DISSERTATION

Energy Accumulation and Starvation Tolerance of the Embayment Copepod *Acartia steueri* Smirnov (Calanoida: Acartiidae)

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ABSTRACT

Fluctuation of phytoplankton biomass in embayment areas changes abruptly because of irregular freshwater inflows, tidal movements, complex submarine topography, and seasonal changes. Copepods are therefore frequently exposed to fluctuations in chlorophyll *a* concentrations and suffer from food-limited environments for a period of days to weeks. The abrupt decrease in food concentrations or starvation conditions is indeed a major obstacle to the survival and reproduction of herbivorous copepods. To combat starvation, however, copepods likely have a mechanism of physiological tolerance to maintain their population in the embayment, which may permit a sustainable supply of the energy fixed by phytoplankton into ecosystems through seasons.

The research aim of this Ph. D. thesis is, therefore, to elucidate the starvation tolerance of the embayment copepod *Acartia steueri*: first, chemical compositions of *A. steueri* were investigated to clarify the characteristics of energy accumulation under food-satiated conditions; secondarily, respiration rate, survival rate, egg production, and fecal pellet production of *A. steueri* raised under starved conditions were investigated to reveal the physiological responses to starvation. *Acartia steueri* is one of the most dominant embayment copepods that has a wide geographical distribution in the western Pacific from South Kuril Bay in Sea of Okhotsk to Kabira Bay in East China Sea, particularly in the northwestern Pacific Ocean. This species has a key role as an essential feed for larvae of commercially important fishes.

In the first experiments, the presence of the extraordinarily heavy embayment copepod *A. steueri* was observed in Sagami Bay, Japan. Similar to the oil sac generally found in overwintering oceanic copepods, *A. steueri* can produce oil droplets in the body cavity under food-satiated condition of 10 days. *A. steueri* also has an ability to accumulate surplus free

amino acids and to convert these to fatty acids as lipid storage. The respiration rate of *A. steueri* raised under the food-satiated condition was about twice as high as that of *A. steueri* raised under the starved condition. Moreover, *A. steueri* survived for 18 days and even produced eggs for 14 days under starved conditions. These results suggest a remarkable starvation tolerance through metabolic regulation when food concentrations are low in their environment. The rapid energy accumulation during food-satiated conditions largely contributes to egg and fecal pellet production during starvation conditions.

Based on the present study, these survival strategies to abrupt changes in food concentrations or starvation support the abundant population of *A. steueri* in embayments, as *A. steueri* can accumulate energy (i.e., food) into its body during short-term sporadic food-satiated conditions, whereas they can endure food-limited conditions using stored energy until the next food-satiated condition.

Chapter Ⅰ

General Introduction

1.1 Role of copepods as stabilization functions in marine ecosystem

Crustaceans are one of the most numerous and diverse groups in the animal kingdom. Most of the approximately 40,000 living species are marine; however, some are found in freshwater and terrestrial environments (Muhlia-Almazán & García-Carreño 2003). Copepods, which are a subclass of the phylum Crustacea, make up 70% or more of all net-collected plankton, and are the most abundant and widely distributed zooplankton in all of the world's oceans (Lalli & Parsons 1997, Finiguerra et al. 2013). Due to their great abundance and diversity, copepods possess physiological and/or behavioral adaptation strategies to survive under a plethora of conditions, and these adaptations are specific to species, feeding habits, gender, food availability, and life cycle (Muhlia-Almazán & García-Carreño 2003).

In marine ecosystems, copepods consume much of the primary production resources and are an essential food source for larvae of commercially important fishes. Thus, they serve as a linkage, principally as heterotrophic producers, by transferring energy from primary producers to higher trophic levels. The failures and success of fisheries in European waters have been related to copepod availability in the North Sea (Hardy & Gunther 1935, Støttrup 2000). Here, hauls of fishes in certain waters where there were many Calanus copepods were double that in other waters where there were few Calanus copepods (Hardy 1956). From analysis of long-term changes in the western North Pacific using the Odate Collection, a historical zooplankton sample and data set (Odate & Maita 1994), it was shown that increases in Japanese pink salmon (Oncorhynchus

gorbuscha) catch tended to coincide with increasing Neocalanus copepod biomass in the 1990s (Chiba et al. 2008; Yatsu et al. 2008).

Meanwhile, primary production fluctuates with changes in environmental conditions, including light intensity and/or nutrient levels (Barranguet et al. 1998, Chavez et al. 2010). Primary production in the north-western Pacific Ocean is limited by extreme environmental conditions such as water temperature, day length, available light intensity, and water column stability (Subba Rao & Platt 1984). Massive phytoplankton blooms occur in spring, and the primary production levels in other seasons are relatively low (Fiala et al. 1998). Many copepods accumulate energy by feeding on large amounts of phytoplankton during the spring bloom (Kattner & Hagen 1995; Hagen & Auel 2001). Thereafter, the copepods survive and reproduce in a food-scarce environment, consuming their energy reserves (Norrbin et al. 1990; Hagen & Auel 2001). Oceanic copepods accumulate large energy reserves within their body to cope with low food environments and can live for about 6 months producing eggs even under starvation conditions (Saito & Tsuda 2000). Thus, as copepods maintain their population and stably transfer energy to the higher trophic levels even under food scarcity, they play a key role in the stabilization of marine ecosystems.

1.2 Embayment copepods and food environment

The environmental factors in the embayment area, which is a part of the neritic zone and is closest to the coastal line, are influenced by irregular inflows of freshwater, tidal movements, complex submarine topography, and seasonal changes (e.g., Toda et al. 2000, Vidal et al. 2017). As embayment ecosystems are affected by the fluctuation of environmental factors, fluctuations in the amount of biological production occur frequently. Therefore, the planktonic species that can continue to reproduce and maintain a stable population in embayments are limited (e.g., Mauchline 1998). In particular, food availability affects the physiological responses of copepods, including

growth, metabolism, egg production, and energy accumulation (Escribano & McLaren 1992, Shin et al. 2003).

Chlorophyll a concentrations, which are an indicator of the amount of phytoplankton, are quite variable, from 10 to 50 μg L^{-1} in the inner part of San Francisco Bay (Durbin & Durbin 1981), from 3.0 to 47 μg L^{-1} in Chesapeake Bay (Cole et al. 1986), and from 5.0 to 73 μg L^{-1} in Narragansett Bay, United States (Ray et al. 1989) as summarized in Table 1. The fluctuation in the chlorophyll a concentration in the spring or autumn bloom is the largest each year. In Otsuchi Bay, a representative embayment in Japan, diatom blooms occur several times during the spring, induced by local wind stress (Furuya et al. 1993). Diatom blooms with chlorophyll a concentrations of 10 to 15 μg L^{-1} last from several to 10 days, followed by a non-bloom period of 1 to 2 μg L^{-1} that lasts from several days to three weeks and is accompanied by an increase in other organisms, such as flagellates and ciliates (Tsuda et al. 1994). Intensive grazing by copepods has been observed just after blooms (Tsuda 1994). Food availability for copepods in embayments has been reported to be diluted and highly heterogeneous, both temporally and spatially (Mullin & Brooks 1976, Dagg & Grill 1980, Marshall et al. 2006, Agboola et al. 2013). Copepods are exposed to fluctuations in chlorophyll a concentration, and they suffer from a food-scarce environment for a period of days to weeks (Huntley & Boyd 1984, Saiz et al. 1993, Runge & Plourde 1996, Niehoff et al. 1999, Niehoff et al. 2000). The abrupt decrease in food concentrations is one of the most probable obstacles to the survival and reproduction of herbivorous copepods in embayments. However, embayments typically have extremely high population densities of copepods, which are a major food source for larval fish. Embayment areas are the most suitable habitats for larval fishes (Ueda 1992). In order to comprehend the biomass of higher trophic organisms, including commercial neritic fish, it is essential to investigate the abundances and/or physiological responses of embayment copepods.

Species living in unstable embayment environments are exposed to adverse conditions for varying periods of time (e.g., unfavorable temperature and food availability) and have therefore developed traits to allow them to cope with these adversities (Holm et al. 2017). It is known that resting egg production as a survival strategy of some embayment copepods provides a means to cope with unfavorable environmental periods (both predictable and unpredictable), and high productivity is restored in favorable periods, reducing genetic drift (Hairston & De Stasio 1988). Embayment species are generally not adapted to low food concentrations (Paffenhöfer & Stearns 1988) although their food environments are often found to be limited in nature (Durbin et al. 1983, Peterson et al. 1991, Breteler & Schogt 1994). As these species lack almost all storage elements (Dagg 1977) and respond to changes in food availability with a short time lag (Tester & Turner 1990), a close coupling between food availability and physiological responses, including metabolism and egg production, would be expected (Calbet & Alcaraz 1996).

The biodiversity of copepods in embayments is extremely low compared to that in oceanic waters (Ueda 1992) and is dominated by a small number of species. The genera *Acartia*, *Oithona*, and *Paracalanus* are the most dominant in neritic-embayment waters (Yamazi 1956, Palomares-Garcia & Gómez-Gutiérrez 1996). Among the calanoids, members of the genus *Acartia* are the major constituents of holozooplankton communities in embayments, neritic zones, estuaries, and other semi-enclosed marine coastal areas (Conover 1959, Abraham 1969, Alcaraz 1983). The embayment copepod *Acartia steueri* Smirnov, 1936 has a wide geographical distribution in the western Pacific, from South Kuril Bay in the Sea of Okhotsk, to Kabira Bay in the East China Sea (Fig. 1-1; Kos 1958, Tanaka et al. 1987, Ueda 1980, Uye 1980, 1981, 1983, Nishida 1985, Kurihara et al. 2004, Onoue et al. 2004, Kang & Kang 2005); this species has a key role as an essential food source for larvae of commercially important fishes (Tanaka et al. 1987). Therefore, many biological parameters, such as growth rate (Uye 1980), egg production (Uye 1981), egg

development (Onoue et al. 2004), production rate (Kang & Kang 2005), and food availability (Natori et al. 2017) have been investigated for this species.

Thanks to medium- to long-term marine observations from 1995 to the present in the neritic-embayment area of Sagami Bay, Japan, *A. steueri* has been identified as one of the most dominant embayment copepods in this area throughout most of the year, except for in summer (Onoue et al. 2006). The density of adults reached 2.3×10^4 inds. m^{-3} at its maximum value and was several hundred times higher than that of other *Acartia* copepods (*A. sinjiensis*, *A. hudsonica*, *A. omorii*, *A. japonica*, *A. negligence*, *A. erythrea*, and *A. danae*) (Shimode unpublished). The population density of *A. steueri* was also extraordinarily high compared with other *Acartia* copepods. Thus, *A. steueri* maintains a high abundance even when exposed to fluctuations in temperature and food availability at the temperate embayment areas. As for adaptation of *A. steueri* to the fluctuations in temperature, this species is known to produce diapausing eggs as a survival strategy under unfavorable temperatures in summer (Uye 1983, Onoue et al. 2004). On the other hand, little is known about the adaptability of *A. steueri* to fluctuations in food concentrations. The chlorophyll a concentration varied from 0.1–12.5 μg L^{-1} in 2002 and from 0.3–18.8 μg L^{-1} in 2003 during the spring bloom, and abruptly decreased for short periods by approximately one-sixtieth-fold for 7 days in Manazuru Port, Sagami Bay (Satoh et al. 2000). The food requirements of copepods in the coastal area of Sagami Bay often exceeded primary production (Ara & Hiromi 2006). At Manazuru Port, Sagami Bay, the food requirements of adult female *A. steueri* to phytoplankton biomass varied from 1.2%–164% in a year according to calculations by Onoue (2006); therefore, they are often exposed to insufficient food or starvation conditions. Hence, the population of *A. steueri* could be maintained through a life strategy of adapting to abrupt decreases in food concentrations and subsequent low food conditions or starvation for a certain period of time.

1.3 Physiological responses of *Acartia* copepods to starvation condition

Laboratory studies have shown a positive relationship between food conditions and physiological responses, such as growth, metabolism, egg production, fecal pellet production, and survivorship (Kiørboe et al. 1985, Jónasdóttir 1994, Kleppel et al. 1998, Dam & Lopes 2003, Koski et al. 2006, Jónasdóttir et al. 2009). Kiørboe et al. (1985) found that the rates of ingestion, excretion, respiration, and egg production in the food-acclimated embayment copepod *A. tonsa* increased in relation to increased food concentrations in embayments. Ingestion and egg production rates increased sigmoidally with food concentrations (Berggreen et al. 1988). Rates of excretion and respiration increased with food concentration in a decelerating manner and decreases in respiration rate and other metabolic activities during starvation have been observed in many organisms (e.g., Raymont 1959, Ikeda 1977, Surendranath et al. 1987). Respiration rates and excretion rates of *A. tonsa* given saturated food concentrations were more than four times higher than those of starved copepods. *A. tonsa* intermediately adapted to fluctuating or patchy food conditions; they survived starvation longer (6–10 days) than did other *Acartia* copepods (about 5 days). The egg production of *A. tonsa* decreased immediately under starvation after exposure to an adequate food concentration (Parrish & Wilson 1978). Thus, starvation is one of the most probable candidates for mortality and population maintenance of copepods.

During starvation, energy reserves within organisms are utilized for survival, metabolism, and egg production (Liu et al. 2010). The dry and carbon weights of crustaceans have been shown to be strongly affected by food conditions (Vidal 1980, Breteler & Gonzalez 1988, Hessen et al. 1989), and to vary seasonally (Hessen et al. 1989, Tanskanen 1994). The carbon weight of female *A. bifilosa* increased with increasing food concentrations (Koski & Kuosa 1999). Rapid increases in the body and carbon weights of *A. tonsa* and *A. bifilosa* in higher food treatments were observed, along with a loss of body weight at starvation (Durbin et al. 1992, Koski & Kuosa 1999).

Additionally, as the carbon content of female *A. bifilosa* seemed to determine egg production, the energy reserves of females further stress the importance of food conditions for egg production.

Energy flows obtained from feeding in adult female copepods were roughly estimated for the first time by Ikeda & Motoda (1978) (Fig. 1-2). After feeding, adult females eliminated 30% of food energy as fecal pellets and assimilated 70% into their body, which was finally distributed as 30% and 40% to metabolism and egg production, respectively. It has been thought that the rate of energy distribution in adult female copepods changes depending on the available food conditions. When under extremely high food concentrations, such as during spring blooms, adult females might accumulate energy in excess of that used in metabolism and egg production. On the other hand, when there is little food or no food, including abrupt decreases in food, the rates of energy distribution for assimilation, metabolism, and egg production might change. Therefore, two hypotheses were proposed as a survival strategy for *A. steueri* copepods in food-scarce embayments: 1) this species can accumulate energy in their body under high food conditions, and 2) they can change their energy distribution under starvation conditions.

1.4 Objectives

Until now, the embayment area facing shore was thought to have higher primary production than those of neritic and oceanic areas because the shore directs nutrient resources into the embayment area. Therefore, the influences of short-term, sporadic fluctuations in the food environment in embayment areas on the physiological responses of copepods have been greatly overlooked. I predict that the embayment copepod *A. steueri* has starvation tolerances as a survival strategy to abrupt food concentration changes and/or starvation in the embayment area.

In this thesis, firstly, to elucidate the function of energy accumulation, the chemical compositions, including the dry, carbon and nitrogen weights of *A. steueri* raised under high food conditions, have been investigated (Chapter II). Second, to determine the energy flow of *A. steueri*

raised under starvation conditions, the physiological responses, including respiration, egg production and fecal pellet production rates, have been investigated (Chapter III). Finally, the metabolic specificity of *A. steueri* and its role in marine ecosystems have been discussed in Chapter IV as the general discussion part of this thesis.

Table 1. Annual variations of chl. *a* concentration in embayment areas.

Sea area	chl. *a* (μg L^{-1})	References
Bedford Basin	1.2— 6.0	a
Chesapeake Bay	3.0—47	b
Hiroshima Bay	2.0—35	c
Narragansett Bay	5.0—73	d
Otsuchi Bay	1.0—15	e
Sagami Bay	0.1—59	f
Sanfrancisco Bay	5.0—50	g
The mouth of Ria de Aveiro	0.1—11	h

a: Côté & Platt 1983, b: Cole et al. 1986, c: Lee et al. 1996, d: Ray et al. 1989, e: Tsuda et al. 1994, f: Satoh et al. 2000, g: Durbin & Durbin 1981, h: Vidal et al. 2017

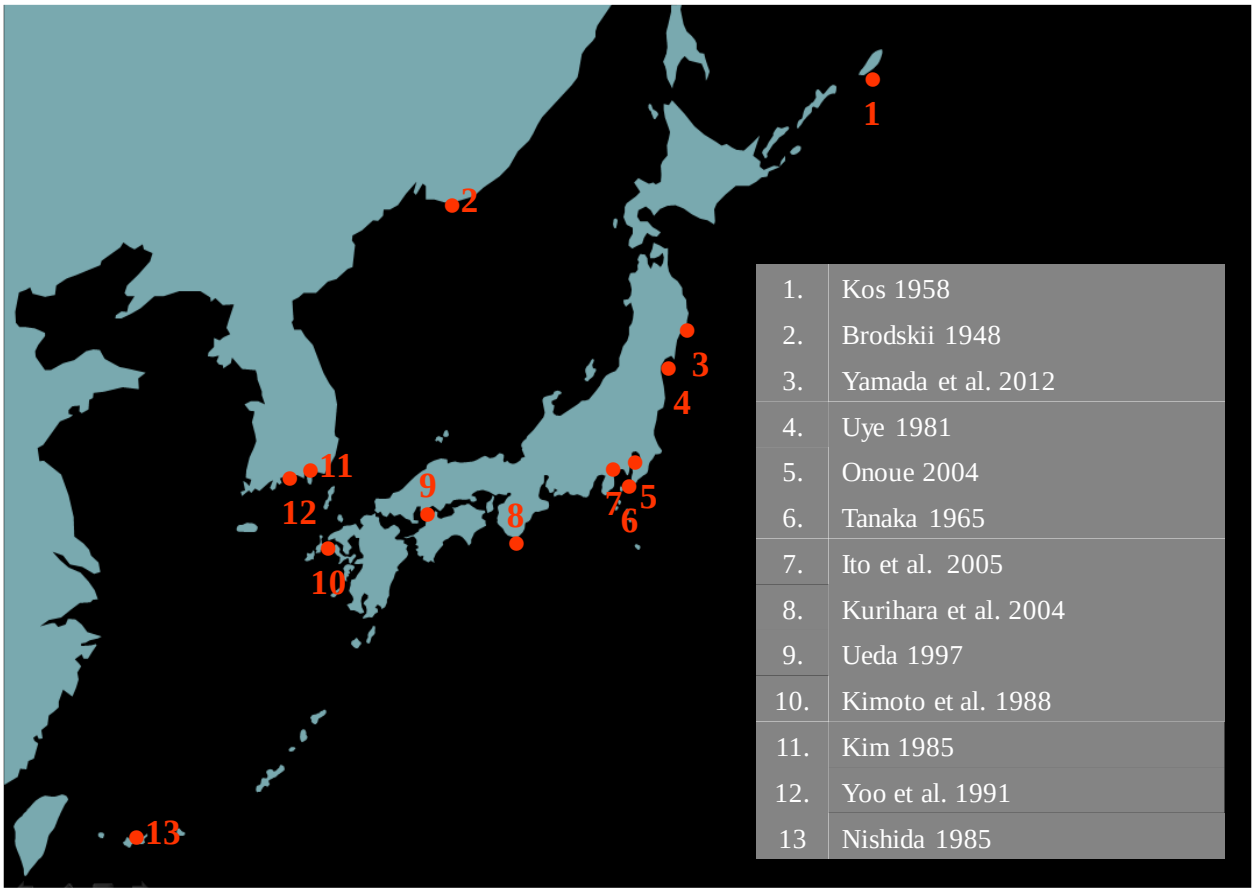


Fig. 1-1 Distributions of an embayment copepod *Acartia steueri* around Japan Islands.

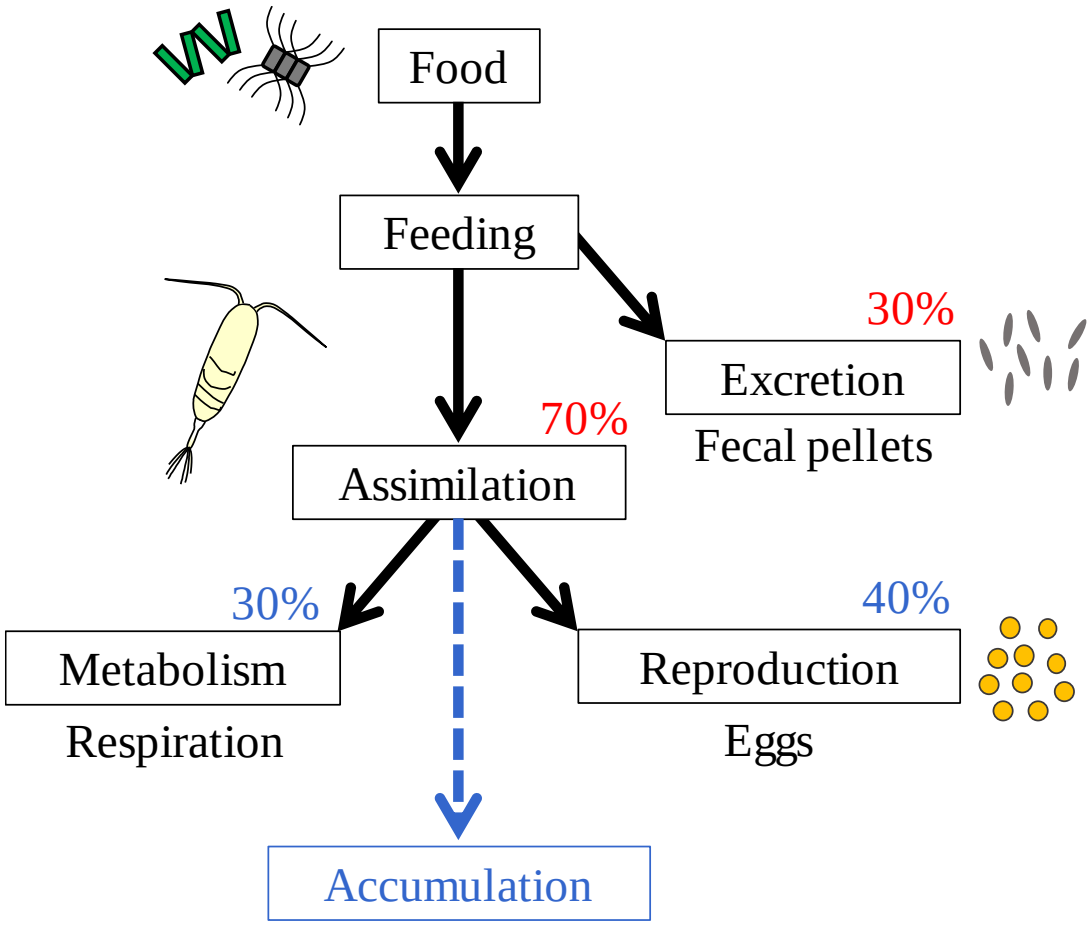


Fig. 1-2 The energy flow obtained by feeding in a copepod female reported by Parsons et al. 1984. The ratio of each energy flow was quoted from Ikeda & Motoda 1978.

Chapter Ⅱ

Characteristics of energy reserve of *Acartia steueri*

2.1 Introduction

Energy reserve would be one of survival strategies of copepods to ensure longevity and to sustain reproduction in unpredictable environments (e.g. Armstrong et al. 1991). The energy reserve may allow copepods to maintain their biomass even when food is scarce. As ecological function of the energy reserve of copepods in marine ecosystem, the energy source is supplied stably to higher trophic level than that of copepods. Copepods have a key role to stabilize the fluctuation of phytoplankton in marine ecosystems (Taniguchi 2008).

Hirahara & Toda (2018) summarized the length-weight relationship of 98 species of oceanic and neritic-embayment planktonic copepods from 123 references (Fig. 2-1; Heinle 1966, McLaren 1969, Omori 1969, Heinle & Flemer 1975, Durbin & Durbin 1978, Breteler et al. 1982, Tande 1982, Uye 1982, Durbin et al. 1983, Gaudy & Boucher 1983, Kimmerer & McKinnon 1987, Breteler & Gonzalez 1988, Mizdalski 1988, Hay et al. 1991, Jerling & Wooldridge 1991, Durbin et al. 1992, Hirche & Mumm 1992, Cataletto & Umani 1994, Karlson & Båmstedt 1994, Thompson et al. 1994, Escribano & McLaren 1999, Yamaguchi & Ikeda 2000, Kobari & Ikeda 2001, Richardson et al. 2001, Kobari et al. 2003, Shoden et al. 2005, Zhang et al. 2005). Even though these copepods have different life histories and life spans, the length-weight relationship of all copepods can be represented by a regression line on the logarithmic graph. Dry weight is needed for the estimation of biomass and secondary production in marine ecosystem and the length-weight relationship is powerful tool for estimating copepod biomass and secondary production in marine ecosystem. In contrast to the measurement of population density, which estimates biomass directly by counting the number of individuals from field samples, usually only

prosome length is measured, and then dry weight is calculated using the relationship. Therefore, it is important for accurate measurement of dry weight but the drying conditions such as temperature and treatment time are same method (at 60^{o}C for 24 h) for a long time from year 1962 (Lovegrove 1962). Water contents in organisms are categorized into free water and bound water. Free water is usually used to express water whereas bound water is bonding substance with water molecule and certain substances, including inorganic minerals and protein by physical bounding force. The bound water evaporates more than 100^{o}C (Imagawa et al. 2011, Nihon Kansoki Co. Ltd.: http://www.kansoki.co.jp/kanso.html.) Therefore, the conventional drying temperature which has been used for a long term should be re-evaluated to dry up water contents completely.

Dry weights were strongly correlated with lipid contents of oceanic and neritic copepods (Nakamura et al. 2017). The survival days of copepods under starvation are related to the energy reserves including lipids in their body (Lee et al. 2006). Oceanic copepods are long-lived large species and accumulate large energy reserves within their body against periods of starvation for 1-2 years (Saito & Tsuda 2000). Conversely, neritic copepods store little energy reserves, withstand only short periods of starvation and are short-lived small species (Peters et al. 2007). It was reported by Ikeda (1974) and Mayzaud (1976) that for marine invertebrates, three types of metabolism have been categorized: (1) lipid-oriented; (2) polysaccharide-oriented, and (3) what might be called protein-oriented, although no biochemical substrate is preferentially stored or used. In the absence of any accumulation of polysaccharide by zooplankton (Mayzaud & Martin 1975), actually two types of lipid- and protein- oriented need to be considered. Lipid-oriented species such as oceanic copepods use their lipids as energy source preferentially and C/N ratios decrease under starvation period, but they appeared to be able to switch several times during the starvation period from a predominantly branching point protein catabolism to a predominantly lipidic catabolism vice versa. On the other hand, protein-oriented species such as neritic copepods might metabolize large amounts of protein because C/N ratios were low and constant throughout the

starvation period (Mayzaud 1976). From these reports, the utilizing of protein might contribute to metabolic characteristics of *Acartia* copepods.

Amino acids are essential biochemical units for protein biomass. Their stable isotopic compositions of ^{15}N /^{14}N ($\delta^{15}N$) within amino acids have recently been employed as a method for estimating the trophic position of organisms in food webs (Chikaraishi et al. 2009), and should be closely related to metabolic processes and their flux in organisms. The $\delta^{15}N$ values for bulk organisms and their tissues increase by~3.4‰ with increasing each trophic level (DeNiro & Epstein 1981, Minagawa & Wada 1984, Post 2002). However, this "bulk method" involves several pitfalls, which often lead to large errors in estimating the trophic level. Recent studies have reported that the nitrogen isotopic composition of some amino acids such as glutamic acid dramatically increases in ^{15}N /^{14}N during the relationship from diet to its consumer, whereas other amino acids (e.g., phenylalanine) show little change in ^{15}N /^{14}N through this relationship. This is because deamination occurs for the first metabolic process of the former amino acids but not for that of the latter amino acids (Chikaraishi et al. 2007). The deamination rate of amino acids in the metabolic flux between diet and consumer thus may be calculated by using the Rayleigh isotopic fractionation model as the following equation (1):

$$\delta^{15}N_{AA\_cunsumer} - \delta^{15}N_{AA\_diet} = 1000 \times [(1 - F)^{(\alpha - 1)} - 1] \qquad (1)$$

where $\delta^{15}N_{AA\_consumer}$ and $\delta^{15}N_{AA\_diet}$ are the isotopic ratios of a single amino acid in the consumer and diet, respectively, α is isotopic fractionation factor (i.e., 0.99405, in Macko et al. 1986; Miura & Goto 2012), and *F* is a metabolic rate of amino acid by enzymatic deamination of consumer. The degree of $\delta^{15}N$-increase has been suggested to be a universal value of ~8‰ for glutamic acid (Chikaraishi et al. 2009), which corresponds to a deamination of approximately 64-86% of glutamic acid derived from diet and remaining 14-36% is used for construction of protein biomass (Macko et al. 1986, Miura & Goto 2012).

In Chapter II, length-weight relationship of *Acartia steueri* was investigated to reveal the accumulation ability of *A. steueri*, and compared to that of other copepods (Fig. 2-1). Evaluation of measurement protocol for the dry weight was conducted to obtain the accurate dry weight removed water contents from samples. Subsequently, the carbon weight, nitrogen weight, C/N ratio, the composition of free amino acids, protein, lipid and carbohydrate were measured to clarify the accumulation characteristics and the accumulation substances of *A. steueri*. Finally, the metabolic flux of an amino acid in *A. steueri* were determined to prove the importance of amino acids to the metabolism of *A. steueri*.

