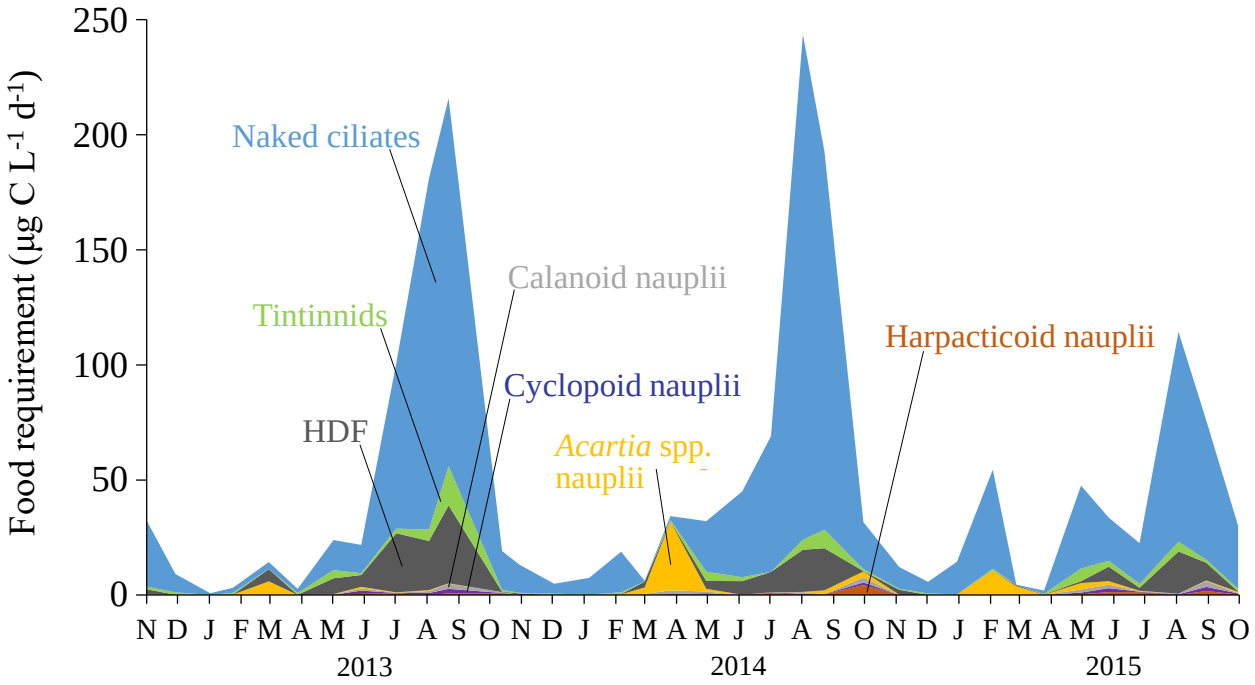


Figure 4-2. Seasonal variations in the food requirements of microzooplankton. HDF; heterotrophic dinoflagellates.

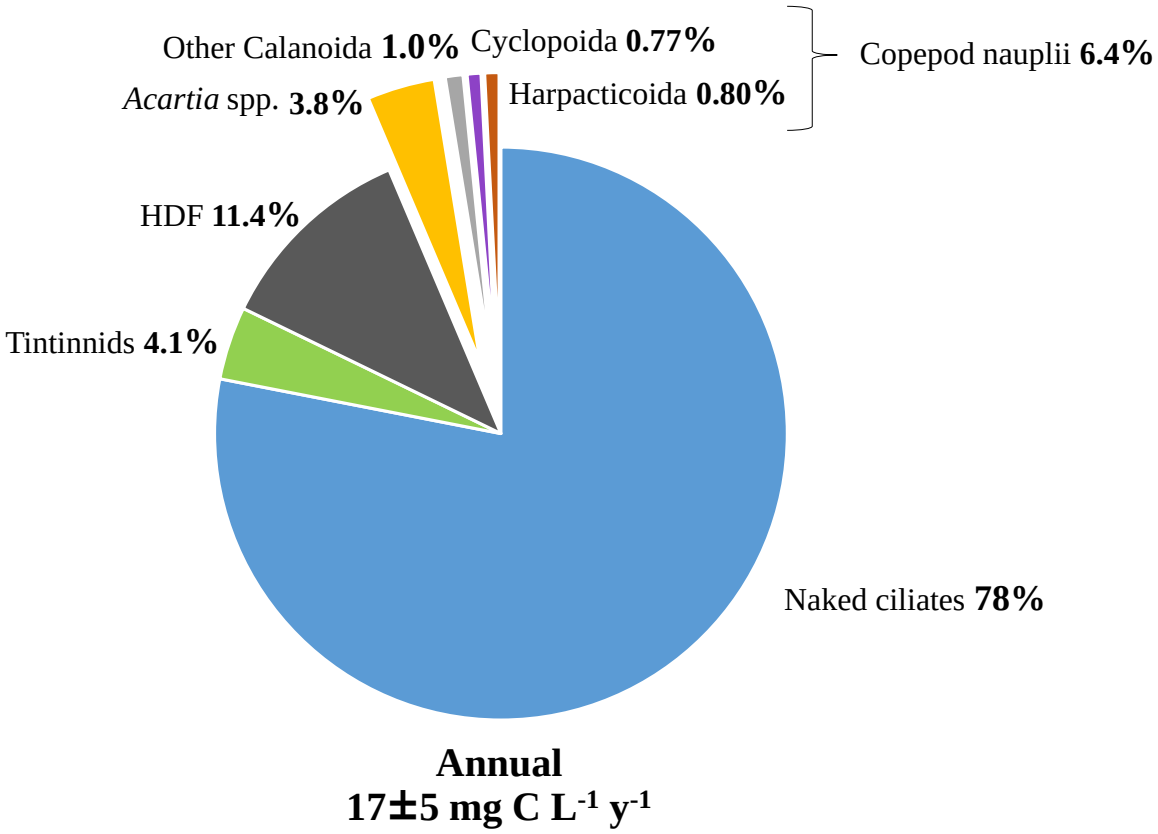


Figure 4-3. Ratio of food requirement of microzooplankton in terms of annual food requirement.

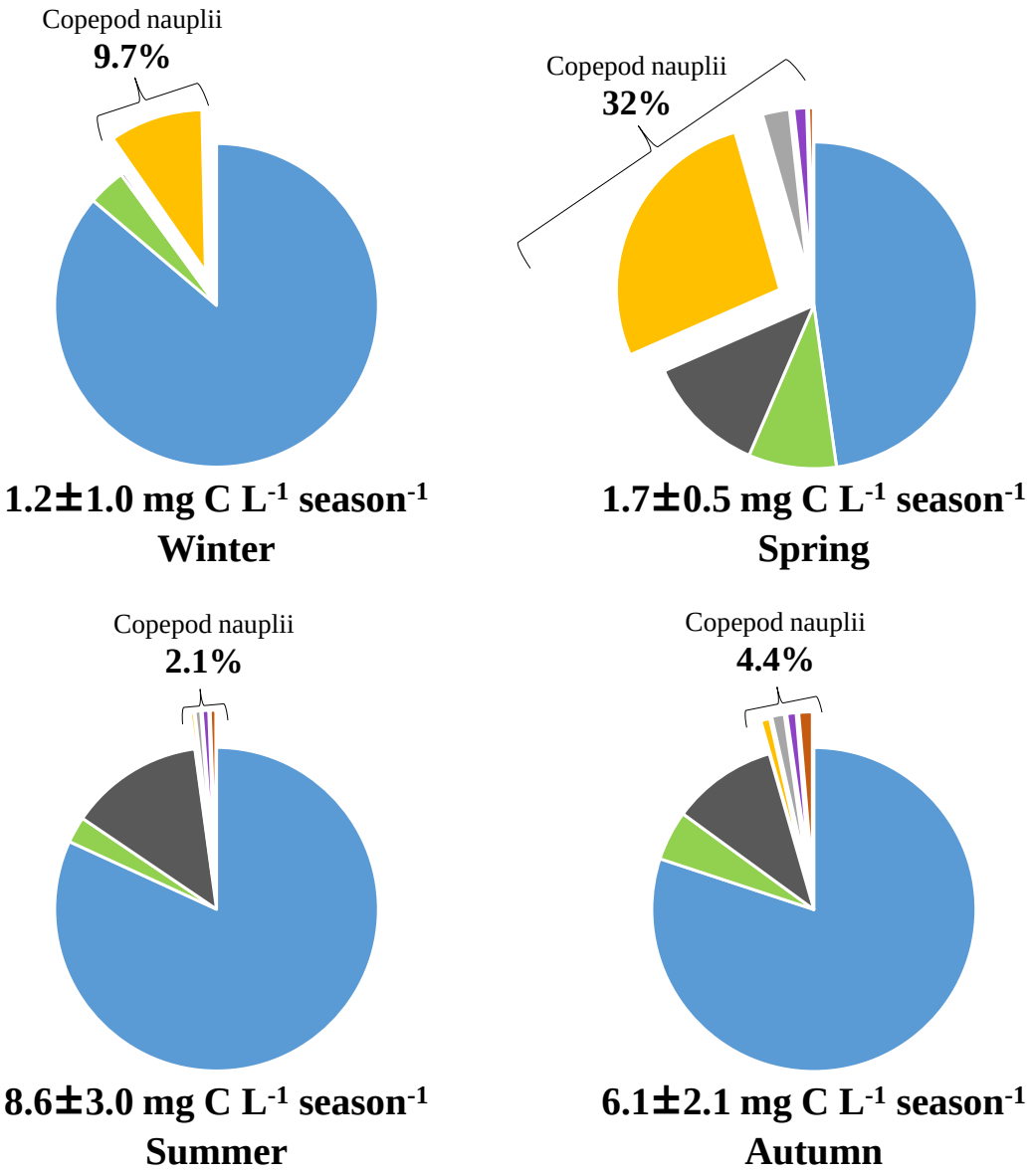


Figure 4-4. Ratio of food requirement of microzooplankton in terms of seasonal food requirement.