2.2 Materials & Methods

2.2.1 Sampling location

Sampling was conducted at a fixed station, Station A (St. A; 35°09’49” N, 139°10’33” E, maximum depth: 6m) of Manazuru Port which is located in the northwestern coast of Sagami Bay, Japan (Fig. 2-2). Manazuru Port is a well-represented temperate site that has been methodically studied for variability in environmental factors and the distribution and abundance of plankton (Shimode et al. 1998, Satoh et al. 2000, Toda et al. 2000, Nagao et al. 2001, Onoue et al. 2004, Tsuchiya et al. 2013). The mouth of Sagami Bay faces the Pacific Ocean, and its hydrography is primarily related to fluctuations of the Kuroshio Current axis and to the waters originating from the Sagami and Sakawa rivers as well as water from Tokyo Bay (Hogetsu & Taga 1977).

2.2.2 Chemical compositions of *in situ* and reared *Acartia steueri*

Zooplankton samples used in this Chapter II were summarized in Table 2-1. Sampling was conducted at St. A (Fig. 2-2) in May to July 2017 and 8th, 21st and 27th April 2018 for measurement of chemical compositions such as dry weight, carbon weight and nitrogen weight. Plankton samples were collected by gently towing a plankton net (diameter: 30 cm; length: 150

cm; mesh aperture: 180 μm) obliquely from 5 m depth to the surface. The plankton samples were immediately transferred to the Manazuru Marine Center for Environmental Research and Education (MMCER), Yokohama National University. Live adult females of *A. steueri* were sorted from plankton samples under a dissecting microscope (WILD M10, Leica). Adult females were incubated at high food condition (1.0 μg C mL^{-1}; Berggreen et al. 1988) for 5 days to cancel the effect of the prior condition in the field, and subsequently they were replaced on two food conditions: the high food (1.0 μg C mL^{-1}) and starvation conditions (<0.22 μm filtered sea water) for 10 days. Concentration of food in the high food treatment was based on a food concentration at which growth rate and egg production rate in *Acartia tonsa* were saturated (Berggreen et al. 1988). Drillet et al. (2011) also used this concentration for the high food treatment in an incubation experiment with *A. tonsa*. The periods of high food and starvation conditions to the copepods were determined based on the fluctuation of *in situ* spring bloom (Furuya et al. 1993). The diatom *Thalassiosira weissflogii* (64.4 pg C/cell) cultured with f/2 media was used for food algae and placed in <0.22 μm fresh filtered sea water with the food algae every 48 hours. Incubation was conducted under near-ambient temperature (20ºC) and the light cycles (12L: 12D).

Prosome length of *in situ* adult females in May to July 2017 and November 2018 was measured using ocular micrometer under the dissecting microscope. Dry weights of *in situ* adult females and adult females under two different food conditions of high food and starvation for 10 days were measured, respectively. Additionally, the dry weights of females in 8th April 2018 raised under the high food condition were measured at day 5 and day 10 at the end of incubation for the accumulation rate. Firstly, adult females were cleaned with filtered sea water. Five to ten adult females were then placed onto a precombusted glass fiber filter (Whatman, GF/C) and rinsed with distilled water to remove salt. Adult females on the filters were dried at 60 ºC for 24 hours in an electric oven according to the standard method when measuring zooplankton biomass (e.g., Omori 1969, Durbin and Durbin 1978, Uye 1982, Omori and Ikeda 1984, Harris et al. 2000).

Measurement of dry weight were made on an electric microbalance (METTLER TOLEDO: Model UMX2). To determine the dry weight of an adult female, the weight of the filter plus adult females minus the weight of the filter was divided by the number of adult females. To determine carbon and nitrogen contents of adult females on the filters, the filters were treated with HCl fumes for 24 hours to remove inorganic carbon, dried at 60°C for 12 hours in a dry oven, and stored in a desiccator until analysis. Carbon and nitrogen contents of adult females on the filters were determined using an elemental analyzer (Instruments NA-1500 CNS, FISONS) according to Nagao et al. (2001). Elemental compositions including silicate (S), phosphorus (P) and sulfur (S) of *A. steueri* under *in situ* and the high food condition, and *in situ Pseudodiaptomus nihonkaiensis* Hirakawa as a comparison species collected at Manazuru Port in July 2018 were measured by a Cartesian-geometry energy-dispersive X-ray fluorescence (EDXRF) spectrometer (Rigaku, NEX CG, Applied Rigaku Technologies, Inc., Austin, TX, USA). The fundamental parameter methods (FPs) were adopted as quantitative analysis for copepods samples. This method is commonly used for matrix correction in quantitative analysis of samples of less than infinite thickness (Sitko 2009).

2.2.3 Evaluation of measurement protocol for dry weight

The filter samples of *A. steueri* in November 2018 raised under the high food condition for 15 days were dried on the muffle furnace (DENKEN: KDF-S70) at 60ºC for 24 h as the conventional method (Lovegrove 1962). And then, the filter samples were dried on the muffle furnace at 80 ºC, 100ºC and 120ºC for 12 h and 24 h as drying conditions of higher temperature. The reason of novel temperature setting is based on the drying condition of bound water【>100ºC; Imagawa et al. (2011), Nihon Kansoki Co. Ltd.: http://www.kansoki.co.jp/kanso.html.】. After drying, the dry weight of filter samples was measured by the same methods as described above in 2.2.2.

2.2.4 Biochemical compositions of *in situ* and reared *Acartia steueri*

Sample collection was conducted at St. A (Fig. 2-2) in 8th, 21st and 27th April 2018 by the same method as 2.2.2. Samples of ten adult females raised under *in situ*, the high food condition and starvation onto a precombusted glass fiber filter (Whatman, GF/C) were prepared by the same method as 2.2.2. After measuring dry weight, the filter samples were preserved at -80℃ for biochemical analyses of free amino acids, protein, carbohydrate and lipid until analysis. Samples for free amino acids were extracted in 500 μL of 6% tri-chloro acetic acid (TCA) for 24 h at 4°C according to Helland et al. (2000). The extracts were centrifuged for 5 min at 5000 × g, and the supernatant used for free amino acids analysis. The amount of free amino acids was determined using the photometric nin-hydrin method by Moore and Stein (1948). In terms of the mechanism of ninhydrin reaction, the detection and the quantitative estimation of amino acids has long been accomplished by their reaction with ninhydrin (2,2-dihydroxy-1,3,-indandione). The reaction products include an aldehyde with one carbon atom less than the amino acids and carbon dioxide in stoichiometric amounts and varying amounts of ammonia, hydrindantin and a chromophoric compound, Ruhemann's Purple (diketohydrindylidenediketohydrindamine). This pigment serves as the basis of detection and quantitative estimation of amino acids. Light absorption at 570 nm is recorded on a spectrophotometer. Sample for protein was extracted in 0.5N NaOH at 80°C for 1 h. The amount of protein was determined using folin-phenol reagent according to the Lowry method (Lowry et al. 1951). Lowry method is the most common colorimetric method to determine protein content. Under alkaline conditions, cupric ions (Cu^{2+}) chelate with nitrogen atoms of the peptide with the consequence of a reduction of cupric (Cu^{2+}) to cuprous ions (Cu^{+}). The cuprous ions (Cu^{+}) are reduced by folin's reagent (phosphomolybdic/phosphotungstic acid) to form tungsten blue. Light absorption at 750 nm is recorded on a spectrophotometer. Samples for carbohydrate and lipid were extracted using the chloroform-methanol-water system of Bligh &

Dyer (1959). Samples were homogenized in 3mL of chloroform plus 6 mL methanol to which 3mL of chloroform and 3 mL of distilled water were added. It was then transferred to a centrifuge tube, stirred 1min on a Vortex mixer, and left calmly at a few minutes. Upper layer of the extracts was used for carbohydrate analysis, and lower layer of the extracts was used for lipid analysis. The amount of lipid was determined using method of Kochart (1978). Lipid is quantified by charring with either dichromate or H_2SO_4 and then analyzed by spectrophotometer. Dichromate is reduced by lipid which have a role as reducer and the absorbance increase with decreasing the reduced dichromate. Light absorption at 350 nm is recorded on a spectrophotometer. The amount of carbohydrate was determined using the phenol-sulfuric acid method by Dubois et al. (1956). Phenol-sulfuric acid method is used for determining carbohydrate concentration as it is one of the most widely used colorimetric methods. The basic principle of this method is that carbohydrates, when dehydrated by reaction with concentrated sulfuric acid, produce furfural derivatives, which further reacts with phenol to develop detectible color. Then, light absorption at 490 nm is recorded on a spectrophotometer.

2.2.5 Metabolic flux of amino acids in *Acartia steueri*

Sampling was conducted at St. A (Fig. 2-2) in October 2014 using the plankton net. Fifty adult females were sorted from the plankton samples under a dissecting microscope. In order to illustrate the metabolic flow of an amino acid, glutamic acid of adult females under the high food condition, $\delta^{15}N_{AA}$ values of amino acids in adult females, food algae, eggs, and fecal pellets were investigated. Adult females were incubated at high food condition (1.0 μg C mL^{-1}; Berggreen et al. 1988) for 10 days. The diatom *T. weissflogii* (64.4 pg C $cell^{-1}$) cultured with f/2 media was used for food algae. Incubation sea water was changed every day. Nine hundred eggs were collected for three days from day 8 to day 10. Fecal pellets were collected for the same three days and combined into a sample. Twenty-one females of *A. steueri* were collected on day 10. Those samples were

immediately fixed with 1% buffered formalin. These eggs, fecal pellets, adult females and food item *T. weissflogii* were prepared for the measurement of stable nitrogen isotope of amino acid. Ingestion rate of adult female was determined by changes in food concentration between the start and end of the experimental incubation period for the control (only the food algae) and feeding conditions (the food algae and adult females). Five 250 mL glass bottles were prepared for the control and feeding conditions, respectively. All bottles were filled with the prey suspension, three adult females were sorted from the cohort, washed with 0.22 μm FSW to remove the ambient prey, and added to each feeding bottles.

The amino acids and nitrogen isotopic analysis were extracted by after HCl hydrolysis and N-pivaloyl/isopropyl (Pv/iPr) derivatization, according to the procedure in Chikaraishi et al. (2009). The isotopic composition was determined by gas chromatography/isotope ratio mass spectrometry (GC/IRMS) using a 6890N GC (Agilent Technologies) instrument coupled with a DeltaplusXP IRMS instrument through combustion (950ºC) and reduction (550ºC) furnaces via a GC-C/TC III interface (Thermo Fisher Scientific). The isotopic discrimination was expressed relative to atmospheric nitrogen (δ15N, ‰ vs. AIR) on a scale normalized to the known $\delta^{15}N$ values of nine isotopic reference amino acids from −25.9‰ to +45.6‰ (Indiana University and SI Science co., Sato et al., 2014). The accuracy and precision for the isotope measurements of the reference amino acids were 0.0‰ (mean of Δ) and 0.34‰ (mean of 1σ), respectively. The $TP_{Glu/Phe}$ values were calculated using equation (2).

$$TP_{Glu/Phe} = [(\delta^{15}N_{Glu} - \delta^{15}N_{Phe} - 3.4)/7.6] + 1 \qquad (2)$$

These samples of *A. steueri* in November 2018 were also prepared for analysis of fatty acids. According to modified Bligh & Dyer method (1959), the total fatty acids were extracted in a 20 mL glass tube containing 15 mL of chloroform: methanol (2:1 v/v) and were extracted using ultrasonic cleaning. The combined extracts were partitioned with purified water (Milli-Q system) to remove salts and water soluble materials. The extracts was completely evaporated under

nitrogen gas, and then transmethylated using 1 mL of acetyl chloride: methanol (1:1 v/v) to form fatty acid methyl esters (FAME) by heating at 100 °C for 30 min. The subsamples were immersed in 3 µL hexane, and were subsequently analyzed on a gas chromatograph-mass spectrometer (GC-MS, Agilent 6890 with PTV inlet and Agilent 5973 mass selective detector) on an Agilent DB-5MS column (30 m in length; 0.25 mm in inner diameter) using helium as the carrier gas. The retention times for each sample were compared with those of the known FAME mixtures.

2.3 Results

2.3.1 Chemical compositions of *Acartia steueri* under high food condition

Zooplankton samples, tables and figures used in this Chapter II were summarized in Table 2-2. In May to July 2017, prosome length of *in situ A. steueri, A. steueri* raised under the high food condition and *A. steueri* raised under starvation was 708 ± 6 µm, 680 ± 18.5 µm and 728 ± 114 µm on an average, respectively (Table 2-3; Hirahara & Toda 2018). *In situ A. steueri* collected in Sagami Bay showed a wide range of dry weight from as a minimum of 1.0 µg to as a maximum of 210 µg, and 56.2 ± 58.8 µg on an average. Dry weight of *A. steueri* raised under the high food condition for more than 5 days was a minimum of 33 µg, a maximum of 290 µg, and 125.4 ± 85.2 µg on an average. Dry weight of *A. steueri* raised under starvation was a minimum of 26 µg, a maximum of 240 µg, and 114.6 ± 79.9 µg on an average. Dry weight of *A. steueri* under the high food condition was significantly higher than that under *in situ* (ANOVA, $p<0.001$). Carbon weight of *in situ A. steueri, A. steueri* raised under the high food condition and *A. steueri* raised under starvation was 5.4 ± 3.0 µg, 7.7 ± 5.0 µm and 3.9 ± 1.3 µm on an average, respectively. Nitrogen weight of *in situ A. steueri, A. steueri* raised under the high food condition and *A. steueri* raised under starvation was 0.8 ± 0.2 µg, 0.9 ± 0.3 µm and 0.5 ± 0.1 µm on an average, respectively. C/N ratio of *in situ A. steueri, A. steueri* raised under the high food condition and *A. steueri* raised under starvation was 6.8 ± 2.2, 8.7 ± 5.5 and 8.3 ± 2.4 on an average, respectively.

Individual dry weights of *A. steueri* under both high food and starvation conditions were significantly higher than that under *in situ* (Student's *t*-test, $p<0.05$, Fig.2-3; Hirahara & Toda 2018). Because *A. steueri* incubated under both the high food and the starvation conditions were raised at the high food condition for 5 days after the sampling, the amount accumulated into the body of *A. steueri* incubated under both the high food and the starvation conditions was higher than that under *in situ*.

In terms of *A. steueri* in April 2018, the dry weights were 2.8 ± 1.6 μg ind.$^{-1}$ in *in situ A. steueri*, 14.8 ± 6.1 μg ind.$^{-1}$ in *A. steueri* raised under high food condition for 5 days and 70.0 ± 30.3 μg ind.$^{-1}$ in *A. steueri* raised under high food condition for 10 days (Fig. 2-4). There was a significant difference of dry weight between *in situ A. steueri* and *A. steueri* raised under the high food condition for 5 days (Student's *t*-test, $p<0.01$). There was also a significant difference of dry weight between *A. steueri* raised under the high food condition for 5 days and for 10 days (Student's *t*-test, $p<0.01$). The dry weight of *A. steueri* in April 2018 raised under the high food condition increased by 12.0 μg ind.$^{-1}$ from day 0 to day 5 and 55.2 μg ind.$^{-1}$ from day 5 to day 10. The accumulation rates of females in April 2018 raised under the high food condition were 2.4 μg ind.$^{-1}$ day^{-1} from day 0 to day 5 and 11.1 μg ind.$^{-1}$ day^{-1} from day 5 to day 10.

There was no significant difference of individual carbon weight between under *in situ* and the high food or the starvation conditions (Welch test, $p<0.05$, Fig. 2-5; Hirahara & Toda 2018). Owing to mixture of some populations *in situ* environment, both individuals of the satiation and starvation conditions might be coexisted in the environment. Individual carbon weight of *A. steueri* under the high food condition was significantly higher than that under starvation condition (Welch test, $p<0.05$, Fig. 2-5; Hirahara & Toda 2018). In addition, individual nitrogen weights of *A. steueri* under *in situ* and the high food condition were significantly higher than that under the starvation condition (Welch test, $p<0.05$, Fig. 2-6; Hirahara & Toda 2018). There was no significant difference of C/N ratio of *A. steueri* raised under among *in situ*, high food and starvation conditions

(Fig. 2-7; Hirahara & Toda 2018). Because *A. steueri* might use the carbon as energy source and continued to metabolism under the beginning of starvation condition, the carbon weight of *A. steueri* under the starvation condition was lower than that under the high food condition.

As for results of elemental compositions (Fig. 2-8), aluminum (Al), phosphorus (P), sulfur (S), zinc (Zn) bromin (Br) of *A. steueri* in July 2018 raised under the high food condition for 10 days were much higher than those of *in situ A. steueri* and *in situ P. nihonkaiensis.* The amount of phosphorus of *A. steueri* raised under the high food condition for 10 days (0.017 µg ind.$^{-1}$) was much higher than those of *in situ A. steueri* (0.009 µg ind.$^{-1}$) and *in situ P. nihonkaiensis* (0.009 µg ind.$^{-1}$). Silicate (Si) which was a major component of diatom such as *Thalassiosira* spp. was not different between *in situ A. steueri* and *A. steueri* raised under the high food condition for 10 days. Al and Si of *in situ P. nihonkaiensis* were not detected.

2.3.2 **Evaluation of measurement protocol for dry weight**

In terms of *A. steueri* in November 2018, the dry weights under all of the higher drying conditions (Temperature: 80 ºC, 100 ºC and 120 ºC, Time: 12 h and 24 h) decreased than those under the conventional drying condition at 60ºC for 24 h (Table 2-4). The amount of weight loss at 80 ºC for 12 h ranged from 2.2 µg ind.$^{-1}$ to 2.7 µg ind.$^{-1}$ (Table 2-4). The amount of weight loss at 80 ºC for 24 h ranged from 2.6 µg ind.$^{-1}$ to 2.9 µg ind.$^{-1}$ (Table 2-4). The amount of weight loss at 100 ºC for both 12 h and 24 h ranged from 7.0 µg ind.$^{-1}$ to 9.3 µg ind.$^{-1}$ (Table 2-4). The amount of weight loss at 120 ºC for 12 h ranged from 2.2 µg ind.$^{-1}$ to 14.1 µg ind.$^{-1}$ (Table 2-4). The amount of weight loss at 120 ºC for 24 h ranged from 2.4 µg ind.$^{-1}$ to 29.3 µg ind.$^{-1}$ (Table 2-4). There is no marked difference between the drying time for 12 h and 24 h. Additionally, the increase in drying temperature did not affect the amount and the rate of weight loss. Carbon weight of *A. steueri* in November 2018 was 8.01 ± 1.20 µg ind.$^{-1}$ on an average (Fig. 2-9). Nitrogen weight was 1.46 ± 0.15 µg ind.$^{-1}$ on an average. C/N ratio was 5.47 ± 0.57 on an average. Conversion factor

from amount of carbon to dry weight at the highest temperature of 120°C of *A. steueri* in November 2018 was 8.42 ± 5.42 on an average.

2.3.3 **Biochemical compositions of *Acartia steueri* under high food condition**

The amounts of carbohydrate were 0.9 ± 0.3 μg ind.$^{-1}$ in *in situ A. steueri*, 0.8 ± 0.4 μg ind.$^{-1}$ in *A. steueri* raised under the high food condition, and 0.9 μg ind.$^{-1}$ in *A. steueri* raised under starvation (Fig. 2-10). There was no significant difference in the amount of carbohydrate between *in situ A. steueri* and *A. steueri* raised under high food conditions. The amounts of lipid were 4.7 ± 2.4 μg ind.$^{-1}$ in *in situ A. steueri*, 8.7 ± 6.7 μg ind.$^{-1}$ in *A. steueri* raised under the high food condition, and 2.0 ± 0.9 μg ind.$^{-1}$ in *A. steueri* under starvation (Fig. 2-10). There was a significant difference in the amount of lipid between *in situ A. steueri* and *A. steueri* raised under the high food condition (*f*-test, $p<0.01$). There was also a significant difference in the amount of lipid between *A. steueri* raised under the high food condition and starvation (*f*-test, $p<0.05$). The amounts of protein were 3.3 ± 0.8 μg ind.$^{-1}$ in *in situ A. steueri*, 4.0 ± 0.4 μg ind.$^{-1}$ in *A. steueri* raised under the high food condition, and 1.9 ± 0.3 μg ind.$^{-1}$ in *A. steueri* under starvation (Fig. 2-10). There was a significant difference in the amount of protein between *A. steueri* raised under the high food condition and starvation (Student's *t*-test, $p<0.01$). The amounts of free amino acid were 1.8 ± 2.7 μg ind.$^{-1}$ in *in situ A. steueri*, 2.6 ± 1.6 μg ind.$^{-1}$ in *A. steueri* raised under high food condition, and 1.8 ± 1.7 μg ind.$^{-1}$ in *A. steueri* raised under starvation (Fig. 2-10). There were no significant differences in the amount of free amino acids among *in situ A. steueri*, *A. steueri* raised under the high food condition and starvation. The ratio of free amino acids to individual dry weight in *A. steueri* was varied among each condition (Table 2-5). The ratios of free amino acids to dry weigh were 4.8%dw in *Artemia franciscana* under high food condition, 2.3%dw in *Artemia parthenogetica*, 1.4-4.4%dw in *Brachionus rotundiformis*, and 9.1%dw in *Temora longicornis*. In

the present study, the ratios to the dry weight were 15.0%dw in *in situ A. steueri*, 7.1%dw in *A. steueri* raised under the high food condition, and 23.5%dw in *A. steueri* raised under starvation.

2.3.4 **Metabolic flux of amino acids in *Acartia steueri* under high food condition**

Values of glutamic acid for females at day 10 and day 11 were 8.1 nmol ind.$^{-1}$ and 3.9 nmol ind.$^{-1}$ respectively. The values of glutamic acid for eggs, fecal pellets, and ingested food algae were 0.04 nmol ind.$^{-1}$ day^{-1}, 0.03 nmol ind.$^{-1}$ day^{-1}, and 1.03 nmol ind.$^{-1}$ day^{-1}. The δ^{15}N values of glutamic acid were +8.8‰, +8.9‰, +8.8‰, +3.3‰, and +0.3‰, and those of phenylalanine were −3.2‰, −3.1‰, −3.2‰, −3.6‰, and −3.7‰ for the female at day 10, that at day 11, eggs, fecal pellets, and ingested food algae (Fig. 2-11; Hirahara et al. 2015). According to the analytical error in the δ^{15}N value in this study (1σ=0.34‰), a variation in the δ^{15}N value of glutamic acid (from +0.3‰ to +8.9‰) was significantly large among these samples within a controlled-feeding experiment, whereas that of phenylalanine (from −3.1‰ to −3.7‰) was substantially small or negligible among them. Based on these δ^{15}N values and equation (1), the $TP_{Glu/Phe}$ values are calculated to be 2.1, 2.1, 2.1, 1.5, and 1.1 for the female at days 10 and 11, eggs, fecal pellets, and food algae. According to previous findings on the isotopic discrimination of amino acids in a grazing process, glutamic acid trends to have significant enrichment in ^{15}N by 8.0 ± 1.2‰, whereas phenylalanine has little enrichment in ^{15}N by 0.4 ± 0.5‰ from prey to consumer in trophic position (Chikaraishi et al. 2009). This is illustrated by how, on the cross plot for δ^{15}N glutamic acid and δ^{15}N phenylalanine values, consumer and prey organisms would be plotted on lines of the integer-based number of trophic positions (2.0 and 1.0, respectively) with enrichment in ^{15}N by 8.0‰ for glutamic acid and by 0.4‰ for phenylalanine from resource to consumer species if the consumers feed only on uniform primary producers as their food item (Fig. 2-11; Hirahara et al. 2015). In this study, although the plots of the bodies and eggs of *A. steueri* and of its food algae seem to be slightly, positively shifted from the lines of TP=1.0 and 2.0, respectively (Fig. 2-11; Hirahara et al.

2015), these data are likely to be consistent with the standard model according to both errors in isotope analysis of this study (i.e., 0.34‰) and trophic position estimation in this approach (i.e., 0.12 unit, Chikaraishi et al. 2009). Indeed, the isotopic discriminations between consumers and food algae were +8.5‰, +8.6‰, and +8.5‰ for glutamic acid and +0.5‰, +0.6‰, and +0.5‰ for phenylalanine in the bodies at days 10 and 11 and in the eggs, respectively. No substantial differences are found among these three samples. Based on these results, we conclude that the isotopic discrimination in amino acids in *A. steueri* is consistent with the general values reported in the previous study (Chikaraishi et al. 2009). In a previous controlled-feeding experiment of *A. steueri* with a different algal species, the haptophyte *Isochrysis galbana* (Nakatomi et al. 2013), the isotopic discriminations between consumers and the food algae were +8.7‰ and +8.4‰ for glutamic acid and +0.4‰ and +0.1‰ for phenylalanine in the bodies at days 6 and in the eggs, respectively. As a result, the effects of food algae (haptophyte *vs*. diatom) and the isotopic discrimination of eggs in *A. steueri* was very small or almost negligible. The $\delta^{15}N$ values of fecal pellets are +3.3‰ and −3.6‰ for glutamic acid and phenylalanine, respectively, which are plotted into an intermediate zone between bodies and the food algae. The $TP_{Glu/Phe}$ of fecal pellets in *A. steueri* was calculated as 1.5 in Fig. 2-11, which was characterized as 0.4 units higher than the 1.1 for food algae. These results may indirectly reflect the microbial activities on the food-derived amino acids through the gut of *A. steueri* and/or in the fecal pellets, but this assumption is not yet confirmed. Although we have only one set of data in the present study, it is a value integrated for a large number of fecal pellets from 50 individuals of *A. steueri* within an experiment. This $TP_{Glu/Phe}$ value is very consistent with the expected value of marine snow, 1.4 ± 0.1, in the western north Pacific (Miller et al. 2013) and with the determined value of sediment traps (450 m depth), 1.4, in the Santa Barbara basin (Batista et al. 2014). Based on the data in this study and several previous studies, we predict that the trophic position of fecal pellets would be approximately 0.4 units higher than that of their foods and that these phenomena may be universal in many cases of

aquatic environments. Because fecal pellets supply basal food resources for many organisms living in aphotic zones, our data may provide a better understanding of their trophic position. It appears that food chains in aphotic zones and the sea floor start from the trophic position of 1.4-1.5 for many organisms, unlike the 1.0 in photic zones of the ocean.

Values of total fatty acids for ingested food algae, eggs and fecal pellets were 0.08 nmol ind.$^{-1}$ day^{-1}, 0.14 nmol ind.$^{-1}$ day^{-1}, 0.29 nmol ind.$^{-1}$ day^{-1}, respectively (Table 2-6, Fig. 2-15). Detected saturated fatty acid compositions were C14, C15, C16, C17, C18 and C20 in the ingested food algae, eggs and fecal pellets (Table 2-6). C12 was detected in the ingested food algae and fecal pellet only and C21 was detected in the ingested food algae only. Values of saturated fatty acids for ingested food algae, eggs and fecal pellets were 0.04 nmol ind.$^{-1}$ day^{-1}, 0.11 nmol ind.$^{-1}$ day^{-1}, 0.27 nmol ind.$^{-1}$ day^{-1} (Table 2-6, Fig. 2-16). Detected unsaturated fatty acid compositions were C16:1n-7 in only the ingested food algae and eggs, C16:3 in only the ingested food algae, C18:1n-9, C18:3n-3 in only eggs, C20:5n-3 in only the ingested food algae and C20:5n-3 in only the ingested food algae (Table 2-6). Values of unsaturated fatty acids for ingested food algae, eggs and fecal pellets were 0.04 nmol ind.$^{-1}$ day^{-1}, 0.03 nmol ind.$^{-1}$ day^{-1}, 0.01 nmol ind.$^{-1}$ day^{-1} (Table 2-6, Fig. 2-16).

2.4 Discussion

The dry weight, carbon weight, and C/N ratio in *A. steueri* of all conditions were extremely higher than that of the other *Acartia* copepods while the prosome length and the nitrogen weight of *A. steueri* were almost similar to that of the other *Acartia* copepods (Table 2-3). Prosome length ranged from 837 µm (*A. steueri* in Ilkwang Bay, the Sea of Japan) to 1200 µm (*A. tonsa*). There is no clear difference in prosome length between *A. steueri* in Sagami Bay and other *Acartia* copepods. Dry weight of other *Acartia* copepods ranged from 4.9 µg (*A. clausi*) to 12.5 µg (*A. tonsa*). Average value of the dry weight in *A. steueri* in Sagami Bay was about 10 times higher

than that in the other *Acartia* copepods. Carbon weight of other *Acartia* copepods ranged from 2.80 µg (*A. tonsa*) to 5.10 µg (*A. clausi*). Average value of the carbon weight in *A. steueri* in Sagami Bay was about 4 times higher than that in the other *Acartia* copepods. Carbon weight of *A. steueri* in Sagami Bay was significantly higher than other *Acartia* copepods. Nitrogen weight of other *Acartia* copepods ranged from 0.4 µg (*A. tonsa*) to 1.4 (*A. erythrea* CVI). There is no significant difference in nitrogen weight between *A. steueri* in Sagami Bay and other *Acartia* copepods. C/N ratio of other *Acartia* copepods ranged from 3.5 (*A. erythrea* CVI) to 7.0 (*A.tonsa*). C/N ratio of *A. steueri* in Sagami Bay was significantly higher than the other *Acartia* copepods.

Many copepods are able to accumulate large reserves of energy-rich lipids, exhibiting some of the highest lipid levels in organisms on earth (Kattner & Hagen 2009). Energy reserves play an important role in the life history of oceanic copepods, providing energy for reproduction, ontogeny, and diapause during food scarcity (Lee et al. 2006). Oceanic copepod *Neocalanus flemingeri* has 1-2 years life cycle (Tsuda et al. 2001). This species starts to accumulate lipids within their body from the developmental stage of copepodite II in early spring and the lipid accumulation increased with the development stage in the environment: 50% of copepodite III were medially-stored lipid individuals in late summer and over 80% of copepodite IV were fully stored lipid individuals in fall to winter (Tsuda et al. 2001). Thus, oceanic species relatively slowly accumulate energy into their body for about 10 months from early spring to winter. In contrast, neritic copepods, have a few months life cycle, store little or no lipids (Mayzaud 1976, Dagg 1977, Lee et al. 2006, Peters et al. 2007). In the present study, *A. steueri* demonstrated remarkable ability to rapidly accumulate a large energy reserves within their body when food concentration is high (Hirahara & Toda 2018). In embayment environment, copepods are often exposed to fluctuations in chlorophyll *a* concentration, and they suffer from a food-scarce environment for a period of days to weeks as mentioned earlier in Chapter I (Huntley & Boyd 1984, Saiz et al. 1993, Runge & Plourde 1996, Niehoff et al. 1999, Niehoff et al. 2000). The function of rapid energy accumulation

in *A. steueri* acts as energy supply source when the abrupt decrease in food concentration or starvation in embayment (Hirahara et al. 2018) so that *A. steueri* has extremely high population density (>20,000 inds. m^{-3}) in contrast to other *Acartia* copepods in Sagami Bay, Japan (Onoue 2006, Shimode, personal communication data).