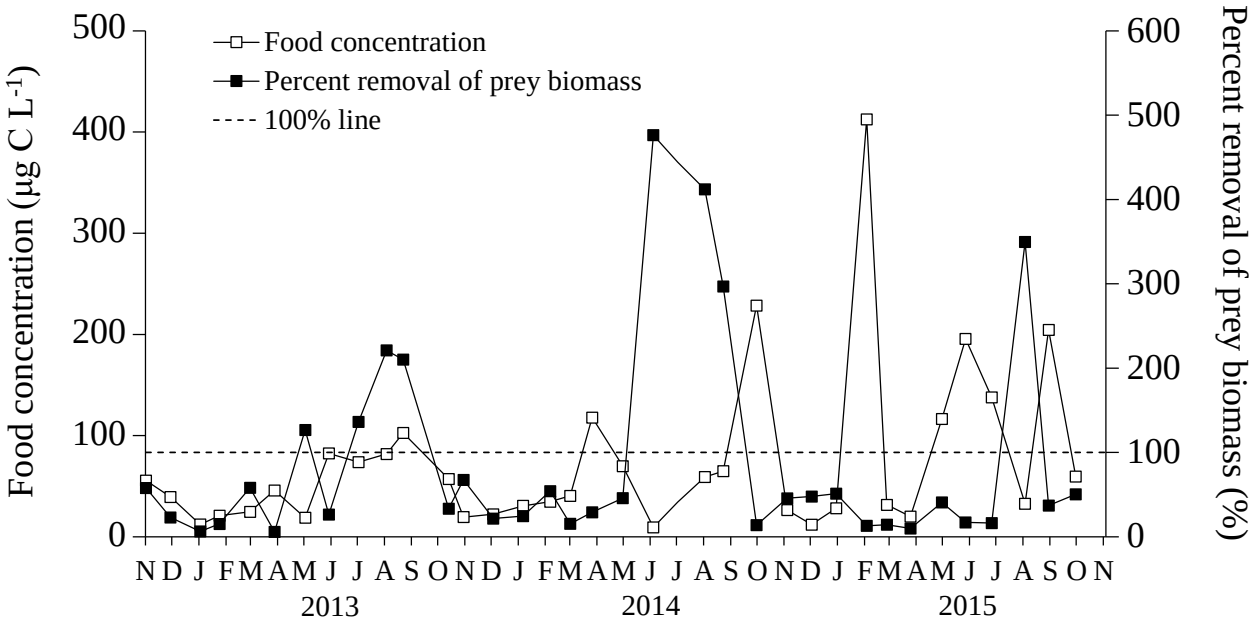


Figure 4-5. Annual variations in the estimated feeding impact of microzooplankton on <20 µm phytoplankton and heterotrophic nanoflagellates.

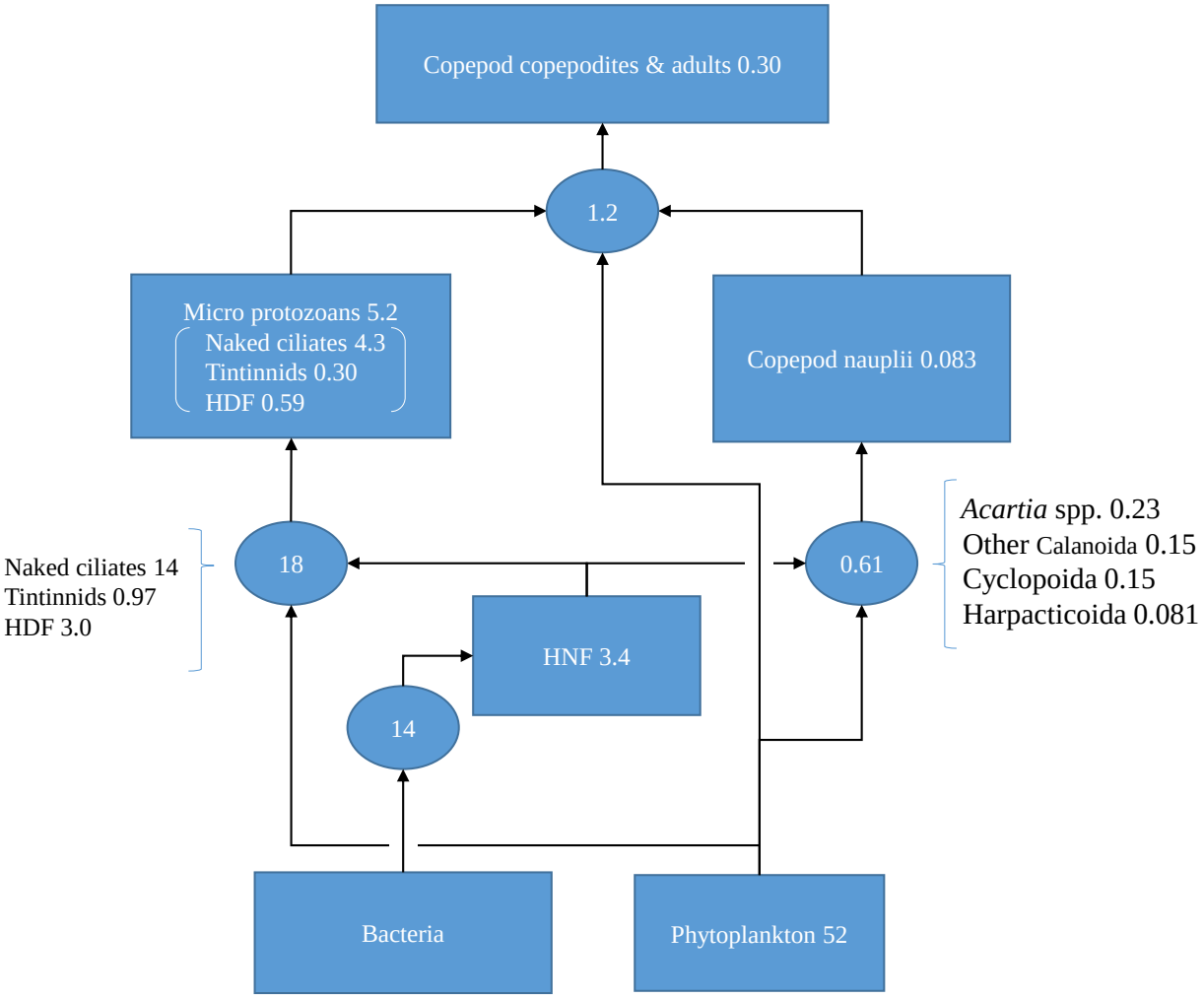


Figure 4-6. Trophodynamics in terms of carbon flow of planktonic organisms from December 2012 to November 2013. HNF; heterotrophic nanoflagellates, HDF; heterotrophic dinoflagellates, number in circle; food requirement, number in square; production rate, number in circle and square; mg C L^{-1} y^{-1}.