The length–weight relationship of most marine planktonic copepods has been well described by the allometric model (Huxley 1932, Ara 2001, Andrade & Campos 2002). The allometric model using length as the independent variable (weight = $a \times \text{length}^b$) is suitable for most species of fish (Frota et al. 2004, Karakulak et al. 2006). The parameter (a) is called the condition factor and the exponent (b) is usually designated as the allometry coefficient and assumes values around 3 (Andrade & Campos 2002). The allometry coefficient for fish is described as isometric growth. The allometry coefficient for marine planktonic copepods in Fig. 2-1 was 2.5, which indicates that copepods exhibit near isometric growth. However, the dry weight of *A. steueri* in the present study was much greater than that of other neritic copepods of approximately the same body length, so this species deviates from this length–weight relationship (Hirahara & Toda 2018). The carbon weight of wild *A. steueri* in Sagami Bay was higher than that in Ilkwang Bay (Kang & Kang 1997). Samples of *A. steueri* in Ilkwang Bay were preserved in 5% formalin for long periods, such as 44–52 months while the samples of dry weight in most studies summarized into Table 2-3 and Fig. 2-1 were measured within a few days after sampling without preservation in formalin. It has been known that more than 29.5% of organic matter is lost from organisms when preserved in formalin for 1.5 months (Durbin & Durbin 1978, Omori 1978). The original carbon weight of *A. steueri* in Ilkwang Bay, which was sample before preserved in 5% formalin, was estimated as 6.14 μg $ind.^{-1}$. This estimated carbon weight in Ilkwang Bay was higher than the carbon weight of *A. steueri* in Sagami Bay so that *A. steueri* in Ilkwang Bay may have the ability of energy storage same as *A. steueri* in Sagami Bay. Another possible reason was thought that food environment between Ilkwang Bay and Sagami Bay might cause the difference in dry

weight of *A. steueri*. The range of chlorophyll *a* concentration was 1.0–13 μg L^{-1} in Ilkwang Bay and 0.1–60 μg L^{-1} in Manazuru Port of Sagami Bay (Kang & Kang 2005, Satoh et al. 2000). Ilkwang Bay where occupies approximately 1.8 km^2 is an open bay facing Sea of Japan. There are beaches and rocky shores in Ilkwang Bay so that this bay is kept in an almost natural state. On the other hand, Manazuru Port of Sagami Bay where occupies approximately 0.1 km^2 is a semi-closed harbor. The food environment is very susceptible to inflows of waste water from the fishery market and terrestrial water. *A. steueri* in the embayment area of Sagami Bay may be requested to have ability to highly fluctuating food environments unlike *A. steueri* in Ilkwang Bay.

Weight loss dried under the temperature higher than 60ºC for 24 h ranged from 7.2% to 47.3% at 80ºC, from 7.4% to 95.6% at 100 ºC and from 6.1% to 8.3% at 120 ºC for 24 h. Kobari et al. (2003) reported that water content of *Neocalanus* copepods was 92.8% at a maximum. There is not only free water but also bound water in the body of organisms (Akiba 1961, Mullin & Evans 1974, Heys et al. 2008). Madin et al. (1981) calculated that 45% of ash-free dry weight in oceanic salps was bound water. In the present study, moisture contents such as bound water might be contained in the body of *A. steueri* dried under 60ºC for 24 h. *A. steueri* might have the body structure which captures moisture content within their body so that the conventional drying protocol was insufficient for removing water from the filter samples of *A. steueri* completely. Protein cannot work without the bound water (Nakagawa & Kataoka 2010). The bound water covered the surface of protein molecules 1) to adjust the function of surface of protein molecules by friction and 2) to support the bind with protein molecules and other certain substances. In the present study, the amount of protein and free amino acids was much higher in *A. steueri* raised under the high food condition than *A. steueri* raised under starvation and other zooplankton. Lovegrove (1962) found that weight loss of plankton dried at 100ºC was 5-10% to that dried at 50ºC; Curl (1961) has suggested that this could have been caused by temperatures above 60ºC to 80ºC volatilizing lipids. However, lipids in oceanic salps are less likely to be affected by high

temperature as microscopic examination which shows no evidence of volatile lipid storage globules common at times in Crustacea (Schindler et al. 1971). These considerations suggested that drying at 100^{o}C does not significantly volatile lipids in oceanic salps, although it does remove all the free water (Heron et al. 1988). Weight of *A. steueri* dried under the conventional method (60^{o}C for 24 h) might include weight of bound water because the bound water does not be dried up less than 100^{o}C. Therefore, in the future study, the drying condition for dry weight have to be re-evaluated using other zooplankton.

Estimated value of dry weight of *A. steueri* in May to July 2017 was obtained to multiply the amount of carbon by 8.42 ± 5.42 of conversion factor derived from *A. steueri* in November 2018 (Fig. 2-11). Estimated dry weight of *in situ A. steueri* in May to July 2017 multiplied by the conversion factor was 45.46 µg ind.$^{-1}$ (Fig. 2-12). Estimated dry weight of *A. steueri* in May to July 2017 raised under the high food condition was 64.84 µg ind.$^{-1}$. Estimated dry weight of *A. steueri* in May to July 2017 raised under starvation was 32.83 µg ind.$^{-1}$. Dry weight of other *Acartia* copepods ranged from 4.0 to 16.3 µg ind.$^{-1}$ (Table 2-3) so that not only the measured value of dry weight but also the estimated value of dry weights of *A. steueri* in May to July 2017 was also much higher than other neritic copepods (Fig. 2-12).

Surprisingly, *A. steueri* raised at the high food concentration translucent droplets in their body cavity similar to the oil sac common in overwintering oceanic copepods (Fig. 2-13; Hirahara & Toda 2018). There was no the translucent droplet in the body of *A. steueri* under starvation conditions (Fig. 2-14; Hirahara & Toda 2018). Oil droplets are often noted in zooplankton ovaries, and a part of these droplets can be transferred to developing oocytes (Stübing 2004). The oil droplet might contribute to the energy source of *A. steueri*. The dry weight of *A. steueri* in the high food concentration was significantly higher than that in *in situ A. steueri* (Fig. 2-3), but there was no significant difference between the carbon weight of *A. steueri* under high food concentration and those in the wild (Fig. 2-5). Eggs of *Artemia salina* have yolks containing lipoprotein with

approximately equal amounts of protein and lipid (de Chaffoy & Kondo 1980). In the present study, the amounts of lipid and protein in female raised under high food condition were significantly higher than those in female under *in situ* condition. Free amino acids in *A. steueri* under conditions of not only *in situ* but also starvation were contained at a high rate compared to other zooplankton (Table 2-5; Helland et al. 2000, Helland et al. 2003a, Helland et al. 2003b, Aragão et al. 2004). In addition, phosphorus in *A. steueri* raised at the high food condition was much higher than that of *in situ A. steueri* and *P. nihonkaiensis* as comparision species (Fig. 2-8). From these results, it was suggested that *A. steueri* in the present study accumulates lipoprotein-like substances with not only neutral lipids (which consist mainly of carbon), but also amino acids and phospholipids. This might be a reason why the dry weight of *A. steueri* was much higher than those of other neritic species.

Energy flow of glutamic acid in *A. steueri* under the high food condition was constructed based on the values of glutamic acid and $\delta^{15}N$ values among female, eggs, fecal pellets, and ingested food algae (Fig. 2-15). The amounts of glutamic acid for ingested food algae and fecal pellets were 1.03 nmol day^{-1} and 0.03 nmol day^{-1}, respectively. F_1 value (the equation 1) derived from the isotopic discrimination between $\delta^{15}N_{glutamic\ acid\_fecal\ pellet}$ and $\delta^{15}N_{glutamic\ acid\_food\ \mathrm{algae}}$ was a metabolic rate by enzymatic deamination of consumer, and this value was 0.08. Multiplied the amount of glutamic acid for ingested food algae (1.03 nmol day^{-1}) by the F_1 value (0.08), the calculated value of glutamic acid consumed by microorganisms attached to the fecal pellets was 0.08 nmol day^{-1}. Calculated value of glutamic acid for assimilation into *A. steueri* was 0.92 nmol day^{-1} by subtracting total amount of glutamic acid for the fecal pellets (0.03 nmol day^{-1}) and the consumption by the microorganisms attached to the fecal pellets (0.08 nmol day^{-1}) from the ingested food algae (1.03 nmol day^{-1}). F_2 value derived from the isotopic discrimination between $\delta^{15}N_{glutamic\ acid\_body\ of\ A.\ steueri}$ and $\delta^{15}N_{glutamic\ acid\_food\ \mathrm{algae}}$ was 0.76. Multiplied the amount of glutamic acid for the assimilation (0.92 nmol day^{-1}) by the F_2 value (0.76), the calculated value of glutamic acid consumed by metabolic activities of *A. steueri* was 0.7 nmol day^{-1}. The amounts of glutamic

acid for eggs was 0.04 nmol day^{-1}. Calculated value of remaining glutamic acid into the body of *A. steueri* was 0.18 nmol day^{-1} by subtracting total amount of the glutamic acid for the metabolism (0.7 nmol day^{-1}) and egg production (0.04 nmol day^{-1}) from the amount of glutamic acid for assimilation (0.92 nmol day^{-1}).

On the other hand, the amounts of total fatty acids for the ingested food algae, egg and fecal pellet were 0.08 nmol day^{-1}, 0.14 nmol day^{-1}, and 0.29 nmol day^{-1} respectively (Fig. 2-15). The total amount of egg and fecal pellet exceed 0.35 nmol day^{-1} from the amount of the ingested food algae. The amounts of saturated fatty acids for the ingested food algae, egg, and fecal pellet were 0.04 nmol day^{-1}, 0.11 nmol day^{-1}, and 0.27 nmol day^{-1}, respectively (Fig. 2-16). The total amount of saturated fatty acids in egg and fecal pellet exceed 0.34 nmol day^{-1} from the amount of the ingested food algae. The amounts of unsaturated fatty acids for the ingested food algae, egg and fecal pellet were 0.04 nmol day^{-1}, 0.03 nmol day^{-1}, and 0.01 nmol day^{-1}, respectively (Fig. 2-16). The total amount of unsaturated fatty acids in egg and fecal pellet did not exceed from the amount of the ingested food algae. The amount of unsaturated fatty acids between the ingested food algae and the total of egg and fecal pellet was almost balances in contrast to the amount of saturated fatty acids because copepods usually can not synthesis the unsaturated fatty acids, only taking up from food algae.

In summary, 11% and 89% of glutamic acid in ingested food algae were used for excretion and assimilation, respectively. Among 89% assimilated energy into their body, 68%, 4%, and 17% were used for metabolism, egg production and body accumulation, respectively (Fig. 2-17). On the other hand, it was revealed by the results of fatty acids compositions that 362% and 175% of lipid in food was used for excretion and egg production, respectively (Fig. 2-17). In terms of accumulation rate of energy sources provided by the results in energy flow of amino acids by isotopic discrimination (Fig. 2-11), the accumulation of amino acids within the body of female was clarified, while the total amount of lipids of egg and fecal pellet productions exceeded the lipid

amount of uptake by *A. steueri*. Free amino acids are important fuels for fish larvae during the yolk sac stage (Rønnestad et al. 1999) and a high free amino acids content is typically found in marine invertebrates (Yancey et al. 1982). Excess free amino acids which are present throughout the body, for example, muscle and body fluid, are known to be stored as form of amino acids pool, not protein in some zooplankton (Table 2-5; Helland et al. 2000, Helland et al. 2003a, Helland et al. 2003b, Aragão et al. 2004). The amino acids pool is supplied by three sources: amino acid provided by degradation of body protein, from dietary protein, and synthesis of nonessential amino acids from metabolic intermediates. Because many amino acids are easily changed to the metabolic intermediates of TCA by the deamination, which are α-ketoglutaric acid, Succinyl-CoA, Fumarate, Pyruvate, and Acetyl-CoA (Fig. 2-18; Mazelis 1980), the amino acids pool is used for energy source and synthesis of fatty acids. The Acetyl-CoA was converted to Palmitic acid (C16 fatty acid) by following equation.

$$8CH_3CO\text{-}CoA+7ATP+14NADPH+14H^+ \rightarrow C_{15}H_{31}COOH+8CoA+7ADP+7P_i+14NADP^++6H_2O \quad (3)$$

where CH_3Co-CoA is acetyl-CoA, ATP is adenosine triphosphate, NADPH is nicotinamide adenine dinucleotide phosphate (reduced form), CoA is coenzyme A, ADP is adenosine diphosphate, P_i is inorganic phosphate and $NADP^+$ is nicotinamide adenine dinucleotide phosphate (oxidized form).

Phytoplankton has been reported to have high contents of free amino acids (Raymont 1963, Fyhn et al. 1993). The ingested phytoplankton may have contributed to the higher free amino acids content in microalgae-enriched copepod (Brown 1991, Aragão et al. 2004). Free amino acids are converted to fatty acids. In starved *Temora longicornis*, the increase in total free amino acids observed at the beginning of the starvation period is consistent with the reduced protein content, suggesting that the amino acids were rapidly catabolized under starvation (Helland et al., 2003a). It was thought that free amino acids within the body of *A. steueri* might be a key role as buffering

effect. *A. steueri* might synthesize constantly free amino acids to fatty acids for metabolism and egg production under adequate food condition. From these things, *A. steueri* might be able to accumulate energy reserves rapidly. And subsequently, *A. steueri* raised under starvation condition are able to catabolize the protein predominantly and obtain free amino acids for energy sources. Moreover, *A. steueri* may be able to continuously produce eggs under the starvation condition by biosynthesizing fatty acids in the body from protein.

The presence of extra ordinary heavy embayment copepod *A. steueri* in Sagami Bay, Japan was revealed in the present study. The ability of energy accumulation in embayment copepod was revealed firstly. It was suggested that assimilated free amino acids into their body was converted to lipid such as main energy reserves. From these things, *A. steueri* might be able to accumulate energy reserves rapidly. In the wild, by accumulating energy during short periods of sporadic, high food concentration, *A. steueri* would be able to endure low food conditions until they encounter favorable food conditions again. These survival strategies may allow *A. steueri* to maintain their dominant population even when food is scarce and stably transferred to higher trophic level. In the future study, we must elucidate the incomparable ability of *Acartia* copepods which are adapted to the occurrence of sudden unfavorable environmental conditions in the embayment.

Table 2-1 Zooplankton samples used in this chapter II.

Sampling date	Prosome length	Dry weight	Dry weight for accumulation rate	Dry weight for evaluation of protocol	Carbon and nitrogen content	Element content	Biochemical content	Nitrogen isotope	Fatty acid composition
October 2014								✔	
May to July 2017	✔	✔			✔				
8th April 2018			✔						
8th, 21st, 27th April 2018		✔					✔		
July 2018						✔			
November 2018	✔			✔	✔				✔

Table 2-2 Zooplankton samples used in each figure and table of this chapter II.

	Tables				Figures														
Sampling date	2-3	2-4	2-5	2-6	2-1	2-3	2-4	2-5	2-6	2-7	2-8	2-9	2-10	2-11	2-12	2-13	2-14	2-15	2-16
October 2014														✔				✔	
May to July 2017	✔				✔	✔		✔	✔	✔					✔	✔	✔		
8th April 2018	✔						✔												
8th, 21st, 27th April 2018	✔		✔										✔						
July 2018											✔								
November 2018	✔	✔		✔								✔						✔	✔

Table 2-3 Length, dry weight, carbon weight, nitrogen weight and C/N ratio of adult females in *Acartia* species.

Species	Habitat and date	Prosome length (μm)	Dry weight (μg)	Carbon (μg)	Nitrogen (μg)	C/N ratio	Reference
Acartia clausi	Narragansett Bay	881	10.0	5.1	1.2	4.3	Durbin & Durbin 1978
Acartia clausi	The North Sea	800	10.0	—	—	—	Breteler et al. 1982
Acartia clausi	Northern Adriatic Sea	865	4.9	2.2	0.6	3.7	Cataletto & Umani 1994
Acartia erythrea CVI♀	Inland Sea of Japan	1109	11.5	4.9	1.4	3.5	Uye 1982
Acartia erythrea CV♀	Inland Sea of Japan	903	7.7	3.6	1.0	3.6	Uye 1982
Acartia pacifica	Inland Sea of Japan	912	8.3	3.6	1.0	3.6	Uye 1982
Acartia tonsa	Chesapeake Bay	1200	10.0	—	—	—	Heinle 1966
Acartia tonsa	Narragansett Bay	896	12.5	2.8	0.4	7.0	Durbin et al. 1983
Acartia tonsa	Narragansett Bay	950	16.0	—	—	—	Thompson et al. 1994
Acartia tranteri	Narragansett Bay	638	4.3	—	—	—	Kimmerer & McKinnon 1987
Acartia steueri	Ilkwang Bay	837 ± 123	—	4.3 ± 2.4	—	—	Kang & Kang 1997
Acartia steueri -Wild	Sagami Bay in May to July 2017	708 ± 62.5 (n=10)	56.2 ± 58.8 (n=107)	5.4 ± 3.0 (n=122)	0.8 ± 0.2 (n=89)	6.8 ± 2.2 (n=89)	Hirahara & Toda 2018
Acartia steueri -Under high food	Sagami Bay in May to July 2017	680 ± 18.5 (n=7)	125.4 ± 85.2 (n=70)	7.7 ± 5.0 (n=74)	0.9 ± 0.3 (n=70)	8.7 ± 5.5 (n=70)	Hirahara & Toda 2018
Acartia steueri -Under starvation	Sagami Bay in May to July 2017	728 ± 114 (n=3)	114.6 ± 79.9 (n=55)	3.9 ± 1.3 (n=55)	0.5 ± 0.1 (n=26)	8.3 ± 2.4 (n=26)	Hirahara & Toda 2018
Acartia steueri -Wild	Sagami Bay in 8th, 21st and 27th April 2018	—	12.3 ± 13.3 (n=169)	—	—	—	This study
Acartia steueri -Under high food	Sagami Bay in 8th, 21st and 27th April 2018	—	36.0 ± 33.6 (n=139)	—	—	—	This study
Acartia steueri -Under starvation	Sagami Bay in 8th, 21st and 27th April 2018	—	7.6 ± 6.9 (n=51)	—	—	—	This study
Acartia steueri -Wild	Sagami Bay in 8th April 2018	—	2.8 ± 1.6 (n=59)	—	—	—	This study
Acartia steueri -Under high food for 5 days	Sagami Bay in 8th April 2018	—	14.8 ± 6.1 (n=60)	—	—	—	This study
Acartia steueri -Under high food for 10 days	Sagami Bay in 8th April 2018	—	70.8 ± 30.3 (n=49)	—	—	—	This study
Acartia steueri -Under high food	Sagami Bay in November 2018	781 ± 27.5 (n=29)	56.2 ± 55.0 (n=32)	8.0 ± 1.2 (n=32)	1.5 ± 0.2 (n=32)	5.5 ± 0.6 (n=32)	This study

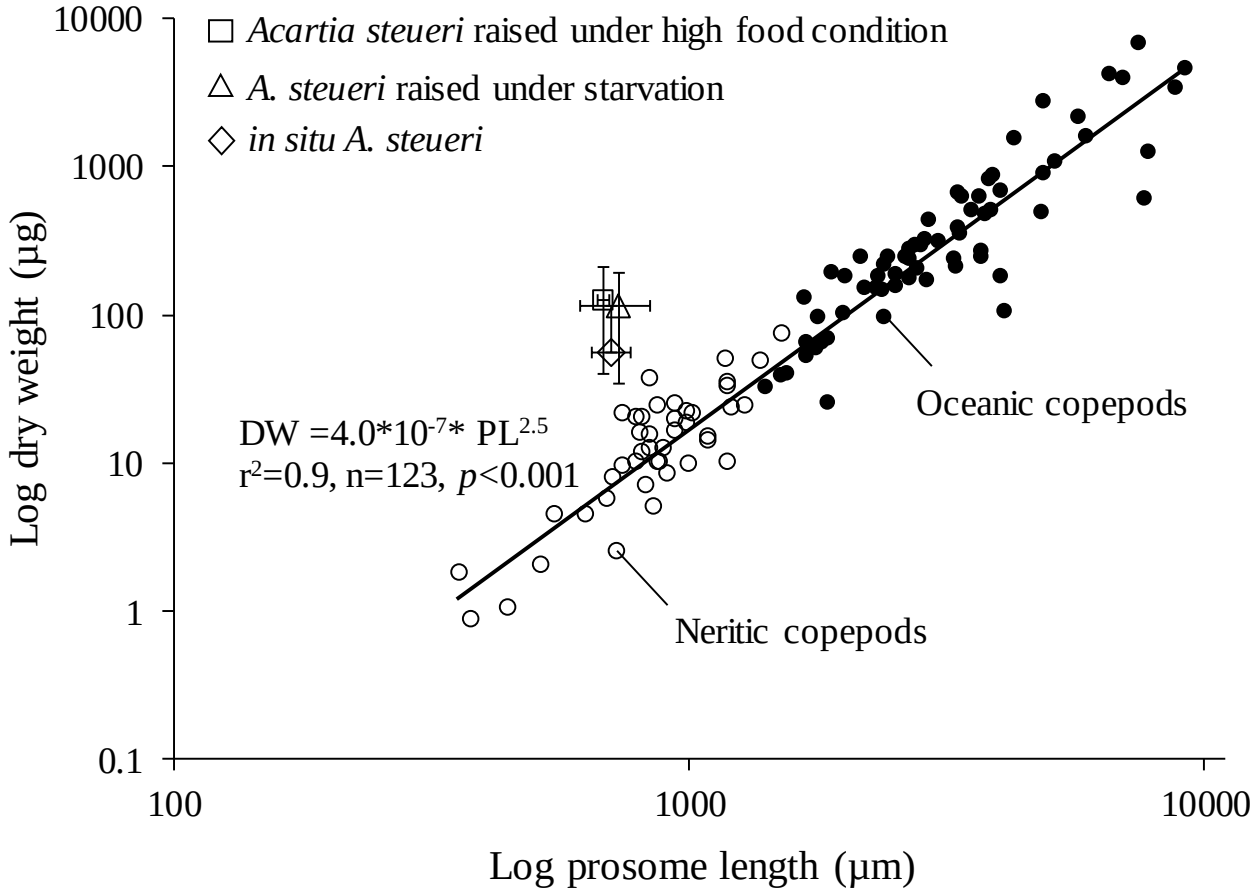


Fig. 2-1 Log–transformed dry weight and log–transformed prosome length relationship for neritic and oceanic copepods (Hirahara & Toda 2018). Open circles represent neritic copepods (Durbin & Durbin 1978, Uye 1982, Durbin et al. 1983, Kimmerer & McKinnon 1987, Breteler & Gonzalez 1988, Durbin et al. 1992, Cataletto & Umani 1994, Richardson et al. 2001). Closed circles represent oceanic copepods (Omori 1969, Tande 1982, Uye 1982, Escribano & McLaren 1999, Yamaguchi & Ikeda 2000, Kobari & Ikeda 2001, Richardson et al. 2001, Kobari et al. 2003, Shoden et al. 2005, Zhang et al. 2005). Diamond symbol represents *in situ Acartia steueri* in May to July 2017. Triangle symbol represents *A. steueri* in May to July 2017 raised under starvation. Square symbol represents *A. steueri* in May to July 2017 raised under high food condition.

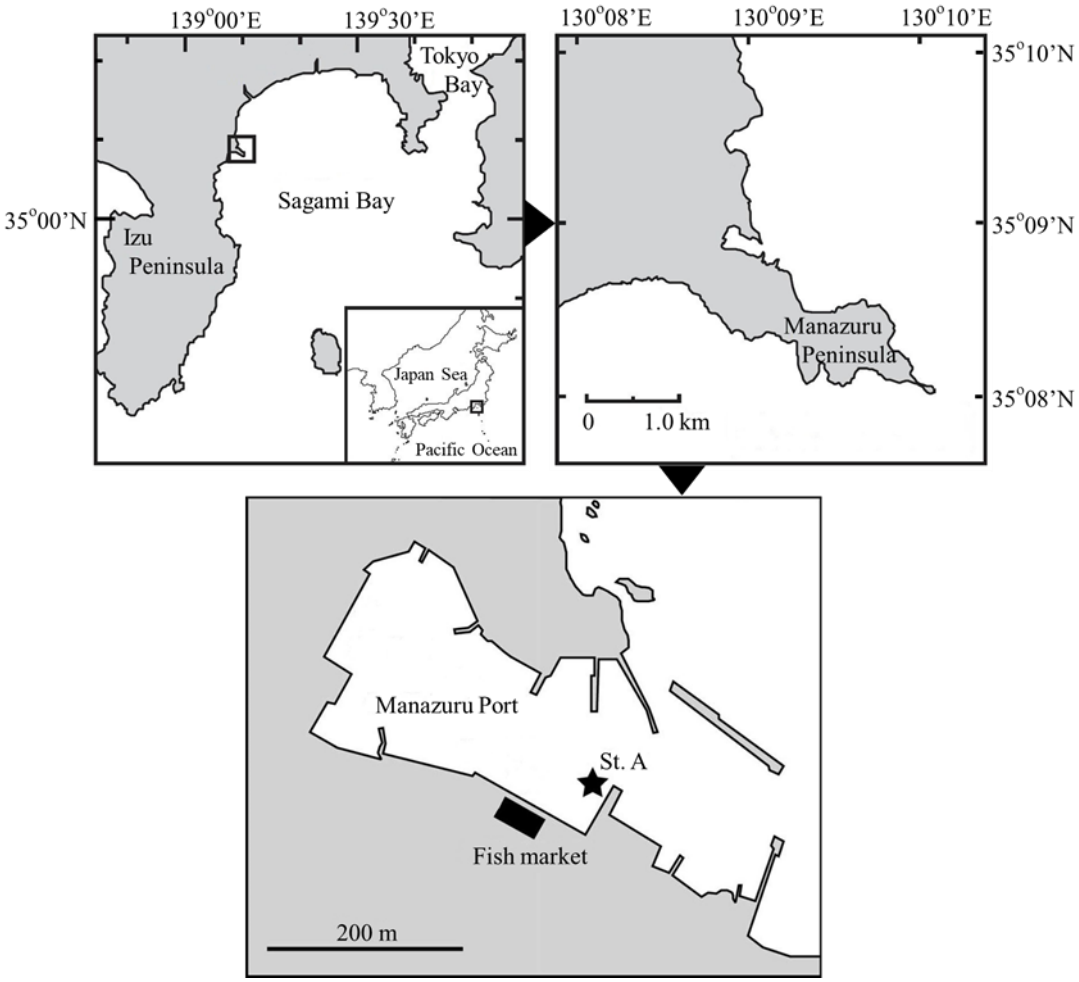


Fig. 2-2 Location of sampling site in this study.

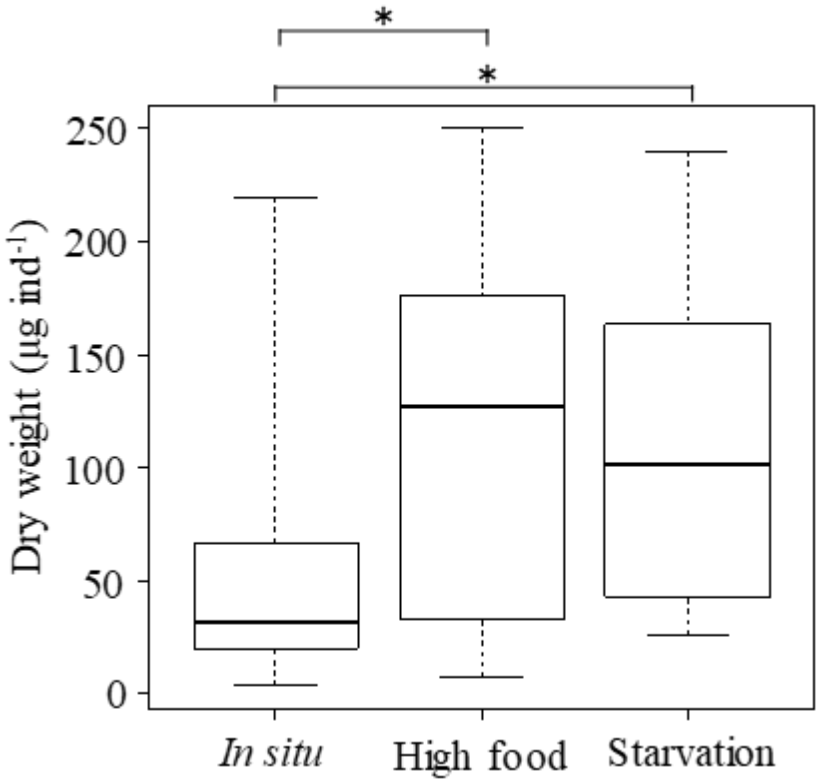


Fig. 2-3 Box plots of dry weights of *Acartia steueri* in May to July 2017 under *in situ*, high food and starvation conditions. Horizontal solid line inside the box indicates median value, the bottom and top of the box indicates first and third quartiles, and whiskers indicate largest and smallest values. *: $p<0.05$ (modified from Hirahara & Toda 2018)

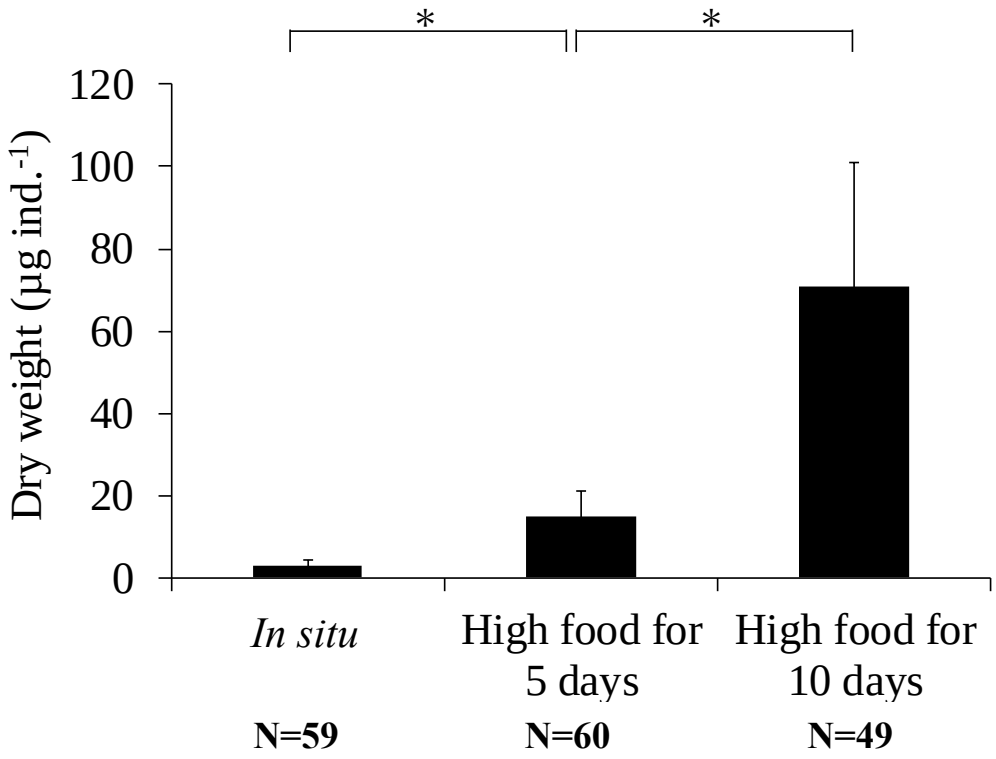


Fig. 2-4 Change in dry weight of *Acartia steueri* in 8th April 2018 raised under high food condition for 10 days. ＊:$p<0.05$

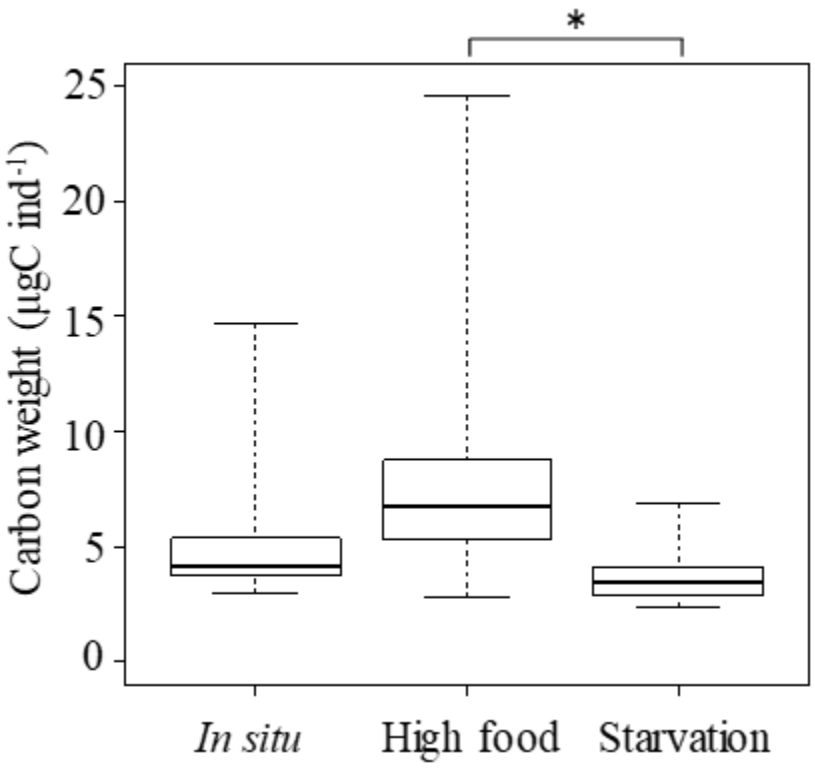


Fig. 2-5 Box plots of carbon weights of *Acartia steueri* in May to July 2017 under *in situ*, high food and starvation conditions. Horizontal solid line inside the box indicates median value, the bottom and top of the box indicates first and third quartiles, and whiskers indicate largest and smallest values. *: $p<0.05$ (modified from Hirahara & Toda 2018)

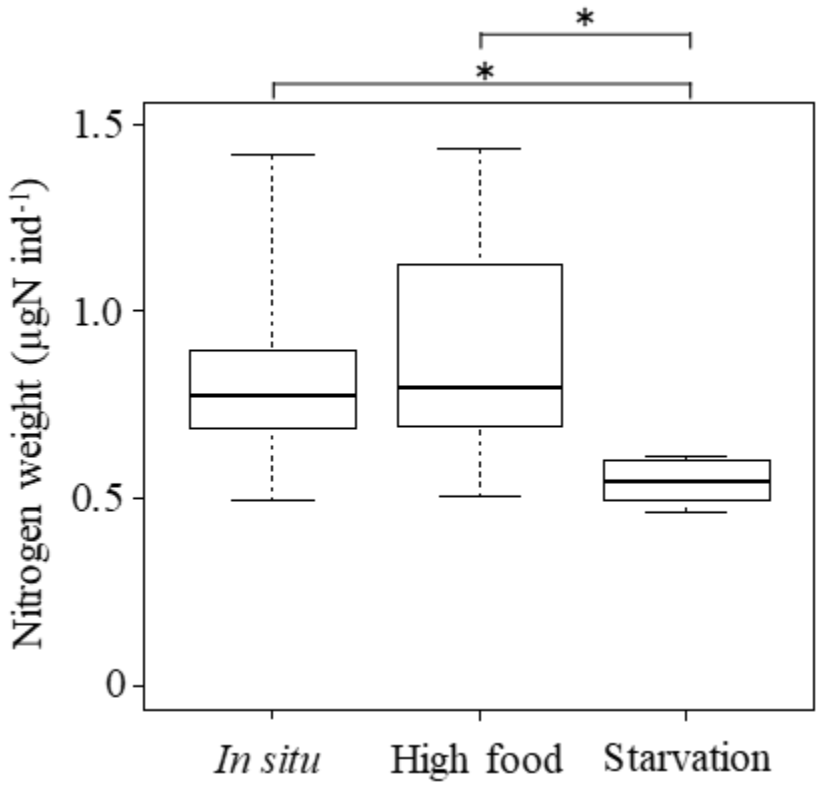


Fig. 2-6 Box plots of nitrogen weights of *Acartia steueri* in May to July 2017under *in situ*, high food and starvation conditions. Horizontal solid line inside the box indicates median value, the bottom and top of the box indicates first and third quartiles, and whiskers indicate largest and smallest values. *: $p<0.05$ (modified from Hirahara & Toda 2018)

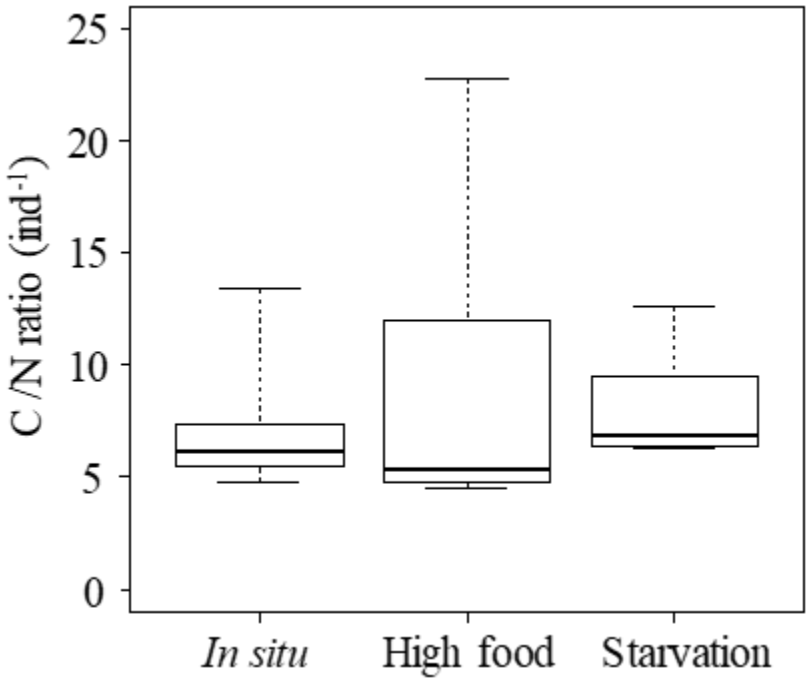


Fig. 2-7 Box plots of C /N ratio of *Acartia steueri* in May to July 2017 under *in situ*, high food and starvation conditions. Horizontal solid line inside the box indicates median value, the bottom and top of the box indicates first and third quartiles, and whiskers indicate largest and smallest values. *: $p<0.05$ (modified from Hirahara & Toda 2018)

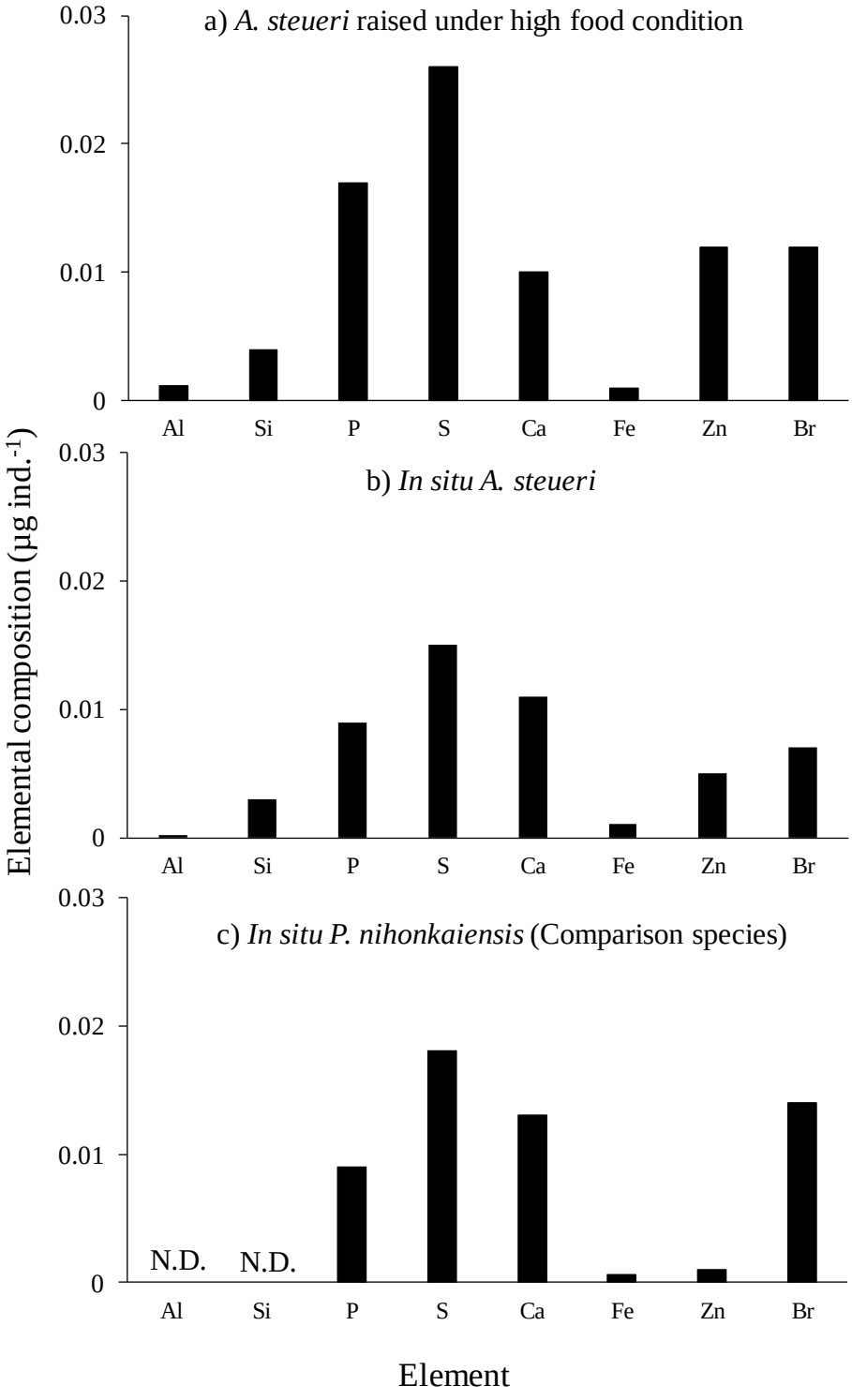


Fig. 2-8 a) Elemental compositions of *A. steueri* in July 2018 raised under high food condition for 10 days, b) *in situ A. steueri* and c) *in situ Pseudodiaptomus nihonkaiensis* as comparison species. ※Aluminum (Al) and Silicate (Si) of *in situ P. nihonkaiensis* were not detected.

Table 2-4 Change of weights (μg ind.$^{-1}$) of *A. steueri* in Novebmer 2018 dried under from conventional condition (60ºC, 24 h) to higher temperature conditions (80ºC, 100ºC and 120ºC, 12 h and 24 h).

Temperature (ºC)	60	80		60	100		60	120	
Treatment time (h)	24	12	24	24	12	24	24	12	24
Dry weight (μg ind.$^{-1}$)	39.46	36.80	36.60	125.76	116.46	116.46	138.84	130.44	130.44
	5.46	3.28	2.88	7.32	0.32	0.32	20.60	19.04	18.90
							125.76	111.66	96.46
							39.46	28.00	28.00

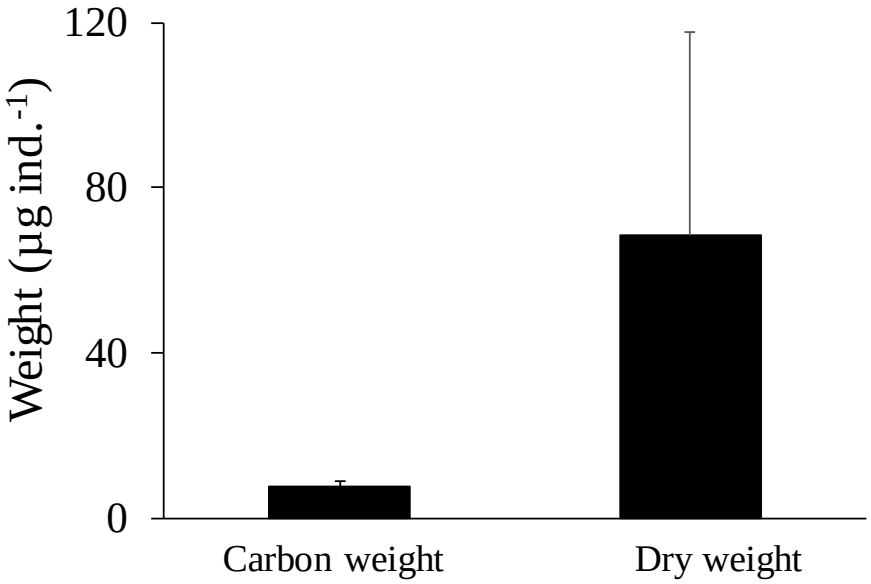


Fig. 2-9 Carbon weight and dry weight (µg ind.$^{-1}$) of *Acartia steueri* in November 2018. Dry weight indicated the weight of *A. steueri* dried under the highest temperature conditions at 120ºC for 24 h. Conversion factor from total amount of carbon and nitrogen to dry weight is 8.42 ± 5.42 (see text).

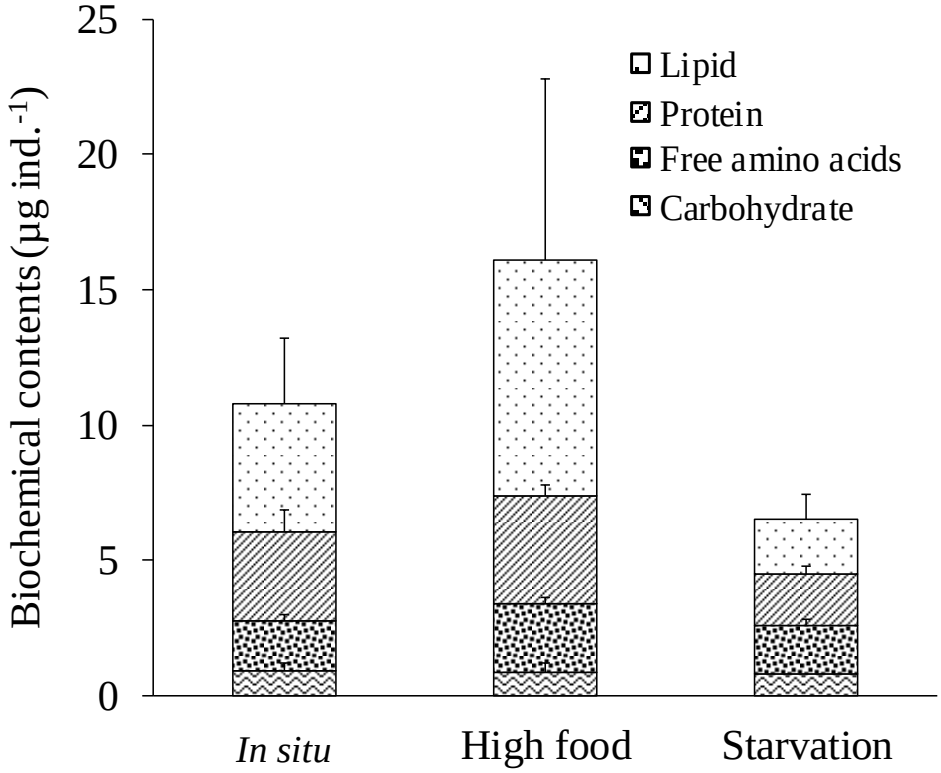


Fig. 2-10 The accumulated biochemical compositions (lipid, protein, free amino acids, and carbohydrate) in *Acartia steueri* of 8th, 21st and 27th April 2018 under *in situ*, high food condition and starvation condition.

Table 2-5 Rates of protein and free amino acid to individual dry weight in some zooplankton and *A. steueri* in 8th, 21st and 27th April 2018 of this Chapter II.

Species	Condition	Protein (%dw)	Free amino acids (%dw)	Reference
Artemia franciscana	enriched	31.6	4.8	Helland et al. 2003a
Artemia parthenogenetica	enriched	33.8	2.3	Aragão et al. 2004
	starved for 1 d	38.5	2.5	
Brachionus rotundiformis	enriched	29.1	4.4	Aragão et al. 2004
	starved for 1 d	37.1	1.4	
Calanus finmarchicus	*insitu*	45.0	11.9	Helland et al. 2003b
	starved for 40 d	6.9	15.7	
Temora longicornis	*insitu*	30.6	9.1	Helland et al. 2003a
Acartia steueri	*insitu*	26.7	15.0	This study
	enriched	11.1	7.1	
	starved for 10 d	25.1	23.5	

Values are expressed in relation to dry weight (dw)

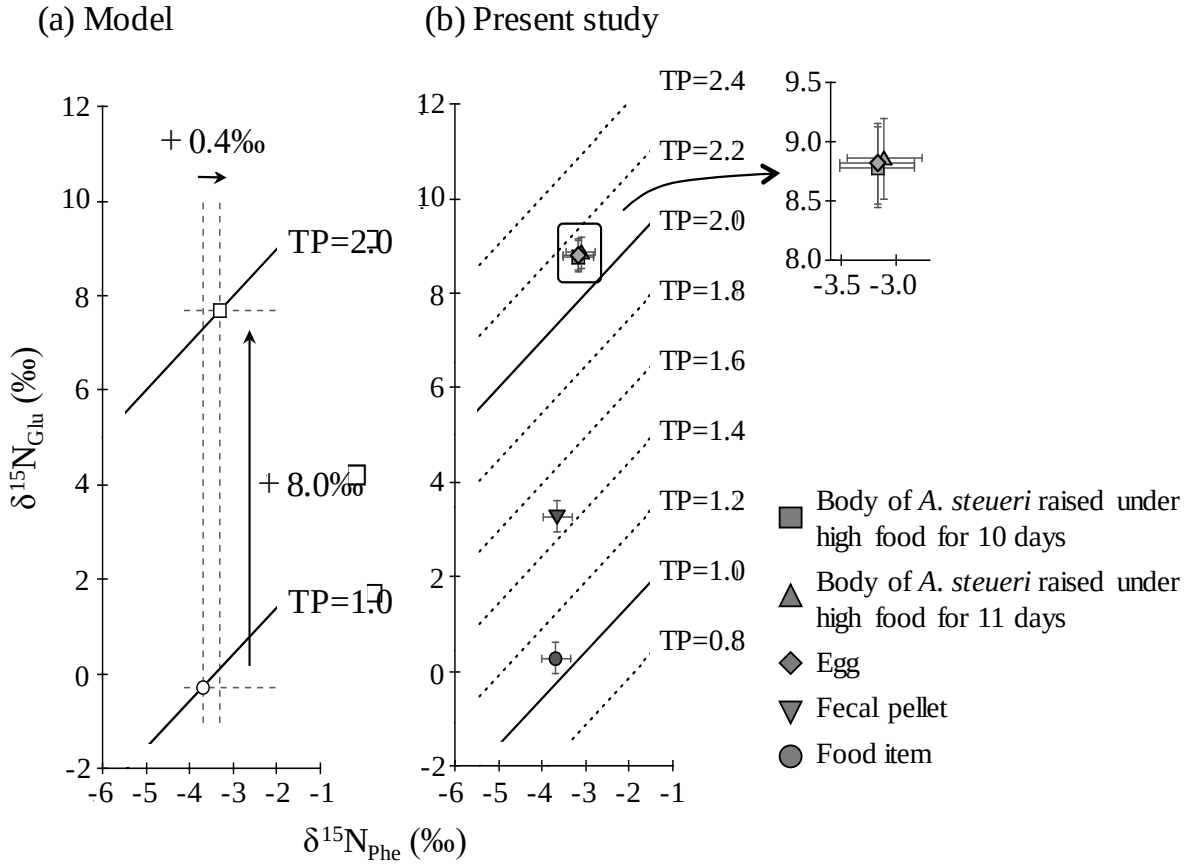


Fig. 2-11 Cross-plots of the $\delta^{15}N_{Glu}$ and $\delta^{15}N_{Phe}$ values: (a) a standard model for the isotopic discrimination in $^{15}N/^{14}N$ in a consumer (open square) feeding only on a primary producer (open circle) (Chikaraishi et al. 2009) and (b) data observed in the present study for bodies at days 10 and 11 (filled square and triangle, respectively), eggs (filled diamond), fecal pellets (filled reversed triangle) of *Acartia steueri* in October 2014, and food item (filled circle), with 0.34‰ error bars as the analytical precision. Solid and dashed lines indicate the trophic isocline for integer-based numbers (1.0 and 2.0) and 0.2 intervals of the trophic position, respectively (modified from Hirahara et al. 2015).

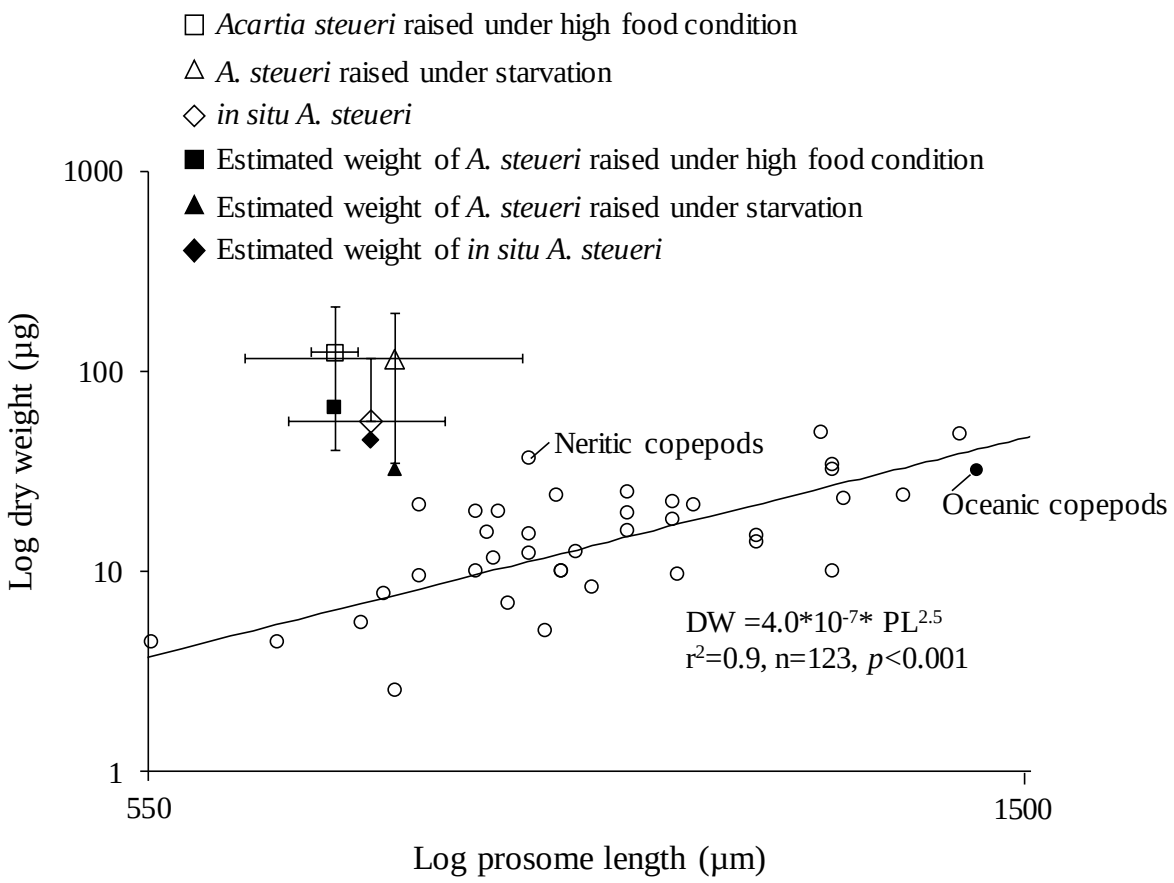


Fig. 2-12 Log–transformed dry weight and log–transformed prosome length relationship for neritic and oceanic copepods obtained from Fig. 2-1. Black square symbol represents estimated dry weight of *A. steueri* in May to July 2017 raised under high food condition derived from total amount of carbon and nitrogen of *A. steueri* in May to July 2017 multiplied by 8.4 as the conversion factor (see text). Black triangle symbol represents estimated dry weight of *A. steueri* in May to July 2017 raised under starvation derived from the amount of carbon multiplied by 8.42. Black diamond symbol represents estimated dry weight of *in situ A. steueri* in May to July 2017 derived from amount of carbon multiplied by 8.42.

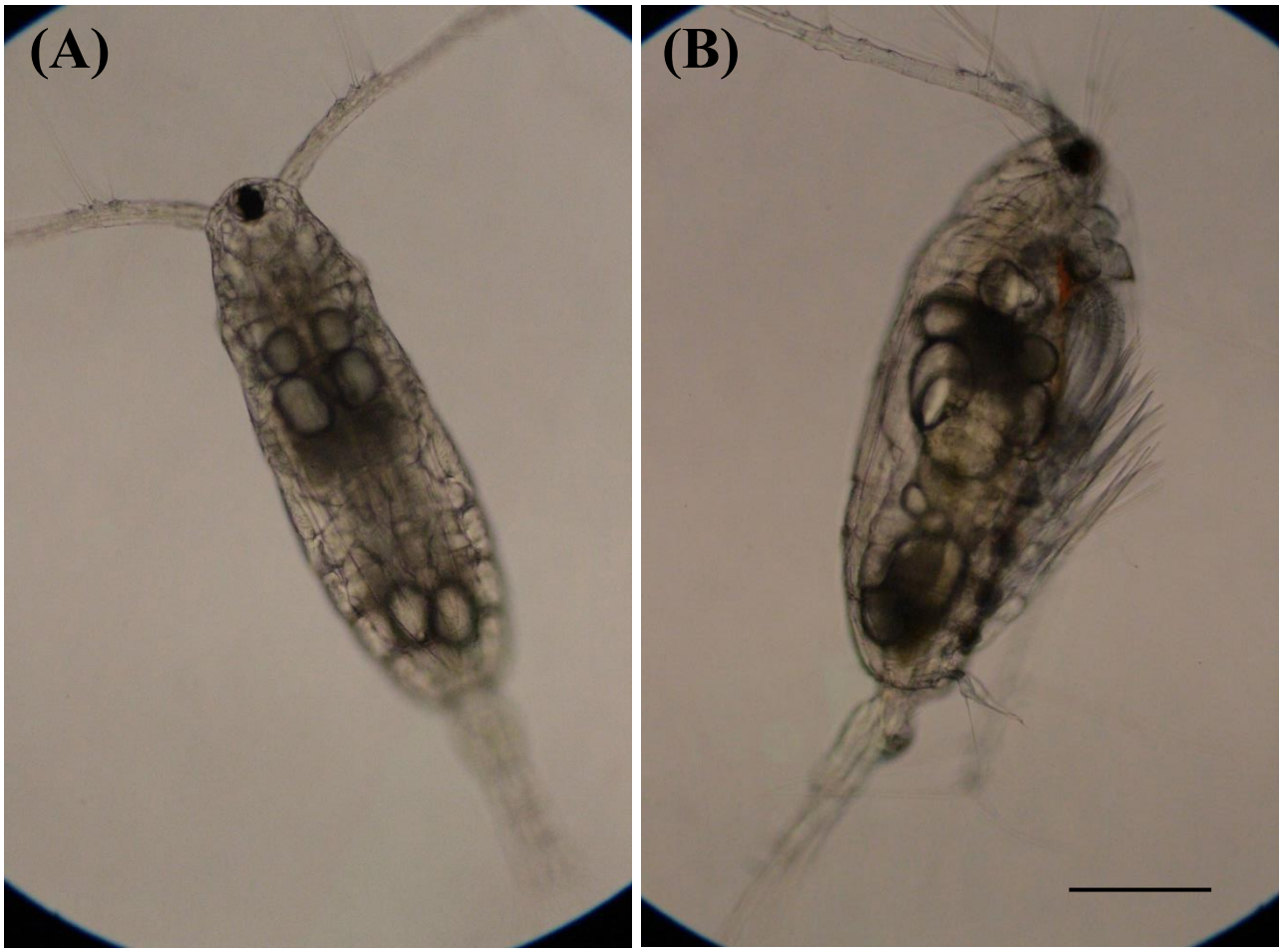


Fig. 2-13 Adult females of *Acartia steueri* in May to July 2017 raised under high food condition for 10 days were observed by a light microscope (modified from Hirahara & Toda 2018). (A) An Image was observed from dorsum of female. (B) An image was observed from ventral part of female. Scale bar = 100 μm.