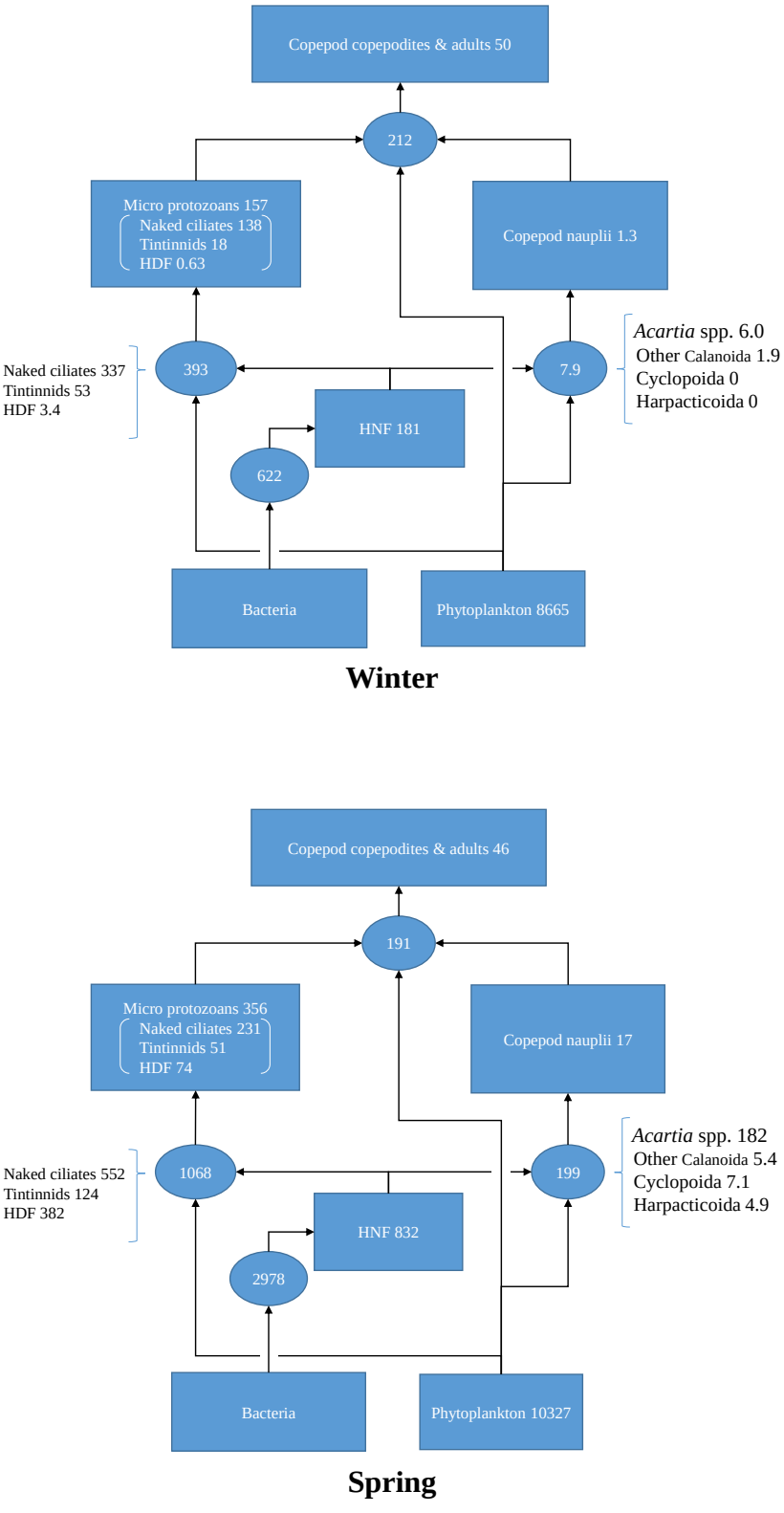


Figure 4-7 A. Trophodynamics of planktonic organisms during winter (from December 2012 to February 2013) and spring (from March 2013 to May 2013). Number in circle and square; μg C L^{-1} $season^{-1}$.