Fig. 2-14 Adult female of *Acartia steueri* in May to July 2017 raised under starvation for 10 days was observed by a light microscope. Scale bar = 100 μm. (Hirahara & Toda 2018).

Table 2-6 Fatty acid compositions in food algae (*Thalassiosira weissflogii*), egg and fecal pellet of *A. steueri* in November 2018. SFA means Saturated Fatty Acid, UFA means Unsaturated Fatty Acid and TFA means Total Fatty Acid. Yellow bars indicated saturated fatty acids.

Fatty acid	Food	Egg	Fecal pellet
C12	0.00089	—	0.01147
C14	0.00637	0.01702	0.04685
C15	0.00106	0.00767	0.03422
C16	0.02171	0.06073	0.12244
C16:1n-7	0.00968	0.02044	—
C16:3	0.02112	—	—
C17	0.00026	0.00170	0.01567
C18	0.01552	0.02310	0.04599
C18:1n-9	0.00071	0.00291	0.01331
C18:3n-3	—	0.01001	—
C20	0.00030	0.00042	0.00131
C20:5n-3	0.01067	—	—
C21	0.00010	—	—
C22:6n-3	0.00112	—	—
SFA	0.04621	0.11065	0.27795
UFA	0.04330	0.03336	0.01331
TFA	0.08951	0.14400	0.29126

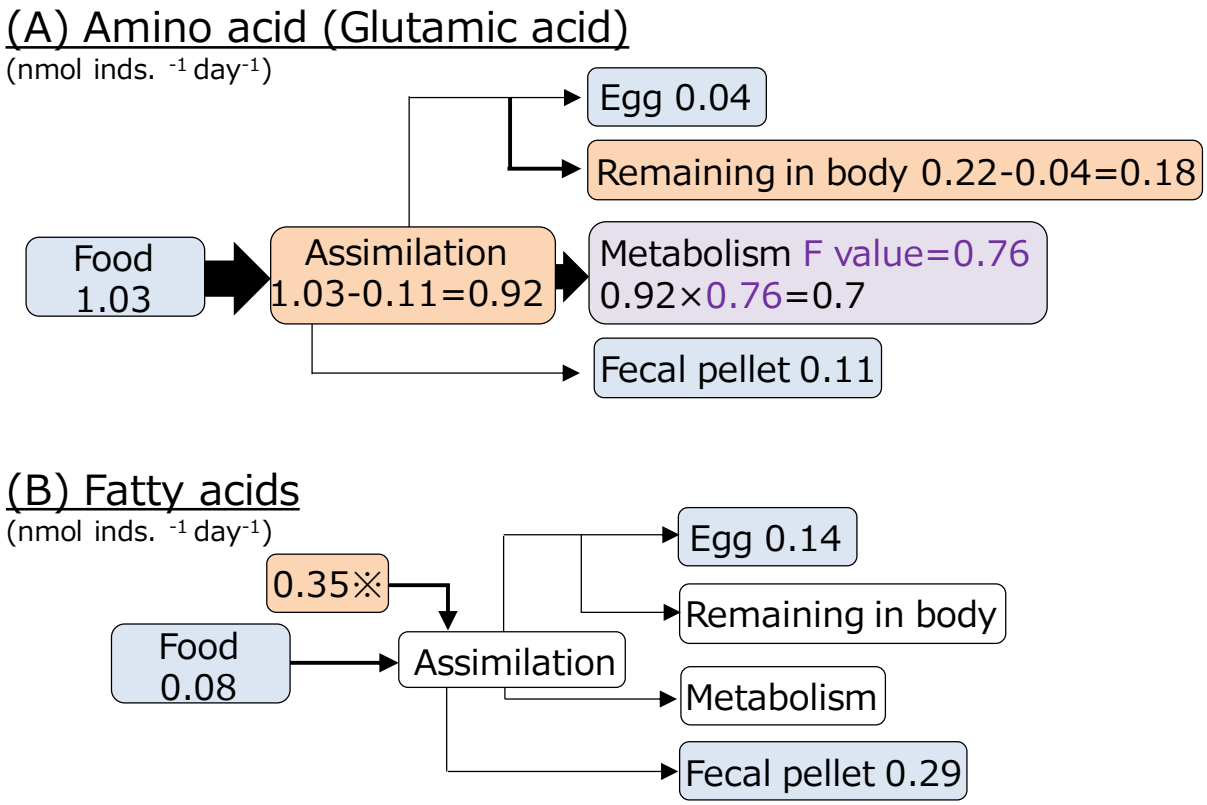


Fig. 2-15 Estimated Energy flows of *Acartia steueri*. Upper figure (A) indicates the energy flow of glutamic acid as representative of amino acids in *A. steueri* of November 2014. Blue squares indicate measured values. Orange squares indicate calculated values. Purple squares indicate F_1 and F_2 values required by nitrogen isotopic ratio. Below figure (B) indicates the energy flow of total fatty acids in *A. steueri* of November 2018. Asterisk (※) indicates predicted value converted remaining in body (0.18 nmol day^{-1}) of amino acids to fatty acids on the basis of carbon.

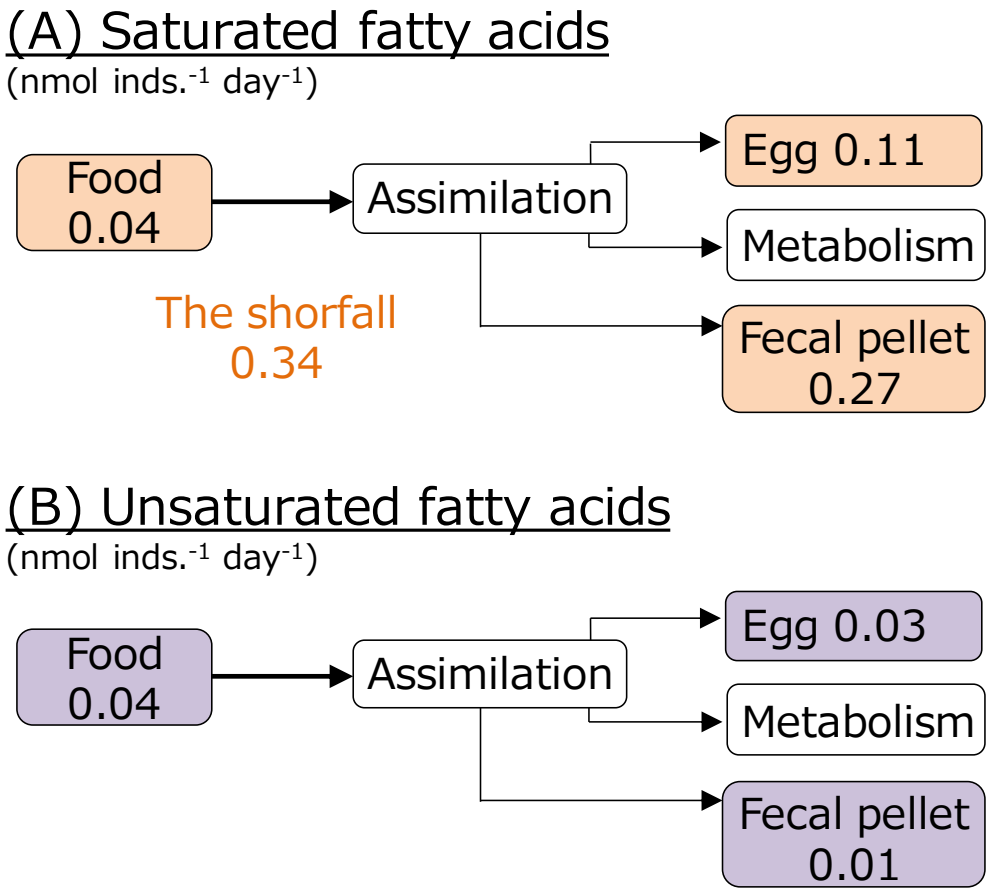


Fig. 2-16 Energy flows of each fatty acid of *Acartia steueri* in November 2018. Upper figure (A) indicated the energy flow of saturated fatty acids and lower figure (B) indicated the energy flow of unsaturated fatty acids.

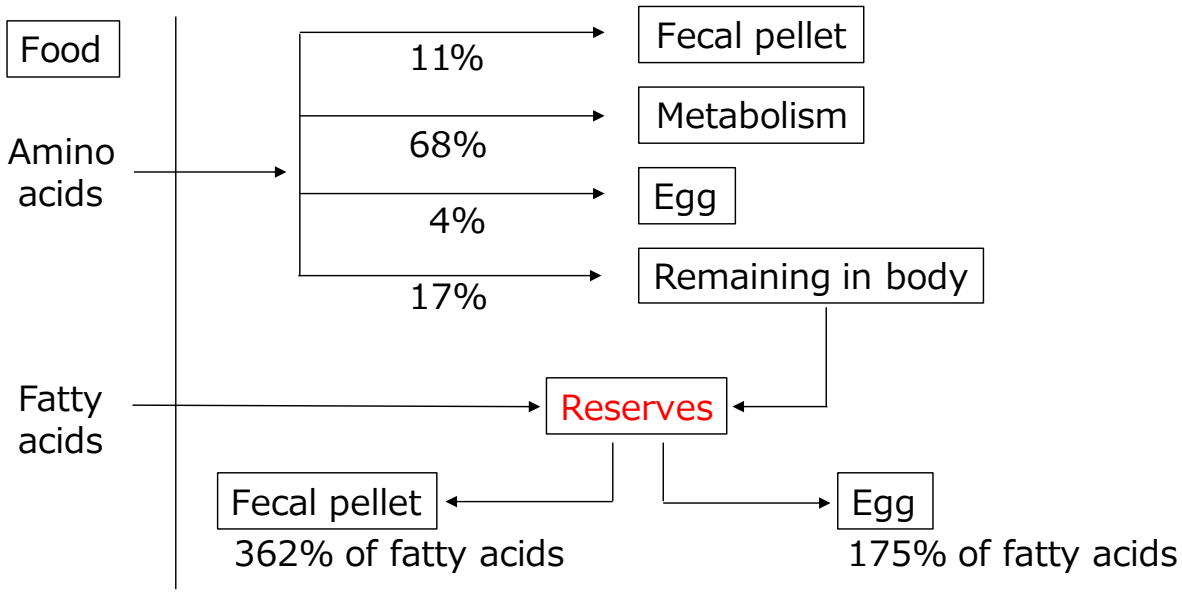


Fig. 2-17 Estimated rate of energy flows both of an amino acid (glutamic acid) and fatty acids in *Acartia steueri*

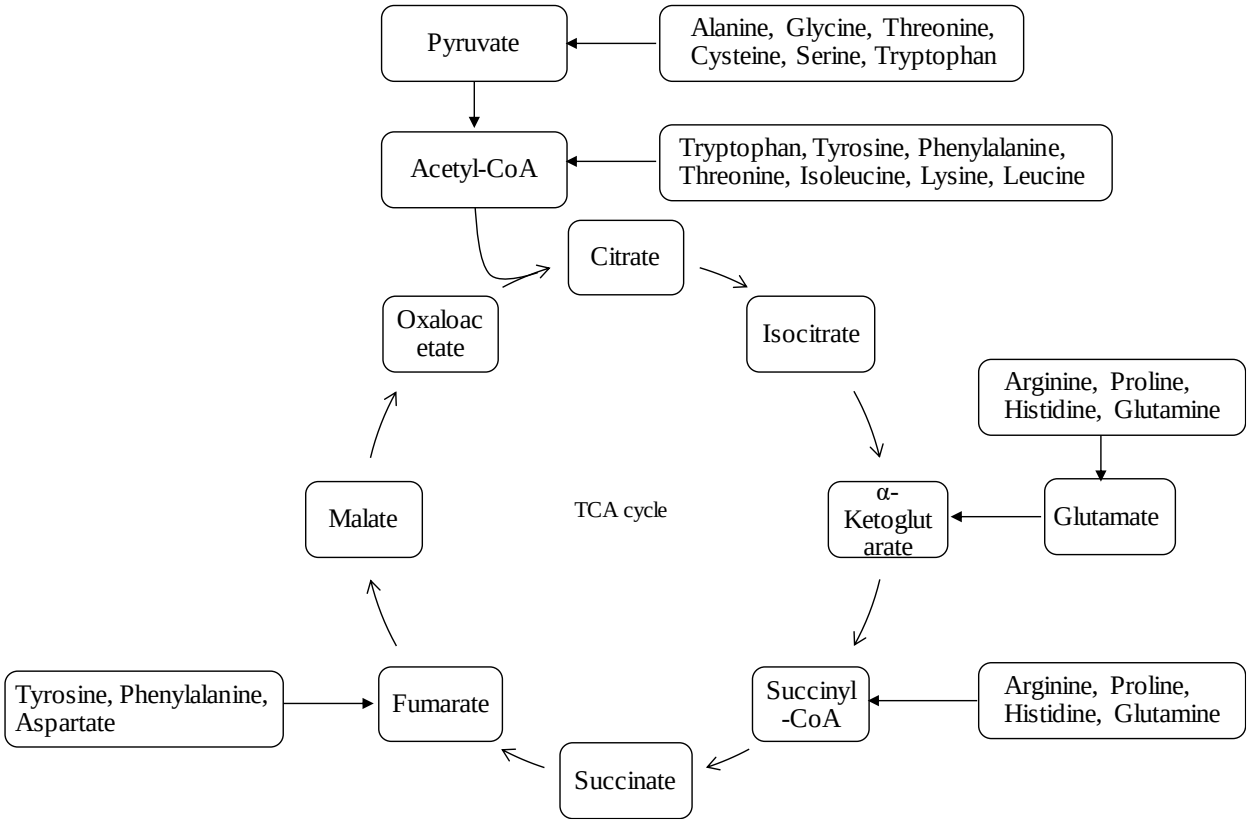


Fig. 2-18 Amino acid catabolism in TCA cycle (Mazelis 1980)

Chapter Ⅲ

Physiological responses of *Acartia steueri* to starvation condition

3.1 Introduction

Food in embayment environments is generally scarce, patchy and fluctuating, often because of tide, irregular inflows of waste water and terrestrial water and the seasonal cycles (Cebrian & Valiela 1999; Winder & Cloern 2010). The embayment copepods were exposed to short-term fluctuations of food environment so that these copepods have to cope with the risk of starvation for short and long periods. Although instantaneous ecological consequences of poor and spotty nutrition are sometimes difficult to distinguish, the survival and reproductive potentials of the embayment copepods experiencing such the extreme low food conditions may become reduced. In fact, starvation is considered one of the main non-predatory causes of mortality in zooplankton (Tang et al. 2014).

Ingestion rate and metabolic rate of copepods has been reported to decrease considerably during prolonged starvation periods (Ikeda 1977; Tsuda 1994; Thor 2000). For small neritic copepods, such as *Acartia* species, low or absent food intake is especially unfavorable condition since they lack the potential of energy storage including lipids (Lee et al. 2006). They are adapted to nearshore or estuarine environments relying on a readily available food supply. *Acartia tonsa* react to periods of low or absent food intake with a compensatory transient increase in clearance rate following these periods (Tiselius 1998). The increase of clearance rate of copepods causes the increases of metabolic and respiration rate. The clearance rate of *A. tonsa* was not changed under the starvation periods for less than 3 hours so that the metabolism of the copepods is able to compensate for the lacking energy input. But after progressively longer periods of starvation for more than 6 hours, the copepods exhibit a hunger response with elevated clearance rates. The

respiration rate of *A. tonsa* finally decreases with 50% within 10 hours of starvation. This decrease to a basal metabolic rate is thought to enhance the survival probability by reducing the energy expenditure (Tsuda 1994). The subsequent recovery of respiration has been investigated in daphnids (Lampert 1986), but never in copepods.

Egg production rate, which has been often examined for secondary production, growth rate, recruitment, and response to environmental conditions, is a key component of population dynamics of copepods. Eggs and nauplii of copepods as well as copepodid and adult stages of copepods are used as the food source for larvae of many commercial fishes so that the reproduction of copepod could affect the recruitment of fishes (Halsband & Hirche 2001). The egg production of copepods can vary with temperature, salinity, season, species, size, feeding, and food condition (Williamson & Butler 1987, Jonasdottir 1989, Kiørboe & Sabatini 1995, Kleppel et al. 1998). Since copepods are often associated with phytoplankton patches and blooms, the reproductive activity is usually restricted to the phytoplankton bloom in spring (Diel & Tande 1992, Plourde & Runge 1993). Correlation, however, between the co-occurrence of phytoplankton and egg production in copepods is difficult to prove in practice (Hansen & Santer 1995, Zaballa & Gaudy 1996). The lack of correlation may be because chlorophyll *a* is an imperfect index of the availability of phytoplankton or, more likely, that the copepods are also feeding on organisms other than phytoplankton. During their reproductive period, females are often temporarily exposed to unfavorable feeding conditions prior to or after the spring bloom (e.g. Runge & Plourde 1996, Niehoff et al. 1999, Niehoff et al. 2000). Therefore, it is difficult to understand the egg production rate of copepods to food concentration change in the natural environment. In order to consider the *in situ* egg production, it is necessary to understand physiological responses to abrupt food concentration change and/or starvation. Though absolute lack of food might not occur in the natural environment, knowledge about responses to starvation can considerably improve general understanding of reproductive processes.

The energy flow obtained by the feeding in the adult female of copepod is illustrated in Fig. 1-3. Firstly, adult female eats foods such as phytoplankton, they eliminate the fecal pellets and assimilate the other energy into their body. Consecutively, the assimilation energy is used for the metabolism and the egg production. When the feed intake decreases, the fecal pellet and assimilation energy also decrease necessarily. As for the distribution of assimilation energy, how much is the energy use for the egg production in comparison with the metabolism? Little is known of how variation in feed intake influences the distribution of energy flow between the egg production and fecal pellet production.

Acartia steueri is one of the most dominant embayment copepods in Sagami Bay, Japan and exists in water column through a year, though their abundance decreases in summer (Onoue et al. 2004). The abundance increases in late autumn. *A. steueri* is known to produce morphologically different two types of eggs, smooth and spiny eggs (Uye 1983, Onoue et al. 2004). The spiny eggs are diapausing eggs with various lengths of refractory phases in our sampling point. The production of diapausing eggs is considered as a surviving strategy for living under the unfavorable seasons. Sagami bay belongs to a temperate coastal environment on the east coast of Japan, is influenced by the seasonal changes (e.g., Satoh et al. 2000; Ara et al. 2007). The water mass in the bay is strongly complicated. It might be expected that the population maintenance of *A. steueri* would be related with the adaptation for the abrupt food concentration change in addition to the production of diapausing egg. It is important to investigate the biological responses to abrupt changes of food concentration or starvation condition for understanding its survival strategy of *A. steueri* and population dynamics of this species.

In this study, the incubation experiments were conducted in order to examine the physiological responses such as metabolic rate and egg production rate of *A. steueri* to the abrupt changes of food concentration. The four experimental conditions were designed by combination of two high food concentration periods (2 or 5 days) and two low food concentrations including starvation,

respectively. Finally, we discussed the metabolic specificity of *A. steueri* under the starvation condition compared with other neritic copepods.

3.2 Materials & Methods

3.2.1 Respiration rate

Plankton samples were collected using a plankton net (mouth diameter: 30 cm; mesh aperture: 180 μm) at Manazuru Port, Sagami Bay, Japan (Fig. 2-2) in June and July 2016 and July 2017. The samples were immediately transferred to a field laboratory. Live adult females of *A. steueri* were sorted from plankton samples into glass bottles containing filtered sea water (<0.22 μm) under a dissecting microscope (WILD M10, Leica). Adult females were incubated at high food condition (1.0 μg C mL^{-1}; Berggreen et al. 1988) for 10 days and starvation condition (<0.22 μm filtered sea water) for 10 days at two different temperatures, 15ºC and 20ºC, respectively. The concentration of the high food condition was set based on a concentration at which growth rate and egg production rate in *A. tonsa* were saturated (Berggreen et al. 1988). Drillet et al. (2011) was also used this reference for the high food condition in incubation experiment of *A. tonsa*. The periods of high food and starvation conditions were determined based on the fluctuation of *in situ* spring bloom (Furuya et al. 1993). The diatom *Thalassiosira weissflogii* (64.4 pg C/cell) cultured with f/2 media was used for food algae and placed in the fresh filtered sea water with the food algae every 48 hours.

Oxygen consumption rate of adult females under the high food and starvation conditions were measured at 15ºC and 20ºC to determine the differences of metabolic responses to the food condition and temperature, respectively. Five gas-tight glass bottles were situated in a water bath placed in an incubator (CN-25C, Mitsubishi, Tokyo, Japan) to keep a constant temperature in the bottles (Fig.3-1). All experimental bottles were cleaned with a neutral detergent without using any antibiotics. The DO concentration in each bottle was measured using a fiber-optic oxygen meter

(Firesting O_2, PyroScience, Aachen, Germany), fitted with a spot-fiber oxygen sensor (SPFIB, PyroScience, Aachen, Germany). This allowed semi-continuous (every minute) measurements using four oxygen sensors (two for experimental bottles with animals and two for control bottles without animals) with a submersible temperature sensor (TSUB21, PyroScience, Aachen, Germany) in the last bottle. An oxygen sensor spot was glued to the inner wall of each experimental bottle to non-invasively and non-destructively measure the DO concentration with oxygen sensors from outside the bottles. This new technology for measuring DO is based on an optical oxygen-detection technique using red light irradiance and a spot-fiber oxygen sensor providing a DO detection limit of 1 $\mu LO_2\ L^{-1}$ with a coefficient of variation of 0.02–0.2%. Adult females were washed five times with Filtered Sea Water (FSW, <0.22 μm). to remove remaining algae and bacteria from the stock culture. Finally, two to four individuals were placed into a gas-tight experimental glass bottle (3-mL) filled with FSW, and DO concentration monitoring was started. Despite the excellent precision of DO detection with the oxygen meter, unstable DO concentrations were detected during the first few hours of the incubation because of low near infrared-emission under high DO concentrations (>4.5 $mLO_2\ L^{-1}$), so that high DO (±0.2%) variation was detected. DO concentrations in the experimental bottles also fluctuated during the first few hours of the incubation, probably due to increasing activity of animals as pointed out by Teuber et al. (2013). Therefore, data used for analyses were taken after hour hours from the start of the incubation. After that DO concentrations decreased linearly with incubation time, always more rapidly in experimental bottles compared to those in controls. The period for estimating oxygen consumption rates with regression analysis was from 3 to 12 hours depending on temperature and size of the animals tested. All the experiments lasted 12-18 hours with 3-6 replicates under dark condition. More than 80% of the initial DO concentration remained at the end of all experiments (Liu & Ban 2016). Adult females were not fed during the experimental

period. At the end of the experiments, the animals were preserved with 5% neutral sugar formalin, and the prosome length was measured with an eyepiece micrometer under a dissecting microscope.

Meanwhile, calculated respiration rate was estimated by the multiple-regression model proposed by Ikeda (Ikeda 1985), expressed as:

$$\text{Respiration}=0.524+0.8354 \ln Wc+0.0601 T \quad (4)$$

Where *Wc* is individual weight (mg C) and T is incubation temperature.

3.2.2 Survival, egg production and fecal pellet production rates

Plankton samples were collected using a plankton net (mouth diameter: 30 cm; mesh aperture: 180 μm) at Manazuru Port, Sagami Bay, Japan (Fig. 2-2) in April 2014. The samples were immediately transferred to a field laboratory. Live adult females of *A. steueri* were sorted from plankton samples into glass bottles containing filtered sea water (<0.22 μm) under a dissecting microscope (WILD M10, Leica). Sorted adult females were immediately used for incubation experiments. Incubation experiments were conducted to examine the production rate of eggs and fecal pellets. Four experimental conditions were designed with two different high-food concentration periods followed by two low-food concentration periods (Fig. 3-1). Adult females were first exposed to a higher-food concentration (1.0 μgC mL^{-1}; Berggreen et al. 1988) for 2 or 5 days and then transferred to two different low food concentrations: an extremely low-food concentration (0.09 μgC mL^{-1}) and filtered seawater (< 0.7 μm) without food. Adult females were put on the higher-food condition at the beginning of incubation to cancel the effect of condition given before. The low-food condition was set as the concentration required for the survival (Berggreen et al. 1988), and the starvation condition was as the negative control for the low-food condition. Ten females were reared for each condition. The periods of high food condition were set based on the fluctuation of the *in situ* spring bloom (Furuya et al. 1993). The adult females were kept individually in an incubation chamber with a 180 μm sieve 1 cm above the bottom,

which was immersed in a 200-mL beaker containing ca 150 near-ambient temperatures (14.5^0C) and natural light cycles (13L: 11D). The number of surviving females, eggs, and fecal pellets were counted every 24 h. The significant difference was determined by Kruskal-Wallis test. A difference with $p< 0.05$ was considered significant. The Kruskal-Wallis test assess for significant difference on a continuous dependent variable by a categorical independent variable with two or more groups.

3.3 Results

3.3.1 Respiration rate

Respiration rates of *A. steueri* raised under excess food condition were 0.29 ± 0.05 nLO_2 μgDW^{-1} h^{-1} at 15℃ and 1.08 ± 0.27 nLO_2 μgDW^{-1} h^{-1} at 20℃ (Fig. 3-2). Those rates of *A. steueri* raised under starvation condition were 0.09 ± 0.04 nLO_2 μgDW^{-1} h^{-1} at 15℃ and 0.80 ± 0.16 nLO_2 μgDW^{-1} h^{-1} at 20℃. There was a significant difference of respiration rate in *A. steueri* raised under the excess food condition at between 15℃ and 20℃ (*t*-test, $p<0.01$). There was a significant difference of respiration rate in *A. steueri* raised even under the starvation condition at between 15℃ and 20℃ (*t*-test, $p<0.01$). Incubation temperature affected remarkably the respiration rate of *A. steueri* raised under either food conditions. of ambient food conditions. There was a significant difference of respiration rate at 20℃ between *A. steueri* raised under excess food and starvation condition (*t*-test, $p<0.05$). There was a significant difference of respiration rate at 15℃ between *A. steueri* raised under excess food and starvation condition (*t*-test, $p<0.01$). Not only the incubation temperature but also the ambient food condition affected respiration rate of *A. steueri*. Food condition Calculated respiration rates by Ikeda's equation (4) were 4.10 nLO_2 μgDW^{-1} h^{-1} at 15℃ and 6.48 nLO_2 μgDW^{-1} h^{-1} at 20℃. The calculated respiration rate at 20℃ was about 6 times higher than the measured respiration rate of *A. steueri* raised under the starvation condition at 20℃. In addition, the calculated respiration rate at 15℃ was 14 times higher than the measured

respiration rate of *A. steueri* raised under the starvation condition at 15℃. The calculated respiration rate of *A. steueri* was likely to be overestimated according to the ambient food condition.

3.3.2 Survivorship

In all experimental conditions, 27.5% of the total females survived for 20 days through the last day of incubation (Fig. 3-4, Table 3-1; Hirahara et al. 2018). Even under the conditions B and D, one female and four females survived to the end of the experiments for 18 and 15 days under starvation, respectively. The average number of days of individuals survived even in conditions A and B, for which copepods were raised at a high-food concentration for 2 days and then a low-food concentration or no food for 18 days, were 14 ± 6.7 and 10.3 ± 6.4 days, respectively. These days were not significantly different (Kruskal-Wallis's test, $p>0.05$), and the total average of both conditions A and B were 11.6 ± 6.7 days. Without being affected by the shortage of food, *A. steueri* is able to maintain its metabolism even under an environment in which the food concentration largely varies for a certain period.

3.3.3 Survival, egg production and fecal pellet production rates

The average egg production rate (eggs female^{-1} day^{-1}) during the high-food periods of 5 days were significantly higher than during the high-food periods of 2 days among the four experimental conditions (Kruskal-Wallis's test, $p<0.05$). Because females at the high-food period for 5 days had a longer period of food accumulation than for 2 days, the average egg production rate during the high-food period for 5 days was higher than during the high-food period for 2 days. Thirty percent of the total females continued to produce eggs for 14 days under the starvation treatment (Fig. 3-3; Hirahara et al. 2018). The egg production of some females continued to increase under the low food or starvation periods under conditions A, C, and D. Under condition B, egg production did

not increase under the low-food period. Under condition B, the females may have had not enough energy allocated to egg production under starvation after the 2-day high-food period.

There was no significant difference in cumulative egg production (eggs female^{-1}) in the low-food period between condition A with the high-food period for 2 days and condition C with the high-food period for 5 days (Kruskal-Wallis's test, $p>0.05$, Fig. 3-5; Hirahara et al. 2018). This indicates that the cumulative egg production of both conditions A and C reached the same value for 15 days from the start of the low-food condition. Therefore, *A. steueri* are able to produce eggs when they have little food. On the other hand, there was a significant difference in cumulative egg production during the starvation period between condition B with the high-food period for 2 days and condition D with the high-food period for 5 days (Kruskal-Wallis's test, $p<0.05$). Under the starvation treatments, the period of exposure to the high-food concentration influenced cumulative egg production.

There was a significant difference in cumulative egg production between condition A with the low-food condition and condition B with the starvation condition (Kruskal-Wallis's test, $p<0.05$). On the other hand, there was no significant difference in cumulative egg production between condition C with the low-food condition and condition D with the starvation condition (Kruskal-Wallis's test, $p>0.05$). The cumulative egg production by the females raised under the high-food period for 5 days at the beginning of the experiment was not different between the low-food period and the starvation period on account of adequate energy accumulation for the 5 days. On the other hand, the cumulative egg production by females raised under the high-food period for 2 days was different between the low-food period and the starvation period on account of the scarce energy accumulation for the 2 days.

There was no significant difference in cumulative fecal pellet production (pellets female^{-1}) in the low-food period between condition A with the high-food period for 2 days and condition C with the high-food period for 5 days (Kruskal-Wallis's test, $p>0.05$, Fig. 3-7; Hirahara

et al. 2018). This indicates that the cumulative fecal pellet production of both conditions A and C reached the same value for 15 days from the start of the low-food condition. Therefore, *A. steueri* are able to produce fecal pellets when they have a little food. On the other hand, there was a significant difference in cumulative fecal pellet production in the starvation period between condition B with the high-food period for 2 days and condition D with the high-food period for 5 days (Kruskal-Wallis's test, $p<0.05$, Fig. 3-7; Hirahara et al. 2018). Under starvation, the cumulative fecal pellet production was influenced by the high-food periods at the beginning of the experiment.

There was no significant difference in cumulative fecal pellet production between condition A with the low-food condition and condition B with the starvation condition (Kruskal-Wallis's test, $p>0.05$, Fig. 3-7; Hirahara et al. 2018). Similarly, there was no significant difference in cumulative fecal pellet production between condition C with the low-food condition and condition D with the starvation condition (Kruskal-Wallis's test, $p>0.05$). The fecal pellet production was not influenced by whether food was present.

The amount of eggs and fecal pellets under the starvation period differed depending on the length of the high-food period, 2 versus 5 days. From these results, the energy accumulation in the high-food period contributed to the egg production and fecal pellet production during the starvation period.

Fig. 3-8 and Fig. 3-9 illustrated the relationship between the cumulative eggs and fecal pellets produced by one female raised on conditions B and D with the starvation condition (Hirahara et al. 2018). The females raised at condition B produced both eggs and fecal pellets in the high-food period for 2 days but then did not produce eggs or fecal pellets, under the starvation condition. On the other hand, the females raised under condition D produced both eggs and fecal pellets even under the starvation condition. The difference in egg production between conditions B and D was considered to be caused by the 3-day difference in the high-food period. The females

under condition B may not have stored sufficient energy during the high-food period to produce both eggs and fecal pellets under starvation. On the other hand, the females under condition D had adequate energy to produce both eggs and fecal pellets under starvation.

3.4 Discussion

The respiration rate of *A. steueri* raised at the high food condition was about twice higher than that of *A. steueri* raised at the starvation condition. These results suggested that *A. steueri* might regulate their metabolism when the food concentration is low at the environment. Ikeda (1970) summarized that there was a significant difference among the respiration rate-dry weight relationships for tropical, temperate, and boreal species taken from the tropical Pacific, the temperate Pacific southeast of Hokkaido, and the Bering Sea, respectively (Fig. 3-10). These results in which metabolism is expressed per weight of organism, clearly show that the smallest organisms have the highest metabolic rate and additionally, there are geographic differences which reflect an environmental difference in basic metabolic processes of organisms from different areas. As the respiration rates of *A. steueri* under not only starvation condition but also the high food condition were relatively low compared to the other temperate copepod species and similar to subarctic species even though *A. steueri* inhabits temperate area, *A. steueri* might usually maintain the low metabolism in the environment.

Previous study reported that respiration rate of *A. tonsa* at 18 ℃ which was measured by the electron oxygen method was 8.24 nLO_2 μgDW^{-1} h^{-1} (Kiørboe et al. 1985) and about eight times higher than that of *A. steueri* at 20°C which was measured by the fiber-optic oxygen meter. In addition, calculated respiration rates by Ikeda's equation (3) were 6-14 times higher than the respiration rates of *A. steueri* measured by the fiber-optic oxygen meter. The electron oxygen method involves sensitive and tedious procedures and require a very large number of organisms for measuring (~200 inds. in each experiment) over a study (Castellani et al. 2005). Therefore, the

respiration rate of *A. tonsa* measured by the electron oxygen method might be included not only the basic metabolism but also activity metabolism while the respiration rate of *A. steueri* measured by the fiber-optic oxygen meter might be included only the basic metabolism. Ikeda (1974) measured the respiration rate in a total of 112 species including 50 species for tropical (25.7-28.5°C), 13 species for subtropical (17.3-22.5°C), 26 species for temperate (11.7-17.5°C) and 27 species for boreal species (4.5-14.3°C) (Fig. 3-10). The respiration rates of *A. steueri* under both the high food and starvation conditions departed from the regression line of temperate species and rather closed to the regression line of boreal species. It was suggested that metabolism of *A. steueri* was lower than other temperate zooplankton, regardless of ambient food conditions.

Of the total females in all the experimental conditions, 27.5% survived for 20 days until the end of the experiment. Thirty percent of the females cultured under the starvation condition continued to produce eggs for 14 days. *A. steueri* showed a remarkable starvation tolerance to the abrupt decrease in food concentration, differing from other embayment species.

The neritic copepods *Paracalanus parvus* and *Acartia tonsa*, which had been raised under adequate food conditions beforehand, ceased their egg production on day 3 and days 1-4 after exposure to starvation conditions, respectively (Table 3-2; Dagg 1977, Parrish & Wilson 1978, Checkley 1980, Finiguerra et al. 2013). Then, all females of *P. parvus* and *A. tonsa* died on day 7 and days 5-10 of the starvation treatment. *Centropages typicus* survived under starvation for 3 to 6 days (Dagg 1977, Kiørboe et al. 1985). The egg production of *Calanus finmarchicus* ceased for 2 days during starvation period and then was resumed for 2 days after the onset of feeding (Hirche 1990). Because the individuals of these species survived several days into the starvation treatment, it is assumed that these species accumulate energy under the adequate food condition and utilize it for metabolism under the starvation condition without exhausting the stored energy immediately. The remaining energy within their bodies was used only for metabolism for survival (Finiguerra et al. 2013).

The biological responses of *A. steueri* under decrease in food or starvation were different from those of other embayment copepods, such as *P. parvus* and *A. tonsa*. *A. steueri* continued to produce eggs for more than 14 days under the starvation period and survived for more than 18 days. It seemed that result of the cumulative eggs is categorized to two groups, showing high and low egg productions (Fig. 3-5; A, C, and D). Such difference in each experimental condition may be caused by the age structure, that is, duration within adult stage. *A. steueri* individuals raised under a high food concentration for 5 days produced more eggs than those raised under the high food concentration for 2 days even after exposure to low food concentrations or starvation. In addition, from the relationship between the cumulative eggs and fecal pellets (Fig. 3-8, Fig. 3-9), the egg production in females raised at the high food concentration for 5 days increased constantly with the fecal pellet production. In the field, by accumulating energy during short periods of sporadic, high food condition, *A. steueri* would be able to endure the subsequent low food condition until they encounter favorable food conditions again.

Storage lipids play an important role in the life history of many copepods. Storage lipids provide energy for reproduction, food scarcity, ontogeny and diapause (Lee et al. 2006). Mayzaud (1976) proposed two copepod groups in terms of storage lipids that were distinguished by the amount of storage lipids and starvation tolerance. Species of the first group accumulate large lipid stores to survive long starvation periods, whereas species of the second group store little or no lipids and can withstand only short periods of starvation.

Lipid-rich species, which live in high latitude areas, have larval forms with large lipid stores to withstand long starvation periods (Lee et al. 1974, Saito & Tsuda 2000). For example, *C. glacialis* and *N. cristatus*, for which lipids accounted for 56% and 69% of the dry mass, live for 270 days and more than 1 year, respectively (Saito & Kotani 2000, Saito & Tsuda 2000). On the other hand, lipid-poor species, e.g., *Acartia tonsa* and *Paracalanus parvus*, live in inshore and estuarine areas. These species grow rapidly during high food periods, but excess food is not

converted into large lipid stores. They are characterized by low amounts of storage lipids and generally cannot withstand more than a few days of starvation (Uye 1981, Gardner & Paffenhöfer 1982). For example, *A. clausi* and *A. tonsa*, for which lipid accounted for 3% and 16-19% of the dry mass, lived for 3-6 days and 6-10 days, respectively. Surprisingly, dry weight of *A. steueri* was more than about 10 times higher than the other *Acartia* copepods, suggesting that *A. steueri* have a function to accumulate large energy reserves in their body even though the genus *Acartia*. In the present study, the carbon weight of egg production under the starvation condition was about 20% of the carbon weight of female body. *A. steueri* might accumulate energy reserves promptly during short periods of high food condition, and then they would be able to utilize their energy reserves for metabolism and egg production at the subsequent low food condition until they encounter favorable food conditions again.

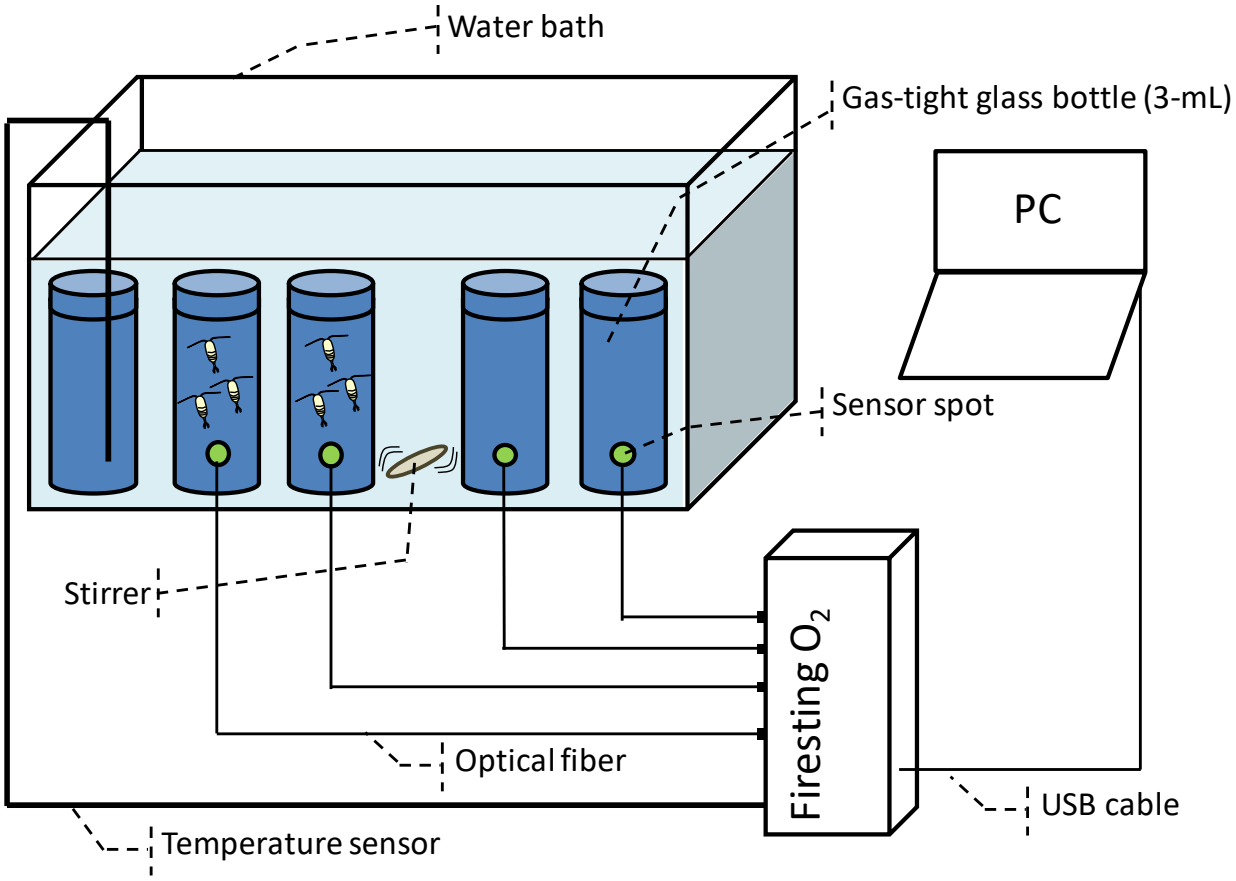


Fig. 3-1 Measurement scheme of respiration rate using fiber-optic oxygen meter followed by Liu & Ban (2016)

Table 3-1 Survivorship of *Acartia steueri* (Hirahara et al. 2018).

Experimental conditions※1	Number of experimental individuals	Average of survival days of individuals (± SD)	Number of survival individuals (inds.)※2
A	10	14 (± 6.7)	3
B	10	10.3 (± 6.4)	1
C	10	12 (± 7.0)	3
D	10	15.9 (± 5.2)	4

※1 See Fig. 3-2

※2 Number of survival individuals at the last day of incubation

Table 3-2 Periods of starvation tolerance and egg production of neritic copepods during starvation (Hirahara & Toda 2018). Periods of starvation tolerance of *A. steueri* mean survival days during the starvation periods after high food conditions for 2 or 5 days. Periods of egg production of *A. steueri* during starvation mean duration of egg production under the starvation periods after high food conditions for 2 or 5 days. nd:no data.

Species	Experimental temperature (°C)	Periods of starvation tolerance (day)	Periods of egg production under starvation (day)	References
Acartia clausi	5	6	nd	Mayzaud 1976
A. tonsa	15	10	nd	Dagg 1977
	18	5	1-4	Parrish & Wilson 1978
	15	8	nd	Finiguerra et al. 2013
Centropages typicus	15	6	nd	Dagg 1977
Paracalanus parvus	18	7	3	Checkley 1980
Temora longicornis	15	4	nd	Breteler & Koski 2003
A. steueri	14	>18	15	This study

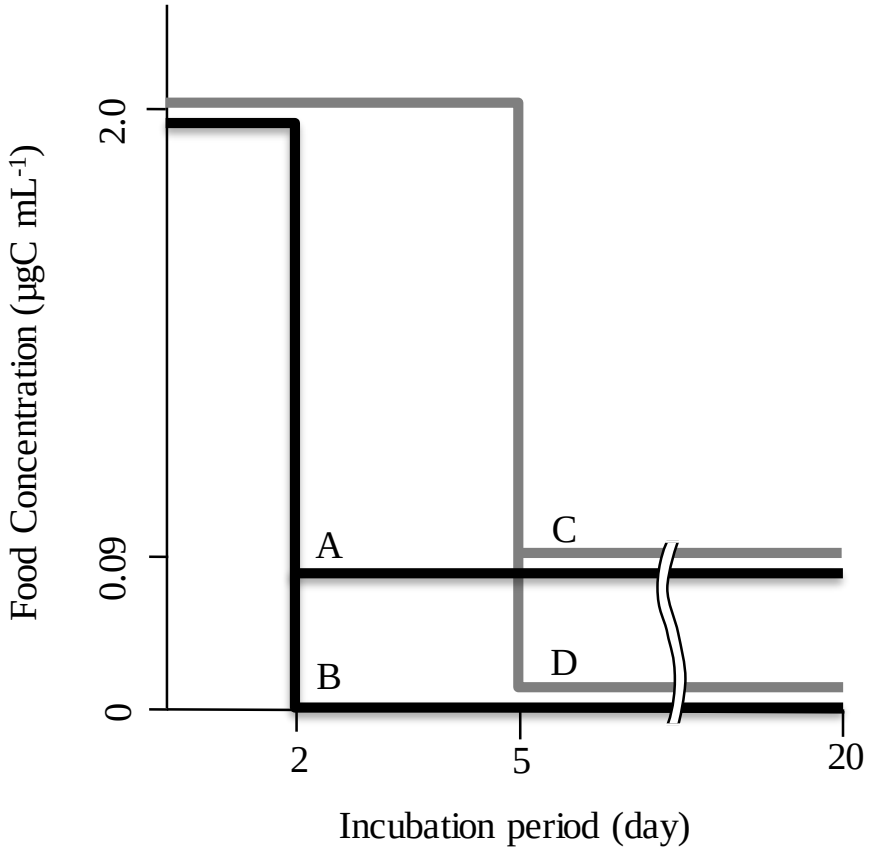


Fig. 3-2 Incubation conditions of food concentrations. Females were exposed to higher food condition (1.0 µgC mL^{-1}) for 2 or 5 days, and transferred to two different food conditions: the low food concentration (0.09 µgC mL^{-1}) and filtered seawater.

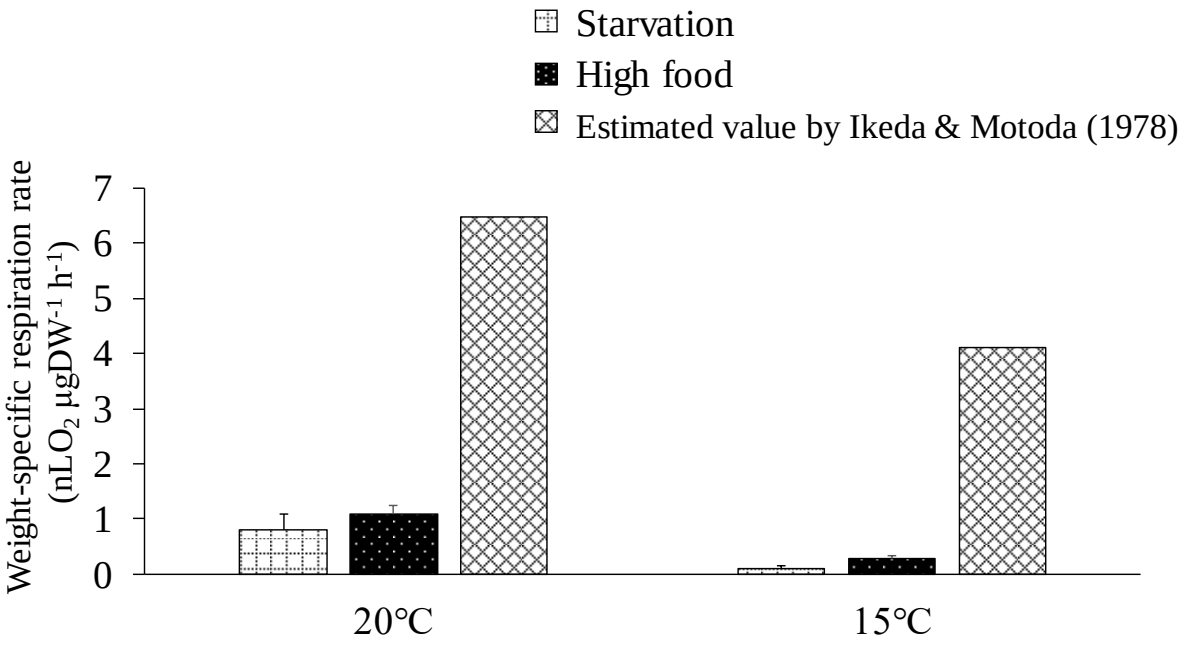


Fig. 3-3 Weight-specific respiration rates at 20℃ and 15℃ of *Acartia steueri* raised under the high food (left bars) and starvation conditions (medium bars). Right bars indicate estimated values of respiration rates at 20℃ and 15℃ by Ikeda & Motoda (1978).

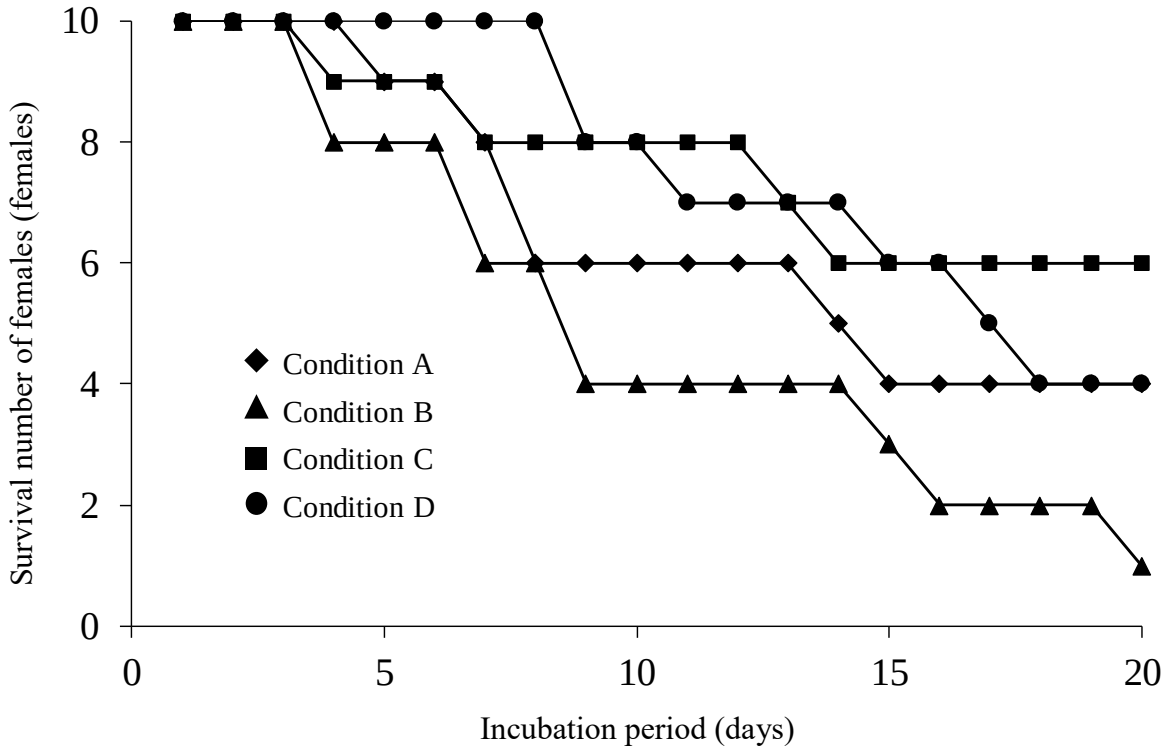


Fig. 3-4 Survival number of females in April 2014 raised at each condition (A～D).

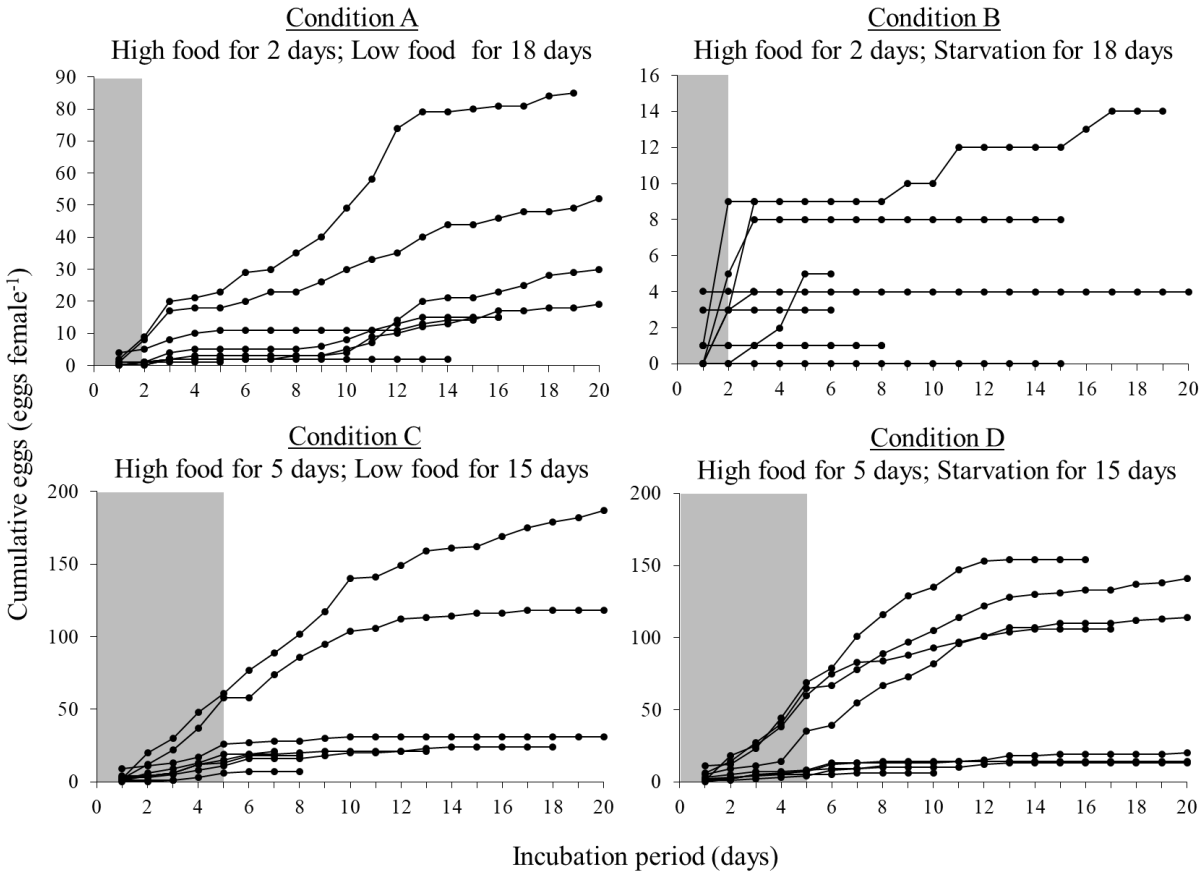


Fig. 3-5 Cumulative egg production rate raised at each condition; condition A: high food for 2 days consecutively transferred to the low food concentration, condition B: High food for 2 days consecutively transferred to the starvation, condition C: high food for 5 days consecutively transferred to the low food concentration, condition D: high food for 5 days consecutively transferred to the starvation. Gray zone indicates the high food duration (2 or 5 days, Hirahara et al. 2018).

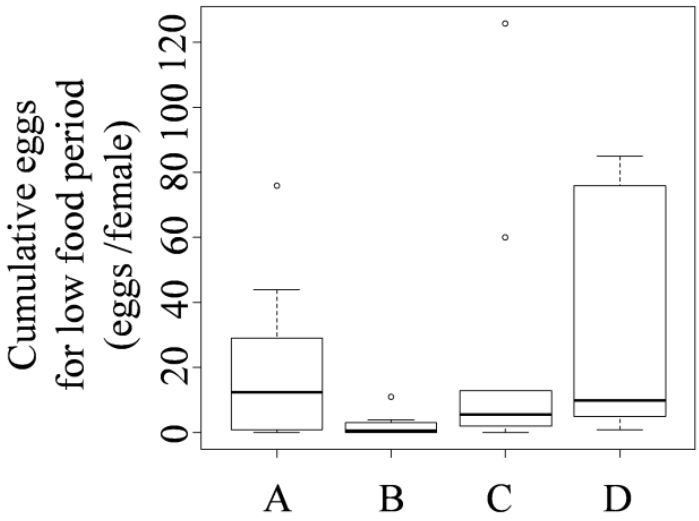


Fig. 3-6 Box plot of cumulative eggs for low food period at each condition; condition A: high food for 2 days consecutively transferred to the low food concentration, condition B: High food for 2 days consecutively transferred to the starvation, condition C: high food for 5 days consecutively transferred to the low food concentration, condition D: high food for 5 days consecutively transferred to the starvation. Open circles indicate the outlier transferred to the starvation (modified Hirahara et al. 2018).

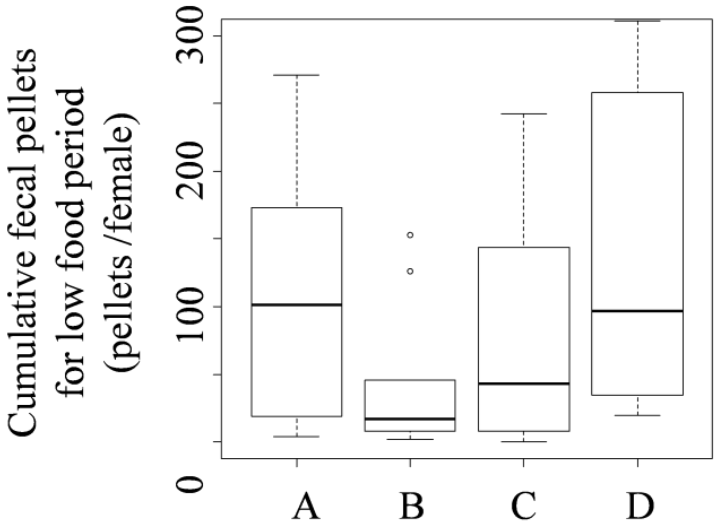


Fig. 3-7 Box plot of cumulative fecal pellets for low food period at each condition; condition A: high food for 2 days consecutively transferred to the low food concentration, condition B: High food for 2 days consecutively transferred to the starvation, condition C: high food for 5 days consecutively transferred to the low food concentration, condition D: high food for 5 days consecutively transferred to the starvation. Open circles indicate the outlier transferred to the starvation (modified Hirahara et al. 2018).