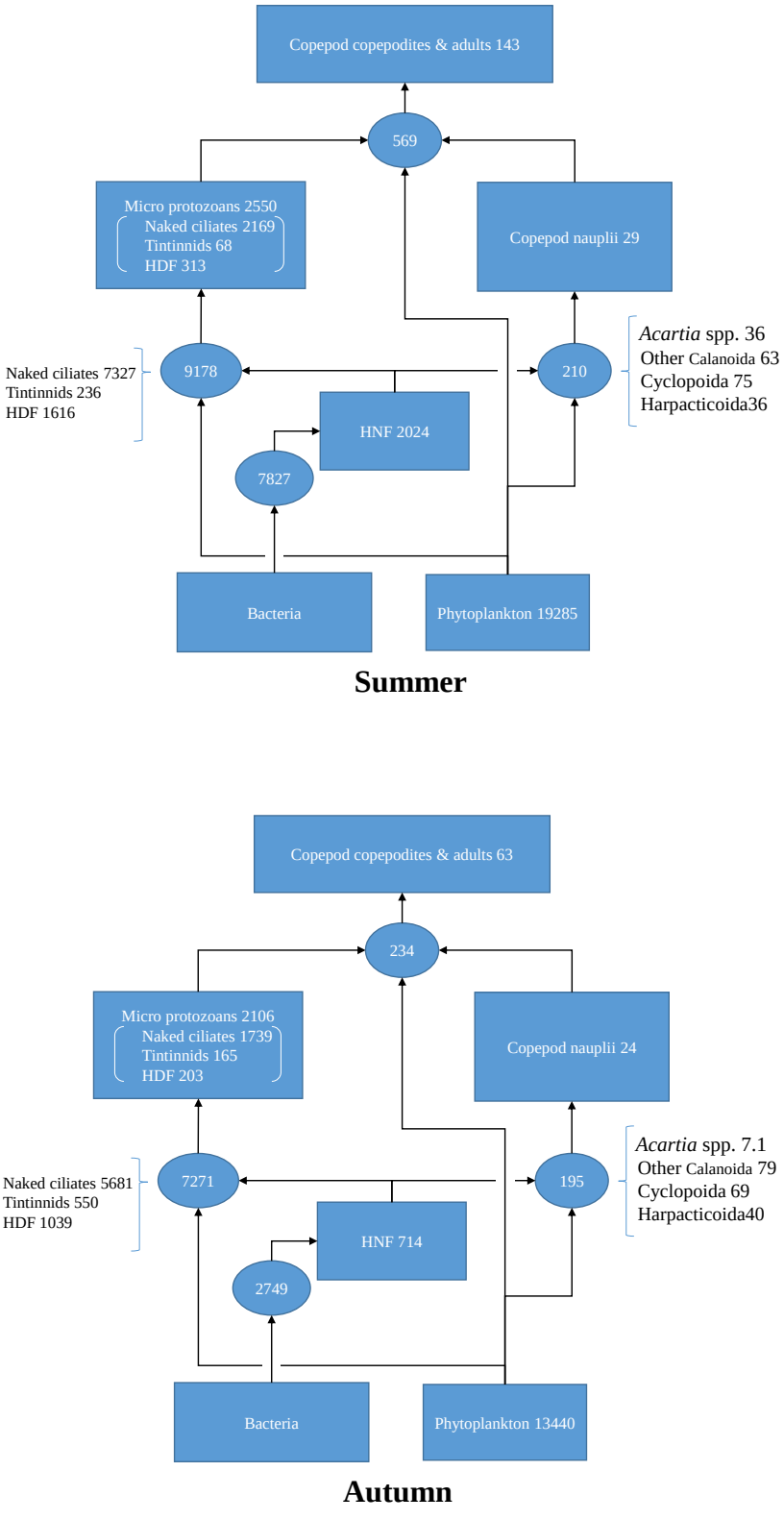


Figure 4-7 B. Trophodynamics (right) of planktonic organisms during summer (from Jun 2013 to August 2013) and Autumn (from September 2013 to November 2013). Number in circle and square; μg C L^{-1} $season^{-1}$.

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Appendix 1-1. Water temperature, salinity, SiO_3 concentration, chl *a* concentrations, bacterial abundnce and abundance of heterotrophic nanoflagellates in the study site in Sagami Bay.

	Temperature (℃)	Salinity	SiO_3 conc.	Chlorophyll *a* concentration (μg L^{-1})				Bacteria ($\times10^9$ cells L^{-1})	Heterotrophic nanoflagellates ($\times10^6$ cells L^{-1})
			μM	<2 μm	<10 μm	<20 μm	<180 μm		
2012/11/16	18.3	33.0	14.8	0.55	0.94	1.4	3.6	0.65	0.09
2012/12/14	16.0	31.6	18.1	0.27	0.91	1.0	1.2	0.45	0.09
2013/1/17	12.9	33.9	21.6	0.11	0.15	0.28	0.42	0.23	0.11
2013/2/8	11.9	34.1	18.4	0.17	0.38	0.48	1.4	0.45	0.22
2013/3/15	14.1	32.9	41.4	0.069	0.42	0.54	1.2	0.71	0.35
2013/4/12	16.5	33.9	9.8	0.22	0.94	1.0	1.8	0.62	0.58
2013/5/17	19.0	32.7	27.3	0.21	0.26	0.39	0.55	1.8	0.4
2013/6/13	20.5	32.4	22.8	0.96	1.4	1.8	2.4	2.5	1.1
2013/7/17	26.1	32.7	7.4	0.30	0.89	1.7	3.2	1.2	0.5
2013/8/18	27.1	33.6	4.6	0.14	0.78	2.0	4.0	1.0	0.2
2013/9/6	26.3	32.8	5.7	0.34	1.5	2.5	4.3	0.76	0.26
2013/10/28	19.9	31.1	32.1	0.81	0.99	1.4	1.4	1.0	0.3
2013/11/14	18.3	33.4	15.9	0.23	0.31	0.48	0.56	0.90	0.06
2013/12/18	10.1	33.8	47.5	0.30	0.46	0.54	0.75	0.62	0.09
2014/1/21	13.5	34.1	40.5	0.31	0.44	0.75	0.86	0.48	0.09
2014/2/21	12.5	34.2	37.6	0.29	0.81	0.85	2.5	0.56	0.11
2014/3/16	12.8	33.4	16.3	0.10	-	0.97	4.3	0.74	0.22
2014/4/10	15.4	33.4	-	0.95	-	2.9	6.4	1.1	0.4
2014/5/15	17.7	33.6	-	0.39	-	1.6	2.4	1.1	0.6
2014/6/19	21.0	32.3	-	0.04	-	0.16	0.16	1.2	0.4
2014/7/17	24.0	31.0	-	-	-	-	3.6	1.7	0.4
2014/8/17	25.6	33.6	-	0.57	-	1.4	1.6	1.3	0.5
2014/9/7	24.7	32.2	-	0.53	-	1.6	1.9	1.3	0.2
2014/10/15	20.5	31.1	74.7	3.7	-	5.7	7.3	1.0	0.3
2014/11/19	18.5	34.0	-	0.24	-	0.59	0.70	0.68	0.32
2014/12/17	12.0	33.7	20.7	0.26	-	0.29	0.79	0.78	0.06
2015/1/14	13.8	34.2	28.3	0.37	-	0.69	1.1	0.74	0.09
2015/2/18	12.5	34.0	10.8	0.55	-	10	10	0.67	0.48
2015/3/13	12.6	34.1	26.0	0.25	-	0.75	0.54	0.77	0.21
2015/4/9	13.0	32.8	28.9	0.15	-	0.36	0.46	1.5	0.6
2015/5/15	18.7	33.7	14.6	0.71	-	2.9	3.3	2.0	0.2
2015/6/11	20.9	32.8	11.8	0.77	-	4.8	7.6	2.1	0.4
2015/7/11	22.3	30.9	18.9	0.27	-	3.3	4.5	1.3	0.5
2015/8/18	25.6	34.0	-	0.26	-	0.73	1.1	1.6	0.4
2015/9/14	24.6	31.0	-	1.6	-	5.1	6.3	1.2	0.2
2015/10/15	22.9	33.6	-	0.59	-	1.4	1.7	0.32	0.35
2015/11/18	19.6	33.8	-	-	-	-	-	0.67	0.26