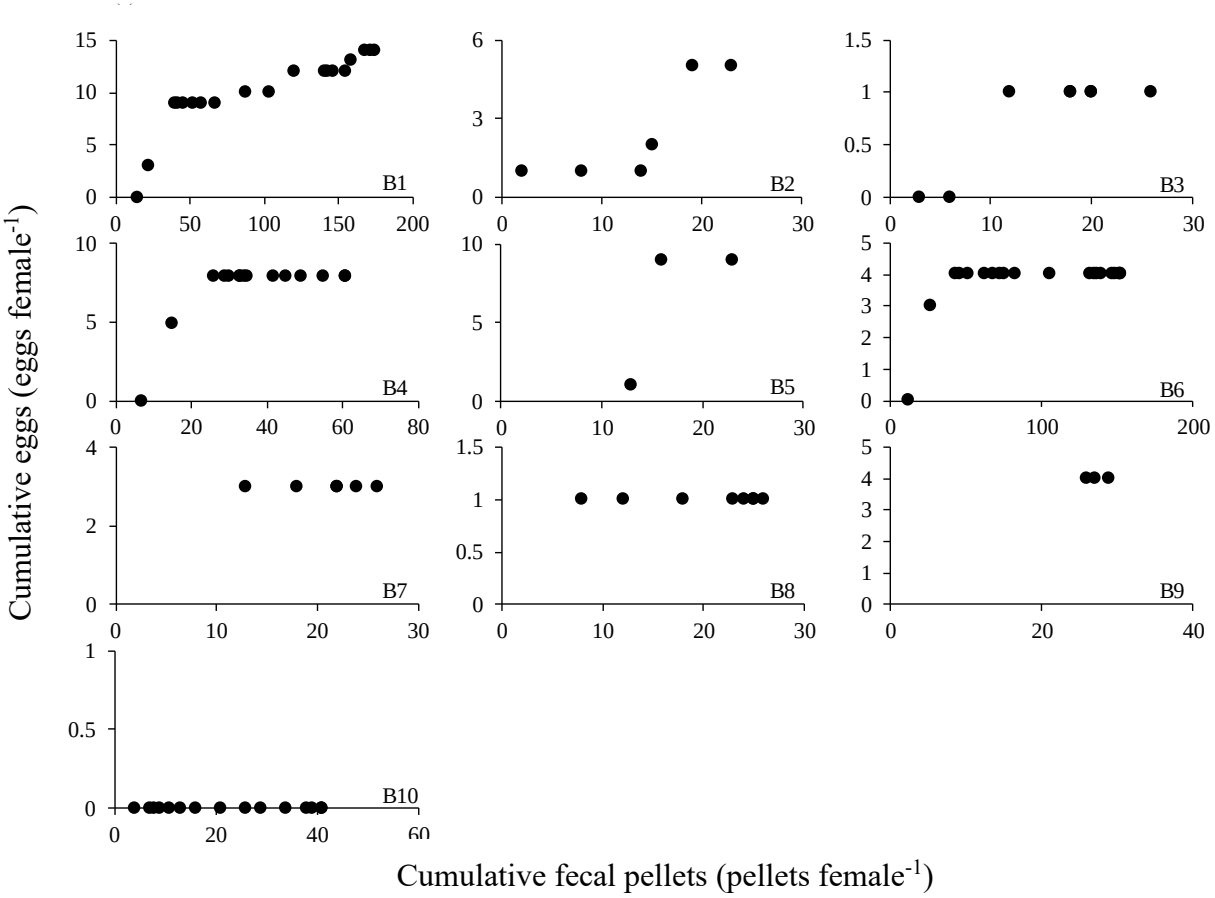


Fig. 3-8 Relationship between cumulative eggs and cumulative fecal pellets of females raised at condition B: High food condition for 2 days consecutively transferred to the starvation condition (modified Hirahara et al. 2018).

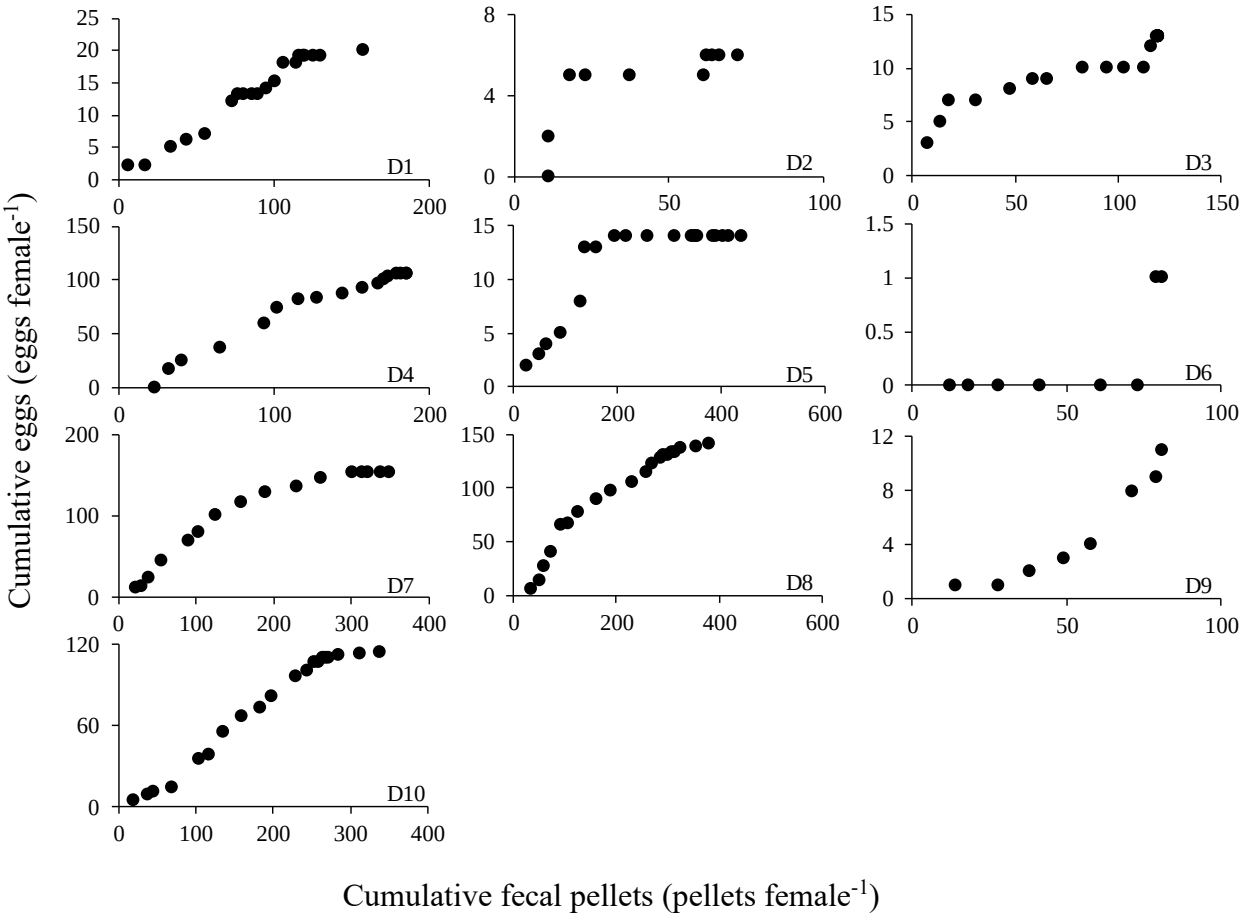


Fig. 3-9 Relationship between cumulative eggs and cumulative fecal pellets of females raised at condition D: high food for 5 days consecutively transferred to the starvation (modified Hirahara et al. 2018).

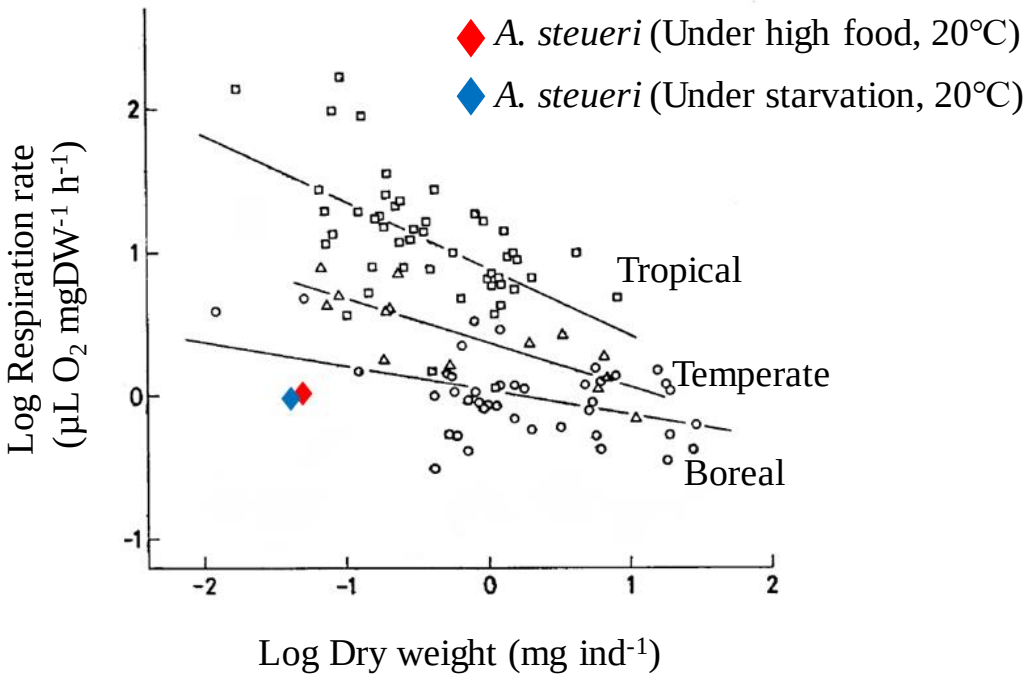


Fig. 3-10 Relationship for boreal, temperate and tropical zooplankton between log weight specific respiration and log dry weight (Ikeda 1970). Diamond symbols of red and blue indicate respiration rate of *Acartia steueri* raised under the high food and starvation conditions, respectively at 20°C

Chapter Ⅳ

General discussion

4.1 Energy flow of *Acartia steueri* under starvation condition

The energy flow obtained by feeding in common adult female neritic copepods were considered by Ikeda & Motoda (1978). After feeding, adult female eliminates 30% of the feed energy as fecal pellets and assimilates 70% of the feed energy into their body, which is finally distributed to 30% and 40% for metabolism (for maintaining their life activity) and egg production, respectively (Fig. 1-2). However, these copepods immediately ceased egg production under starvation conditions after exposure to an adequate food condition; all copepods died after a few days of starvation (Parrish & Wilson 1978). On the other hand, *Acartia steueri* accumulates energy reserves quickly when in high food conditions for short periods. This ensure that they have adequate energy resources for longevity and sustained reproductive capacity in sporadic low food conditions. The rapid accumulation of energy sources within their body might be redounded by the accumulation of not only lipids, but also amino acids, the biosynthesis from amino acids to fatty acids, and low metabolism under not only starvation conditions but also high food conditions (Fig. 4-1).

The energy flows of *Acartia tonsa* were illustrated in Fig. 4-2 and Fig. 4-3 based on the data provided by Kiørboe et al. (1985). In the energy flow of *A. tonsa* raised under high food concentration conditions (5.5×10^5 ngC ind.$^{-1}$ d^{-1}) for 4 d (Fig. 4-2), the feeding rate was 4.3×10^3 ngC ind.$^{-1}$ d^{-1}, and the assimilation rate, which was the total rate of metabolism, egg production, and accumulation, was 2.2×10^3 ngC ind.$^{-1}$ d^{-1}. The rates of metabolic and egg production

attributed from the assimilation rate were 5.3×10^2 ngC ind.$^{-1}$ d^{-1} and 1.7×10^3 ngC ind.$^{-1}$ d^{-1}. In this study, the rates of accumulation and fecal pellet production were not measured, so they were expediently expressed as α_j and n.d. in this energy flow, respectively. In the energy flow of *A. tonsa* raised under starvation conditions for 4 days after exposure to high food conditions for 3 days (Fig. 4-3), the rates of metabolism and egg production were 1.1×10^2 ngC ind.$^{-1}$ d^{-1} and 0 ngC ind.$^{-1}$ d^{-1}, respectively. The metabolic rate of *A. tonsa* raised under starvation conditions decreased to one-fifth of the metabolic rate of *A. tonsa* raised under high food conditions. The energy requirement for metabolism might be distributed by the α_j of the accumulation rate within their body. *A. tonsa* died between day 6 to day 10 of starvation condition with exhausting the energy reserves.

Energy flows of *A. steueri* in the present study were illustrated in Fig. 4-4 and Fig. 4-5. In the energy flow of *A. steueri* raised under high food concentration conditions (1.5×10^5 ngC ind.$^{-1}$ d^{-1}) for 5 days (Fig. 4-4), assimilation rate which was the total rate of metabolism, egg production, fecal pellet production, and accumulation was 1.3×10^3 ngC ind.$^{-1}$ d^{-1}. The metabolic rate provided by the study of respiration rate in Chapter 3 was 1.4×10^2 ngC ind.$^{-1}$ d^{-1}. Rates of egg production and fecal pellet production were provided by the carbon weights of an egg in *A. tonsa* (45.7 ngC egg-1; Kiørboe et al. 1985) and a fecal pellet in *A. omorii* (8.5 ngC pellet^{-1}; Juanita et al. 1998; Uye & Kaname 1994) and the numbers of eggs and fecal pellet production derived from the incubation experiment in Chapter III of the present study. The rates of egg production and fecal pellet production were 2.4×10^2 ngC ind.$^{-1}$ d^{-1} and 1.3×10^2 ngC ind.$^{-1}$ d^{-1}, respectively. Accumulation rate derived from the total amount of biochemical compositions ($A_{biochem}$) of *A. steueri* in April 2018 raised under the high food condition in Chapter II was 7.6×10^2 ngC ind.$^{-1}$ d^{-1} based on the following formula (5) of carbon conversion (National Agriculture and Food Organization, Data base; http://www.naro.affrc.go.jp/org/nkk/soshiki/soshiki07-shigen/01shigen/pdf/sekkeitohyouka/huzoku-1.pdf).

$$A_{biochem}\,(ngC\ ind.^{-1}\ d^{-1}) = (Carbo.\times 0.435 + Protein\times 0.53 + Lipid\times 0.77)\times 1000 \quad (5)$$

Carbo. (μg ind.$^{-1}$ day^{-1}) indicated the accumulation rate of carbohydrate, protein (μg ind.$^{-1}$ day^{-1}) indicated the total accumulation rate of protein and free amino acids, and lipid indicated the accumulation rate of lipid in *A. steueri* raised under the high food condition for 5 days. Additionally, accumulation rate derived from the dry weight (A_{dw}) of *A. steueri* in 8th April 2018 raised under the high food condition for 10 days was 8.1 × 10^2 ngC ind.$^{-1}$ d^{-1} based on the following formula (6).

$$A_{dw}\,(ngC\ ind.^{-1}\ d^{-1}) = (DW_{day10} - DW_{insitu}) / (T_i \times CF) \times R_C / (R_C + R_N) \times 1000 \quad (6)$$

DW_{day10} and DW_{insitu} indicated the dry weight (μg ind.$^{-1}$) of *A. steueri* in April 2018 raised under the high food condition for 10 days and that of *in situ A. steueri*. T_i indicated the incubation time (day) and CF indicated the conversion factor obtained from Chapter II in the present study. R_C and R_N indicated the ratio of carbon and nitrogen in C /N ratio, respectively. In the Fig. 4-4, the accumulation rate derived from the total amount of biochemical compositions ($A_{biochem}$) was adopted. The metabolic rate of *A. steueri* raised under high food conditions was one-fourth of the metabolic rate of *A. tonsa* raised under high food conditions. In the energy flow of *A. steueri* raised under starvation conditions for 15 days after being exposed to high food conditions for 5 days (Fig. 4-5), the rates of metabolism, egg production, and fecal pellet production were 4.1 × 10^1 ngC ind.$^{-1}$ d^{-1}, 9.7 × 10^1 ngC ind.$^{-1}$ d^{-1}, and 8.2 × 10^1 ngC ind.$^{-1}$ d^{-1}, respectively. The accumulation rate, which was the total rate of the metabolism, egg production, and the fecal pellet production, was 2.2 × 10^2 ngC ind.$^{-1}$ d^{-1}. The metabolic rate of *A. steueri* raised under starvation conditions decreased by about one-fourth of the metabolic rate of *A. steueri* raised under high food conditions. In addition, the metabolic rate of *A. steueri* raised under starvation conditions was about one-third of the metabolic rate of *A. tonsa* raised under starvation conditions. The metabolic rates of *A. steueri* under not only starvation conditions, but also high food conditions, were remarkably lower than those of other temperate zooplankton, including *A. tonsa*, suggesting that this species might

be able to accumulate energy promptly. Thanks to the function of prompt energy accumulation, *A. steueri* might be able to ensure both sustained reproductive and metabolism capacities under starvation conditions. Increased amount of carbon within the body of *A. steueri* per a day was estimated as 756 ngC ind.$^{-1}$ d^{-1} from the results of biosynthesis compositions of *A. steueri* under the high food condition in Chapter 2 (if 43.5%, 53% and 77% of carbohydrate, protein and lipid are assumed to be carbon weights, respectively; http://www.naro.affrc.go.jp/). Eventually, the total increased amount of carbon within their body during the high food period for 5 days was calculated as 3780 ngC ind.$^{-1}$. Consumed amount of carbon by *A. steueri* per a day under starvation was estimated as 220 ngC ind.$^{-1}$ d^{-1} from Fig. 4-5 based on the results of respiration rate, egg production rate, and fecal pellet production rate during the starvation period for 15 days in Chapter 3. From these results, *A. steueri* raised at the high food condition for 5 days might exhaust the accumulated energy for 17.1 days during the starvation period. In other words, the 17.1 days was the estimated survival days of *A. steueri* under starvation, and the estimated survival days under starvation was close to the measured survival days under starvation as about 18 days in Chapter III.

The dry weight of *A. steueri* in the present study was extremely higher than those of other embayment copepod. Besides, the dry weight of *A. steueri* raised under high food conditions for 10 days was significantly higher than that of *A. steueri in situ*. The weight of *A. steueri* dried under the conventional drying condition at 60ºC for 24 h might include the bound water which starts to evaporate more than 100ºC. The bound water covers the surface of protein and connects with protein by the physical bonding force in organisms so that the it needs to be dried up under the higher temperature. Other copepods might have the bound water in their body because protein can not work without the bound water. In the future study, re-evaluation of drying condition such as drying temperature and time should be conducted even in other copepods. The amounts of lipids and proteins in females raised under high food conditions were significantly higher than those in females under starvation conditions, respectively. On the other hand, the amount of free amino

acids in *A. steueri* under starvation conditions increased compared to those under *in situ* and high food conditions. It was considered that *A. steueri* accumulate within their body do not accumulate pure lipids, which consist of carbon mainly, but do accumulate some composite substances including lipids. Various polar euphausiids and subpolar copepods appear to utilize phosphatidylcholine, which is a phospholipid, as a storage lipid (Mayzaud 1976; Hagen 1988; Hagen et al. 1996;). Phosphatidylcholine is the major lipid in high-density lipoproteins found in arthropod haemolymph (Lee & Puppione 1988; Lee 1991; Walker et al. 2003). In addition, zooplankton eggs have yolk with lipovitellin, which is a lipoprotein with approximately equal amounts of amino acids and fatty acids. These lipoproteins play an important role in transporting lipids from different tissues, e.g. hepatopancreas/liver to muscle. Some euphausiids may store phosphatidylcholine in lipoproteins within storage tissue (Lee et al. 1991).

Lipids represent the most efficient energy stores because they contain about double the calorific density of proteins or carbohydrates, thus providing a compact source of energy-rich food for higher trophic level organisms. General characteristics of fatty acid biosynthesis have been described extensively in a number of reviews (e.g. Kattner & Hagen 1995; Vance & Vance 1996; Dalsgaard et al. 2003). Fishes, birds, and mammals have the greatest restrictions on fatty acid biosynthesis, and follow the typical animal pattern. Under starvation, some marine invertebrates utilize lipids as an energy source, and other animals metabolize amino acids, including free amino acids and protein (Mayzaud 1976, Hellan et al. 2003). Marine invertebrates generally have very low-carbohydrate diets; some carbohydrates can be converted from free amino acids to enter the usual fatty acid biosynthetic pathways under saturated food conditions (Helland et al. 2003, Iverson 2009). In terms of *Acartia* copepods with small amounts of lipid reserves, amino acids, and protein will be metabolised during the starvation periods to maintain the population. In the present thesis, the carbon weight of *A. steueri* under starvation conditions was significantly lower than that in females under high food conditions (Fig. 2-5; Welch's test, $p < 0.05$), while they

continued to maintain their metabolism and egg production under starvation conditions. Therefore, *A. steueri* might use carbon as an energy source and metabolize it during the beginning of the starvation period (Mayzaud 1976). Meanwhile, the nitrogen weight of females raised under starvation conditions was significantly lower than that of females under high food conditions, while there was no significant difference in the C/N ratio between females raised under high food and starvation conditions (Fig. 2-7). The amounts of lipids and protein in females raised under starvation conditions were significantly lower than those in females under high food conditions (Fig. 2-12). On the other hand, the amount of free amino acids in *A. steueri* under starvation conditions was relatively increased compared to those under *in situ* and high food conditions. Ikeda (1974) and Mayzaud (1976) suggested that *Acartia* species may utilize amino acids, which consist mainly of nitrogen, as an energy source during the latter period of starvation. In the present study, not only lipids, but also amino acids, might be important energy sources for maintaining the metabolism of *A. steueri* under starvation conditions.

A. steueri might have the function to biosynthesized under adequate food conditions, utilizing amino acids effectively and accumulating composite high-density substances such as lipoprotein consisting of free amino acids, protein, and phospholipids. Thus, the dry weight of *A. steueri* was thought to be extremely high, although the carbon weight of *A. steueri* was not high. In Chapter II, oil droplets, such as in the oil sac well-known in overwintering oceanic copepods, were observed in the body cavity of *A. steueri* raised under high food conditions. *A. steueri* may accumulate lipoproteins with approximately equal amounts of protein and lipids as these oil droplets. In a future study, the lipoprotein should be analysed to reveal the composition of the oil droplets.

4.2 Potential of embayment copepods as stabilization function in marine ecosystem

I summarized the characteristics of life strategy of *A. steueri* compared to those of well-studied oceanic species (Fig. 4-6). In polar and subpolar oceanic regions, phytoplankton production is limited by extreme environmental conditions, such as water temperature, day length, available light, and water column stability (Table 6; Arístegui et al. 1997, Marañón et al. 2000, Bode et al. 2001, Mochizuki et al. 2002, Saito et al. 2002, Ohashi et al. 2011). Massive phytoplankton blooms occur in spring, and the phytoplankton production in other seasons is relatively low (Fiala et al. 1998). Many herbivorous copepods in the polar and subpolar regions biosynthesize and accumulate lipids by feeding on large amounts of spring phytoplankton bloom, mainly composed of protein, from early spring to summer (Kattner & Hagen 1995, Hagen & Auel 2001). Thereafter, the copepods have to survive and reproduce for a period of six months to one year under food scarce conditions, consuming their storage lipids (Norrbin et al. 1990, Hagen & Auel 2001).

The amounts of phytoplankton in embayments are larger than in the polar and subpolar regions. However, the environment in embayments is easily affected by an inflow of freshwater, human activity, rainfall, tide, and submarine topography. For these reasons, the amount of chlorophyll *a* in embayments often decreases abruptly for a short term; subsequently, extreme low food conditions continue for a few days to a few weeks (e.g. Onoue et al. 2006, Vidal et al. 2017). Even if the period of extremely low food is short, it might exert a fatal impact on maintaining the population of embayment copepods because the life span of these copepods, which is from a few weeks to a few months, is shorter than that of polar and subpolar oceanic and mid-latitude coastal species. The embayment copepod *A. steueri* might have a peculiar life strategy to low food conditions or starvation and the unique ability to reserve energy in embayments, which differ from those of copepods in the polar and subpolar oceanic regions and the mid-latitude coastal region. The rate of dry weight gain in oceanic copepod *Calanus glacialis* raised under high food conditions

was 2.2 μg day^{-1}, which is equal to 0.3% of the body dry weight (Hirche & Kattner 1993). On the other hand, the rate of dry weight gain in *A. steueri* was 4.6 μg day^{-1}, which is equal to 8% of the body dry weight; the rate of energy accumulation in *A. steueri* was extremely rapid compared to that in a lipid storing species. *A. steueri* can accumulate free amino acids at a high rate for a short period of high food concentration, in addition to lipid and protein. Ambient phytoplankton have been reported to have high contents of free amino acids (Raymont 1963, Fyhn et al. 1993). Free amino acids might be utilized by *A. steueri* as shock absorbers for fluctuating food conditions in embayments. This may explain the starvation tolerance of *A. steueri* in contrast to other neritic copepods. In the wild, by accumulating energy during short periods of sporadic, high food concentration, *A. steueri* would be able to endure low food conditions until they encounter favourable food conditions again. These survival strategies may allow *A. steueri* to maintain their population even when food is scarce, and stably transfer energy to higher trophic levels. Thanks to this survival strategy, *A. steueri* maintain their extremely high abundance in embayments and are expanding their habitat from high latitude to low latitude areas.

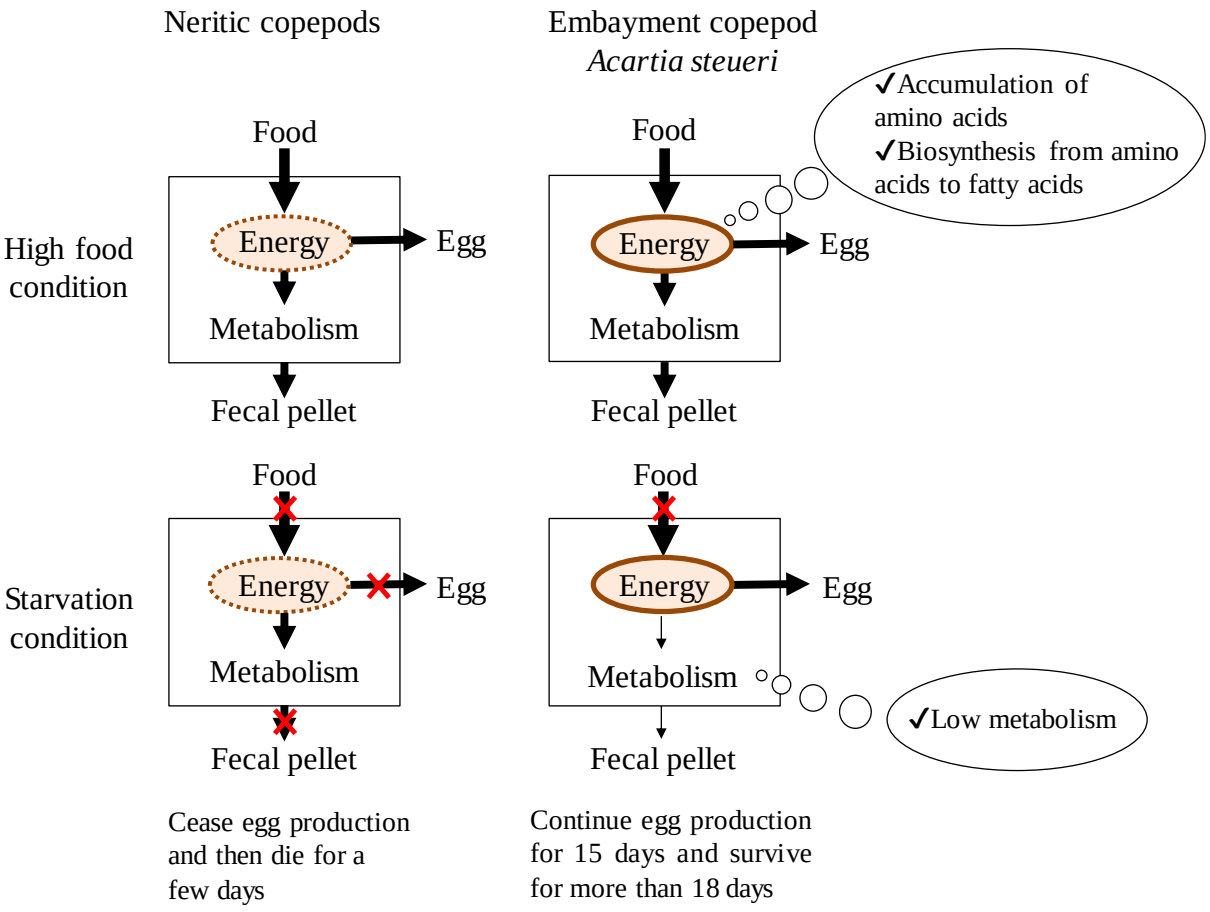


Fig. 4-1 Energy flow of *Acartia steueri* (left figures) and neritic copepods (light figures) at adequate food condition (upper figures) and starvation (below figures), respectively.

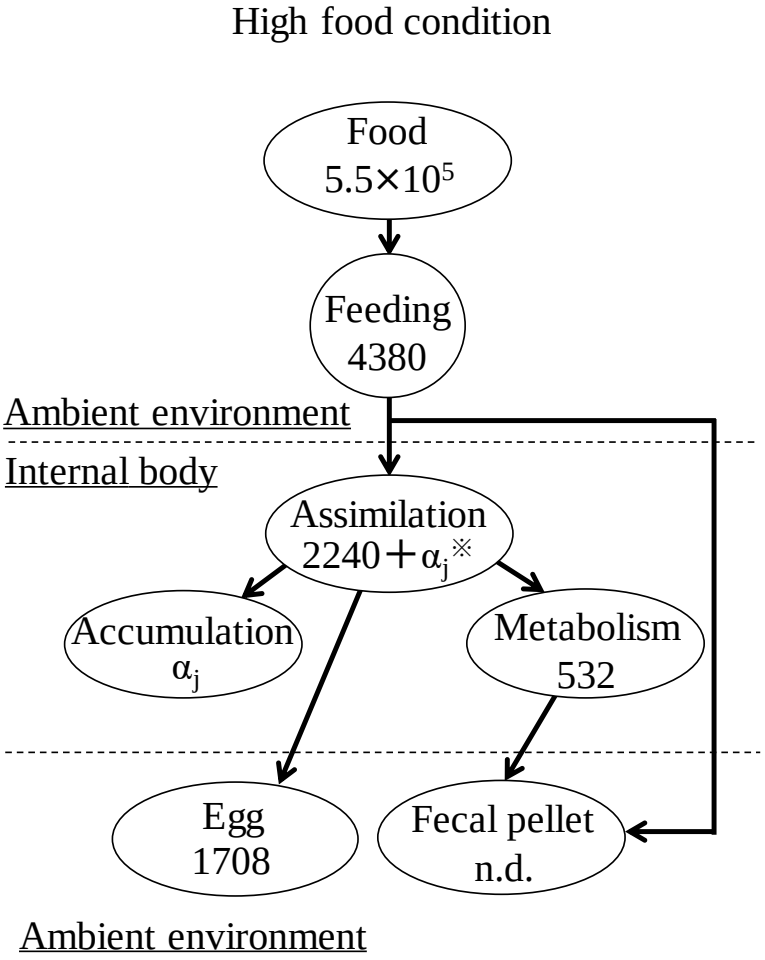


Fig. 4-2 Energy flow (ngC ind.$^{-1}$ d^{-1}) of *Acartia tonsa* raised under high food condition (5.5×10^5 ngC ind.$^{-1}$ d^{-1}) for 4 days (derived by Kiørboe et al. 1985). Incubation temperature was 18 ℃. n.d.: no data. The rates of accumulation and fecal pellet production were not measured so that these rates were expediently expressed as αj and n.d. in this energy flow. ※The amount of assimilation is the total amount of metabolism, egg, fecal pellet, and accumulation.