Appendix 1-1. Results of field water temperature, salinity, concentration of chl a and abundnces of bacteria and heterotrophic nanoflagellates

		Naked ciliates (inds/L)	Total cell volume (μm^3)	Tintinnids (inds/L)	Total lorica volume (μm^3)	Heterotrophic dinoflagellates (inds/L)	Total cell volume (μm3)	Copepod nauplii abundances(inds/L)			
								Acartia spp.	Other Calanoida	Oithonida	Harpacticoida
2012/11/16	18.3	1562	29861	180	29973	280	30298	2.1	7.0	0	0
2012/12/14	16.0	1721	7128	100	84595	80	1456	0	1.8	0	0
2013/1/17	12.9	203	8582	0	0	0	12142	0.70	2.9	0	0
2013/2/8	11.9	142	57886	60	182614	20	11868	6.7	0.6	0	0
2013/3/15	14.1	1060	4934	20	14661	1440	20773	87	6.0	36.4	12.7
2013/4/12	16.5	661	3493	140	27526	80	2806	0	0	1.8	0.0
2013/5/17	19.0	2060	7245	560	25084	3600	3162	3.5	5.6	47.3	1.8
2013/6/13	20.5	1920	6041	140	11695	1240	8574	2.4	8.5	114.5	5.5
2013/7/17	26.1	4244	9915	260	6163	2860	11732	1.3	4.2	14.5	23.6
2013/8/18	27.1	3922	24691	180	67396	1460	21594	2.5	21.1	41.8	1.8
2013/9/6	26.3	4083	27555	1320	23326	2100	28641	1.2	49.7	107.3	21.8
2013/10/28	19.9	2133	8479	216	3863	203	806	0	10.9	49.1	23.6
2013/11/14	18.3	1621	9809	105	5372	296	3999	1.9	1.8	5.5	0.0
2013/12/18	10.1	1688	7128	100	84595	80	1456	0.8	1.6	4.2	0.1
2014/1/21	13.5	1769	8582	20	0	30	12142	3.2	3.8	0	0
2014/2/21	12.5	1031	57886	60	182614	20	11868	5.0	0	1.6	1.6
2014/3/16	12.8	130	4934	20	14661	785	20773	5.0	14.4	6.2	1.1
2014/4/10	15.4	716	3493	97	27526	153	2806	3	10.0	34.0	0.0
2014/5/15	17.7	3968	7245	719	25084	2022	3162	3.5	6.9	58.1	2.2
2014/6/19	21.0	5524	6041	311	11695	1353	8574	25.0	2.7	61.9	6.6
2014/7/17	24.0	4329	9915	0	6163	1220	11732	20.5	2.0	10.3	28.4
2014/8/17	25.6	6583	24691	180	67396	1460	21594	2.8	26.0	46.0	2.3
2014/9/7	24.7	4930	27555	719	23326	1336	28641	25.0	4.0	3.2	26.8
2014/10/15	20.5	2430	8479	216	3863	203	806	3	8.7	60.4	21.7
2014/11/19	18.5	1230	9809	105	5372	296	30298	1.9	4.4	2.7	0
2014/12/17	12.0	1655	7128	100	84595	80	1456	0.8	1.7	2.1	0.1
2015/1/14	13.8	3337	8582	10	0	15	12142	3.2	3.4	0	0
2015/2/18	12.5	2515	57886	60	182614	20	11868	34.0	0.3	0.8	0.8
2015/3/13	12.6	88	4934	20	14661	129	20773	47.0	7.4	15.5	5.1
2015/4/9	13.0	772	3493	55	27526	226	2806	0	5.0	17.9	0
2015/5/15	18.7	5875	7245	878	25084	445	3162	3.5	6.2	52.7	2.0
2015/6/11	20.9	2808	6041	482	11695	1467	8574	2.4	5.6	88.2	6.0
2015/7/11	22.3	1549	9915	310	6163	216	11732	1.3	3.1	12.4	26.0
2015/8/18	25.6	2735	24691	180	67396	1460	21594	2.5	23.5	53.1	2.1
2015/9/14	24.6	1841	27555	118	23326	571	28641	1.2	26.8	72.9	24.3
2015/10/15	22.9	2547	8479	216	3863	203	806	10	9.8	54.7	22.7
2015/11/18	19.6	1771	9809	105	5372	296	3999	1.9	3.1	4.1	0

Appendix 2. Body length (μm) and size (μm) of copepod nauplii and gut contents

Bodey length	Cyanobacteria			Bacteria									Diatoms			Flagellate cells
135	5.5															
149	3															
149	3.6	7.5	6.4													
158	9	4.6	7													
176	4.8	5.7														
178	5.8	7														
178	5.2	8	4.3													
207	3.5															
158				0.6	0.5	1.2	0.5	0.9	0.6							
169				0.8	0.7	0.9										
185				1	1	1	1.2	0.9	1.1	0.8	0.6	0.5				
185				1	0.8	1.3	0.7	1.3								
141													3.4			
145													4.8			
156													6.7	10.2	7.3	
159													4.5	4.2		
167													5.4			
169													6.7			
185													4.3	7.5		
189													9.2			
197													9.9	8.5		
206													8.8			
147																6.7
171																8.6
173																10.7
210																12.2
215																9.3

Appendix 3. Results of ingesting experiments with different food concentrations

Developmental stage	Initial food concentration ug C/L	<C> ugC/L	Clearance rate mL/ind/day	Ingestion rate ug C/ind/day	Carbon specific ingestion rate day^{-1}
NIII	8.1	14	11	0.2	3.7
	9.0	16	5.5	0.1	2.1
	11	15	18	0.3	6.9
	79	133	7.6	1.0	25
	93	157	5.9	0.9	23
	270	350	2.4	0.8	20
	370	426	1.9	0.8	19
NIV	11	13	6.6	0.1	1.6
	12	14	8.5	0.1	2.2
	15	18	26	0.5	8.8
	29	28	25	0.7	13
	42	46	12	0.5	10
	64	85	14	1.2	21
	145	189	3.6	0.7	13
	176	287	3.9	1.1	20
	334	503	2.1	1.0	19
	374	561	1.4	0.8	14
NV	7.3	7.3	4.6	0.0	0.25
	7.4	7.4	2.8	0.0	0.15
	6.3	6.3	20	0.1	0.91
	24	24	3.8	0.1	0.66
	24	24	4.9	0.1	0.86
	24	24	6.2	0.1	1.1
	22	22	14	0.3	2.2
	59	59	2.5	0.2	1.1
	52	52	15	0.8	5.6
	56	56	8.3	0.5	3.4
	91	91	4.9	0.4	3.3
	88	88	8.4	0.7	5.4
	152	152	4.3	0.7	4.7
	151	151	4.9	0.7	5.4
	72	72	8.6	0.6	4.5
	71	71	9.4	0.7	4.9
	138	138	4.1	0.6	4.1
	191	191	3.3	0.6	4.6
	275	275	2.8	0.8	5.5
	276	276	2.1	0.6	4.3
NVI	22	24	0.2	0.0	0.02
	25	27	2.2	0.1	0.17
	40	42	0.9	0.0	0.10
	80	86	4.7	0.4	1.1
	82	88	5.5	0.5	1.4
	88	93	6.5	0.6	1.7
	208	207	3.3	0.7	1.9
	210	211	5.1	1.1	3.0
	351	376	1.8	0.7	1.9

Appendix 4. Results of ingesting experiments with different water temperatures

Water temperature	k	g	<C>	Clearance rate	Cell ingestion rate	Carbon ingestion rate	Carbon specific ingestion rate
			cells/mL	mL/ind/day	cells/ind/day	ug C/ind/day	day^{-1}
12_1	-0.0066	0.0030	24933	4.5	111881	1.1	6.2
12_2	-0.0066	0.0020	17382	3.0	51770	0.51	2.9
12_3	-0.0066	0.0022	19800	3.3	64661	0.64	3.6
17_1	0.0073	0.0041	18579	4.9	91291	0.90	5.0
17_2	0.0073	0.0048	22510	5.7	129123	1.3	7.1
17_3	0.0073	0.0050	22392	6.0	133767	1.3	7.4
22_1	0.0010	0.0047	24420	7.1	172720	1.7	9.5
22_2	0.0010	0.0048	23654	7.2	170706	1.7	9.4
22_3	0.0010	0.0060	21866	9.0	196865	1.9	11
27_1	0.0179	0.0010	32190	1.5	46702	0.46	2.6
27_2	0.0179	0.0006	30428	0.71	21548	0.21	1.2
27_3	0.0179	0.0036	29833	4.3	129090	1.3	7.1