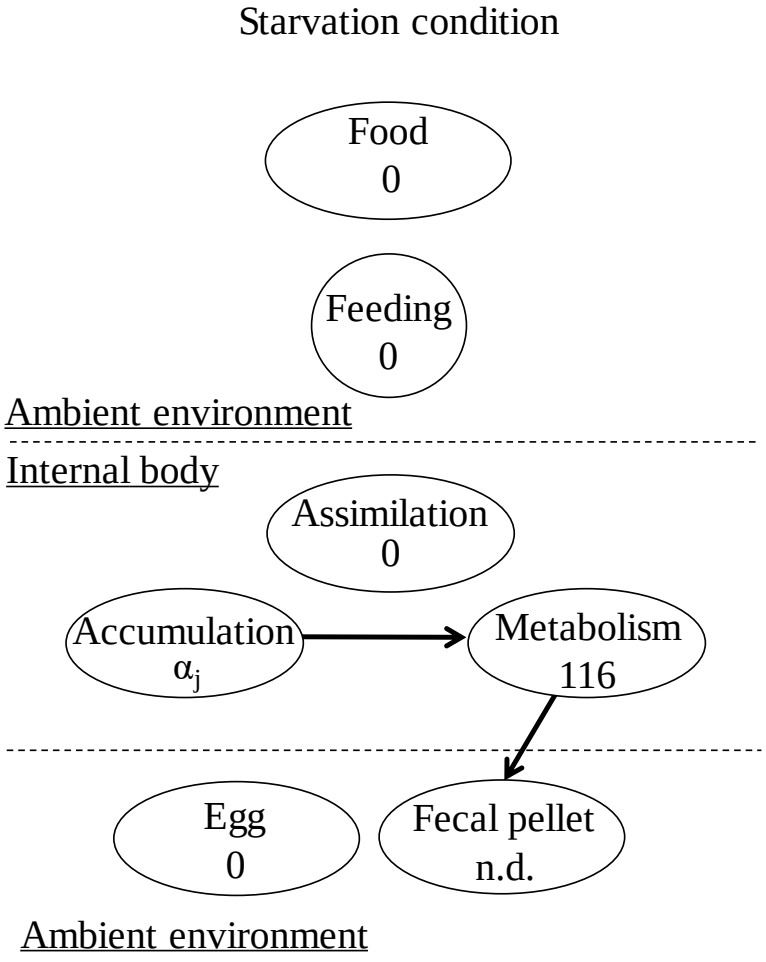


Fig. 4-3 Energy flow (ngC ind.$^{-1}$ d^{-1}) of *Acartia tonsa* raised under starvation condition for 4 days after exposed at high food condition for 3 days (derived by Kiørboe et al. 1985). Incubation temperature was 18 ℃. n.d.: no data. The rates of accumulation and fecal pellet production were not measured, so that these rates were expediently expressed as αj and n.d. in this energy flow.

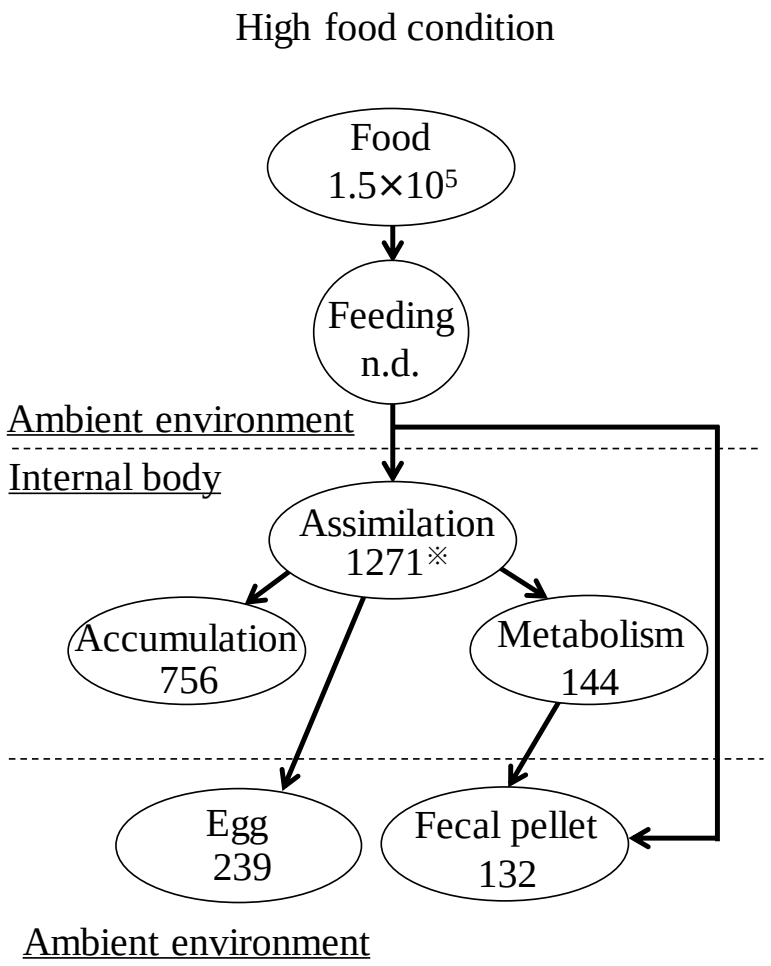


Fig. 4-4 Energy flow (ngC ind.$^{-1}$ d^{-1}) of *Acartia steueri* raised under high food condition (1.5×10^5 ngC ind.$^{-1}$ d^{-1}) for 5 days. Incubation temperature was 14.5 ℃. n.d.: no data. The metabolic rate was provided by the study of respiration rate in Chapter 3. Rates of egg production and fecal pellet production were provided by the carbon weights of an egg in *A. tonsa* (45.7 ngC egg-1; Kiørboe et al. 1985) and a fecal pellet in *A. omorii* (8.5 ngC pellet^{-1}; Juanita et al. 1998; Uye & Kaname 1994) and the numbers of eggs and fecal pellet production derived from the incubation experiment in Chapter 3 of the present study. The rate of accumulation was estimated from the carbon weights of biosynthesis compositions in Chapter 2. ※The amount of assimilation is the total amount of metabolism, egg, fecal pellet, and accumulation.

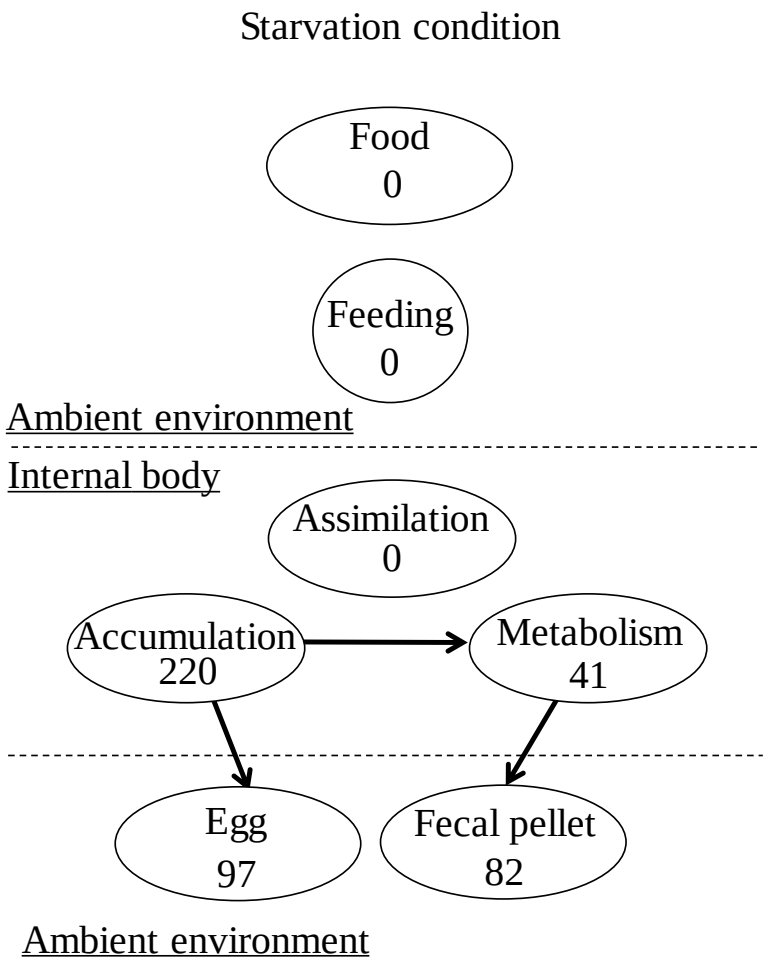
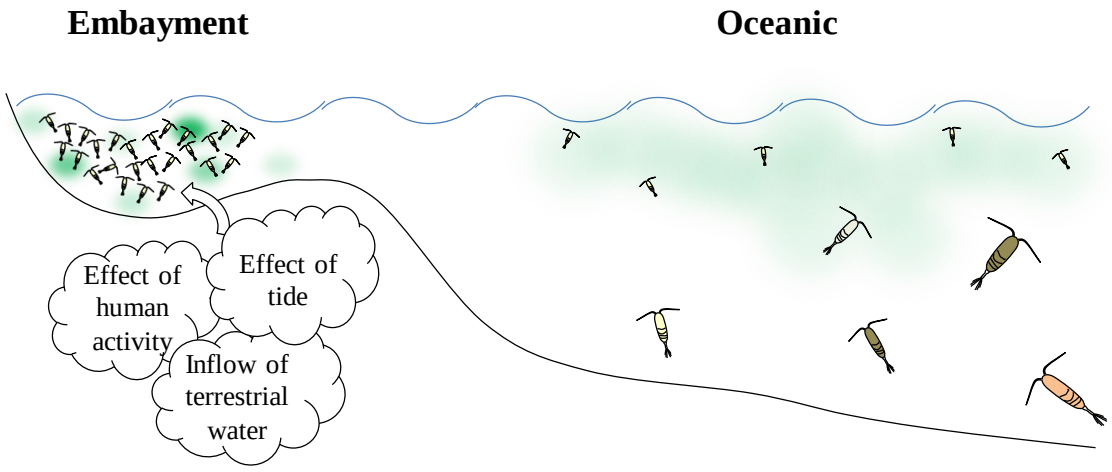


Fig. 4-5 Energy flow of A*cartia steueri* raised at starvation condition for 15 days after exposed at high food condition for 5 days. Incubation temperature was 14.5 ℃. n.d.: no data. The metabolic rate was provided by the study of respiration rate in Chapter 3. Rates of egg production and fecal pellet production were provided by the carbon weights of an egg in *A. tonsa* (45.7 ngC egg-1; Kiørboe et al. 1985) and a fecal pellet in *A. omorii* (8.5 ngC $pellet^{-1}$; Juanita et al. 1998; Uye & Kaname 1994) and the numbers of eggs and fecal pellet production derived from the incubation experiment in Chapter 3 of the present study. The rate of accumulation was estimated from the carbon weights of biosynthesis compositions in Chapter 2.

Table 4-1. Annual variations of chl. *a* concentration.

Sea areas	chl. *a* (mg m^{-3})	References
Oceanic areas		
North Atlantic Ocean	0.05—0.3	a
North Atlantic Ocean	0.01—0.05	b
South Atlantic Ocean	0.2 —0.4	c
western subarctic Pacific Ocean	0.4 —1.4	d
northwest subarctic Pacific Ocean	0.4 —1.6	e
western subarctic Pacific Ocean	0.24—5.6	f
Neritic areas		
Faroe shelf, Faroe Islands	0.3 —4.5	g
soutern East China Sea	0.25—3.0	h
Funka Bay	0.4—10.0	i
Sagami Bay	0.3—20.0	j
southewestern Okhotsk Sea	0.2 —9.5	k
Belgian and southern Dutch coastal areas	0.5—23.0	l
Gulf of Maine	1.0 —6.0	m
Coastal areas of Helgoland, Germany	0.74—6.8	n
Embayment areas		
Bedford Basin	1.2—6.0	o
Chesapeake Bay	3.0—47	p
Hiroshima Bay	2.0—35	q
Narragansett Bay	5.0—73	r
Otsuchi Bay	1.0—15	s
Sagami Bay	0.1—59	t
Sanfrancisco Bay	5.0—50	u
The mouth of Ria de Aveiro	0.1—11	v

a: Arístegui et al. 1997, b: Bode et al. 2001, c: Marañón et al. 2000, d: Ohashi et al. 2011, e: Mochizuki et al. 2002, f: Saito et al. 2002, g: Gaard et al. 1998, h: Gong et al. 2000, i: Odate & Imai 2003, j: Sugai et al. 2016, k: Kasai & Hirakawa 2015, l: Borges & Frankignoulle 1999; m: Glover 1977, n: Lucas et al. 2015, o: Cote & Platt 1983, p: Cole et al. 1986, q: Lee et al. 1996, r: Ray et al. 1989, s: Tsuda et al. 1994, t: Satoh et al. 2000, u: Durbin & Durbin 1981, v: Vidal et al. 2017



	Acartia steueri	Oceanic copepods
Food environment	Aprupt decrease for a short period	Relatively low for a long period
Storage substances	Amino acid pool and lipid	Lipid
Storage period	Short	Long
Accumulation rate	Rapid	Slow
Response to starvation	Continue metabolism and egg production	Continue metabolism and egg production

Fig. 4-6 Food environment and survival strategy of the embayment copepod *Acartia steueri* and oceanic copepods.

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APPENDICES

Appendix 1 Prosome length (PL) and Dry weight of embayment, coastal and oceanic copepods in ChapterII

Species	PL (μm)	Dry weight (μg)	Reference
Embayment species			
Paracalanus parvus	702.0	5.6	Uye 1982
Paracalanus crassirostris	381.0	0.9	Uye 1982
Temora turbinata	877.0	24.0	Uye 1982
Centropages yamadai	1025.3	21.4	Uye 1982
Pseudodiaptomus marinus	816.3	11.7	Uye 1982
Pontellopsis tenuicauda	1185.0	49.2	Uye 1982
Labidocera bipinnata	1523.5	73.1	Uye 1982
Calanopia thompsoni	1387.0	48.0	Uye 1982
Acartia erythrea	1006.0	9.6	Uye 1982
Acartia pacifica	912.0	8.3	Uye 1982
Corycaeus affinis	553.0	4.4	Uye 1982
Microsetella norvegica	361.0	1.8	Uye 1982
Acartia clausi	865.0	5.0	Cataletto and Umani 1994
Acartia tonsa	896.4	12.5	Durbin et al 1983
Temora longicornis	950.0	24.8	Breteler and Gonzalez 1988
Pseudocalanus elongatus	950.0	19.5	Breteler and Gonzalez 1988
Acartia hudsonica	880.6	10.0	Durbin et al 1992
Centropages brachiatus	1215.5	23.0	Richardson 2001
***Acartia steueri* total average**	**708.0**	**109.3**	**This study**
Acartia steueri in situ	**708.0**	**44.0**	**This study**
***Acartia steueri* full**	**708.0**	**166.5**	**This study**
Coastal and oceanic copepods			
Neocalanus cristatus	8837.5	3355.0	Omori 1969
Calanus glacialis	2897.5	170.3	Hirche and Kattner 1993
Calanus finmarchicus	2700.0	235.0	Tande 1982
Calanus finmarchicus	6550.0	4225.0	Marchall et al. 1934
Calanus chilensis	2450.0	245.0	Escribano 1999
Neocalanus cristatus	7510.0	6818.0	Kobari et al 2003
Neocalanus plumchrus	4050.0	4009.0	Kobari et al 2003
Neocalanus flemingeri	3900.0	4022.0	Kobari et al 2003
Eucalanus elongatus	5916.0	1617.0	Richardson 2001
Rhincalanus nasutus	3779.0	473.5	Richardson 2001
Pontellidae spp	3873.0	506.0	Richardson 2001
Calanoides carinatus	2006.0	102.0	Richardson 2001
Calanus agulhensis	2331.5	180.0	Richardson 2001
Eucalanus spp	2533.0	158.0	Richardson 2001
Neocalanus tonsus	2774.0	203.0	Richardson 2001
Calanopia helgolandicus	2411.3	96.6	Escribano 1999
Nannocalanus minor	1560.5	40.0	Richardson 2001
Calanoides carinatus	1705.0	60.0	Richardson 2001
Pleuromamma spp.	1412.0	32.0	Richardson 2001
Scolecithrix spp.	1698.0	53.0	Richardson 2001
Metridia lucens	1517.0	39.0	Richardson 2001
Calanus agulhensis	1780.0	97.0	Richardson 2001
Candacia armata	1826.0	65.5	Richardson 2001
Euchaeta spp	1770.0	59.0	Richardson 2001
Undinula vulgaris	1867.0	69.0	Richardson 2001
Pontella sp.	2173.0	243.3	Uye 1982
Pleuromamma scutullata	2640.0	243.8	Yamaguchi and Ikeda 2000
Heterorhabdus tanneri	2920.0	431.2	Yamaguchi and Ikeda 2000
Calanus sinicus	2295.0	164.3	Zhang et al 2005

Appendix 2 Dry weight, carbon weight and nitrogen weight of *in situ Acartia steueri* in May to July 2017.

In situ Acartia steueri	n	Dry weight	Carbon weight	Nitrogen weight
1705_insitu_1	5	220.0	11.7	1.4
1705_insitu_2	5	18.7	5.4	0.8
1705_insitu_3	5	13.1	4.5	0.7
1705_insitu_4	5	35.3	9.6	1.0
1705_insitu_5	5	3.3	3.8	-
1705_insitu_6	3	70.6	9.0	-
1705_insitu_7	4	23.5	6.7	1.2
1705_insitu_8	5	-	14.7	1.1
1705_insitu_9	5	-	4.6	0.8
1705_insitu_10	5	-	2.9	-
1706_insitu_1	10	10.2	3.8	0.7
1706_insitu_2	10	27.1	3.6	0.8
1706_insitu_3	10	32.3	4.1	0.8
1707_insitu_1	5	26.6	3.2	-
1707_insitu_2	5	14.1	4.0	0.8
1707_insitu_3	5	127.4	3.9	
1707_insitu_4	5	188.1	3.5	
1707_insitu_5	5	21.4	3.7	0.7
1707_insitu_6	5	63.0	3.6	
1707_insitu_7	5	83.6	4.2	0.5
1707_insitu_8	5	39.6	4.5	0.7
1707_insitu_9	5	49.6	4.3	0.7
	Average	56.2	5.4	0.8
	SD	58.8	3.0	0.2

Appendix 3 Dry weight, carbon weight and nitrogen weight of *Acartia steueri* raised under food satiated condition in May to July 2017.

A. steueri raised under food satiated condition	n	Dry weight	Carbon weight	Nitrogen weight
1705_high_1	3	233.3	24.6	1.1
1705_high_2	3	250.0	9.2	1.4
1705_high_3	7	249.0	6.8	0.5
1705_high_4	7	130.8	2.8	0.5
1705_high_5	5	176.4	7.4	-
1705_high_6	7	176.5	9.7	-
1705_high_7	4	-	9.9	0.8
1707_high_1	5	176.2	5.7	1.3
1707_high_2	5	14.9	5.4	1.1
1707_high_3	5	33.4	5.3	1.1
1707_high_4	2	123.0	8.2	-
1707_high_5	6	7.3	8.4	0.7
1707_high_6	5	113.7	3.9	0.8
1707_high_7	5	48.4	5.3	-
1707_high_8	5	22.5	3.2	0.7
Average		125.4	7.7	0.9
SD		85.2	5.0	0.3

Appendix 4 Dry weight, carbon weight and nitrogen weight of *Acartia steueri* raised under starvation in May to July 2017.

A. steueri raised under starved condition	n	Dry weight	Carbon weight	Nitrogen weight
1705_starved_1	6	233.3	6.9	0.5
1705_starved_2	5	240.0	5.7	0.6
1705_starved_3	9	155.6	4.1	-
1707_starved_1	5	155.2	3.9	0.6
1707_starved_2	5	48.6	3.4	0.5
1707_starved_3	5	26.4	3.6	-
1707_starved_4	5	163.2	2.4	-
1707_starved_5	5	42.7	2.8	-
1707_starved_6	5	34.2	3.4	-
1707_starved_7	5	46.8	2.9	0.5
Average	–	114.6	3.9	0.5
SD	–	79.9	1.3	0.1

Appendix 5 Dry weight of *Acartia steueri* under each condition in 8th, 21th and 27th April 2018.

In situ A. steueri	n	Dry weight
180408_insitu_1	10	1.1
180408_insitu_2	10	1.3
180408_insitu_3	10	5.4
180408_insitu_4	10	4.3
180408_insitu_5	10	1.4
180408_insitu_6	9	3.2
180421_insitu_1	10	3.3
180421_insitu_2	10	30.3
180421_insitu_3	10	7.5
180427_insitu_1	10	9.6
180427_insitu_2	10	19.0
180427_insitu_3	10	49.2
180427_insitu_4	10	33.3
180427_insitu_5	10	5.3
180427_insitu_6	10	7.4
180427_insitu_7	10	21.0
180427_insitu_8	10	6.1
Total number of samples	169	–
Average	–	12.3
SD	–	13.3

A. steueri raised under food satiated condition	n	Dry weight
180408_high_1	10	13.3
180408_high_2	10	24.4
180408_high_3	10	8.6
180408_high_4	10	11.3
180408_high_5	10	9.6
180408_high_6	10	22.0
180408_high_1	10	70.1
180408_high_2	9	101.8
180408_high_3	10	103.8
180408_high_4	10	55.3
180408_high_5	10	22.8
180421_high_1	10	52.7
180427_high_1	10	7.2
180427_high_2	10	1.1
Total number of samples	139	–
Average	–	36.0
SD	–	33.6

A. steueri raised under starved condition	n	Dry weight
180421_starved_1	11	19.7
180421_starved_2	10	3.2
180421_starved_3	10	0.3
180427_starved_1	10	10.7
180427_starved_2	10	4.2
Total number of samples	51	–
Average	–	7.6
SD	–	6.9

Appendix 6 Dry weight of *Acartia steueri* under each condition in 8th, 21th and 27th April 2018.

In situ A. steueri	n	Dry weight
180408_insitu_1	10	1.1
180408_insitu_2	10	1.3
180408_insitu_3	10	5.4
180408_insitu_4	10	4.3
180408_insitu_5	10	1.4
180408_insitu_6	9	3.2
Total number of samples	59	–
Average	–	2.8
SD	–	1.6

A. steueri raised under food satiated condition for 5 days	n	Dry weight
180408_high_5d_1	10	13.3
180408_high_5d_2	10	24.4
180408_high_5d_3	10	8.6
180408_high_5d_4	10	11.3
180408_high_5d_5	10	9.6
180408_high_5d_6	10	22.0
Total number of samples	60	–
Average	–	14.8
SD	–	6.1

A. steueri raised under food satiated condition for 10 days	n	Dry weight
180408_high_10d_1	10	70.1
180408_high_10d_2	9	101.8
180408_high_10d_3	10	103.8
180408_high_10d_4	10	55.3
180408_high_10d_5	10	22.8
Total number of samples	49	–
Average	–	70.8
SD	–	30.3

Appendix 7 Dry weight, carbon weight and nitrogen weight of *Acartia steueri* under food satiated condition in November 2018.

Acartia steueri raised under food satiated condition	n	Dry weight	Carbon weight	Nitrogen weight
1811_high_1	5	138.8	8.0	1.6
1811_high_2	5	125.8	9.1	1.7
1811_high_3	5	39.5	8.6	1.4
1811_high_4	7	20.6	5.4	1.2
1811_high_5	5	7.3	8.4	1.4
1811_high_6	5	5.5	8.6	1.4
	Average	56.2	8.0	1.5
	SD	55.0	1.2	0.2

Appendix 8 Survival rate, egg production and fecal pellet production of condition A: high food for 2 days consecutively transferred to the low food concentration in ChapterIV

A	1					2					3					4					5				
Day	Total egg	Nauplius	Subitaneous egg	Diapusing egg	Fecal pellet	Total egg	Nauplius	Subitaneous egg	Diapusing egg	Fecal pellet	Total egg	Nauplius	Subitaneous egg	Diapusing egg	Fecal pellet	Total egg	Nauplius	Subitaneous egg	Diapusing egg	Fecal pellet	Total egg	Nauplius	Subitaneous egg	Diapusing egg	Fecal pellet
1	0	2	2	0	13	0	0	0	0	7	0	0	0	0	13	0	4	4	0	23	0	1	1	0	25
2	0	0	0	0	7	0	1	1	0	8	0	1	1	0	8	0	2	2	0	16	0	0	0	0	4
3	0	1	1	0	3	0	4	4	0	9	0	0	0	0	12	0	2	2	0	11	0	0	0	0	13
4	0	0	0	0	6	0	2	2	0	2	0	2	2	0	2	0	1	1	0	2	0	0	0	0	6
5	0	0	0	0	11	0	0	0	0	6	0	0	0	0	3	0	1	1	0	3	0	0	0	0	13
6	3	0	3	0	13	0	0	0	0	3	0	0	0	0	5	0	0	0	0	5	0	0	0	0	12
7	0	0	0	0	7	0	4	4	0	1	0	0	0	0	10	0	0	0	0	4	0	1	1	0	16
8						0	2	2	0	9	0	0	0	0	18	0	0	0	0	13	0	0	0	0	16
9						0	1	1	0	30	0	2	2	0	24	0	1	1	0	20	0	0	0	0	5
10						1	0	1	0	21	0	0	0	0	24	0	0	0	0	13	0	0	0	0	37
11						0	0	0	0	14	0	0	0	0	18	0	3	3	0	29	0	0	0	0	20
12						0	0	0	0	6	0	0	0	0	8	0	2	2	0	23	0	0	0	0	25
13						0	0	0	0	6	0	0	0	0	9	1	0	1	0	12	0	0	0	0	4
14											0	0	0	0	5	0	2	2	0	12	0	1	1	0	4
15											0	0	0	0	7	0	0	0	0	15	0	0	0	0	5
16											0	0	0	0	21	0	1	1	0	6	0	3	3	0	9
17											0	0	0	0	10	1	0	1	0	8	0	2	2	0	4
18											0	0	0	0	16	0	0	0	0	9	1	1	2	0	3
19											1	0	1	0	10	0	0	0	0	3	0	0	0	0	1
20											0	1	1	0	19	0	0	0	0	3	1	2	3	0	5

A	6					7					8					9					10					
Day	Total egg	Nauplius	Subitaneous egg	Diapusing egg	Fecal pellet	Total egg	Nauplius	Subitaneous egg	Diapusing egg	Fecal pellet	Total egg	Nauplius	Subitaneous egg	Diapusing egg	Fecal pellet	Total egg	Nauplius	Subitaneous egg	Diapusing egg	Fecal pellet	Total egg	Nauplius	Subitaneous egg	Diapusing egg	Fecal pellet	Survival number
1	0	4	4	0	33	0	1	1	0	21	0	3	3	0	30	0	3	3	0	10	0	2	2	0	18	10
2	0	3	3	0	28	2	1	3	0	25	0	19	19	0	40	0	1	1	0	13	0	0	0	0	14	10
3	0	4	4	0	19	0	2	2	0	20	0	28	28	0	47	0	0	0	0	22	0	0	0	0	16	10
4	0	2	2	0	14	0	0	0	0	4	0	10	10	0	8	0	5	5	0	5	0	0	0	0	8	10
5						0	0	0	0	4	1	9	10	0	13	0	0	0	0	0	0	0	0	0	7	9
6						0	0	0	0	3	2	0	2	0	15	0	0	0	0	1	0	0	0	0	8	9
7											0	5	5	0	17	0	0	0	0	10	0	0	0	0	3	8
8											2	9	11	0	10	0	0	0	0	6						6
9											0	16	16	0	12	0	0	0	0	6						6
10											2	6	8	0	14	0	0	0	0	8						6
11											0	8	8	0	26	0	0	0	0	10						6
12											2	1	3	0	30	0	0	0	0	17						6
13											1	4	5	0	9	0	1	1	0	9						6
14											0	5	5	0	34	0	0	0	0	5						5
15											1	1	2	0	23											4
16											0	4	4	0	27											4
17											0	3	3	0	2											4
18											1	4	5	0	2											4
19											0	0	0	0	14											4
20											0	4	4	0	10											4

Appendix 9 Survival rate, egg production and fecal pellet production of condition B: high food for 2 days consecutively transferred to the Starvation condition. in Chapter IV

B	1					2					3					4					5				
Day	Total egg	Nauplius	Subitaneous egg	Diapusing egg	Fecal pellet	Total egg	Nauplius	Subitaneous egg	Diapusing egg	Fecal pellet	Total egg	Nauplius	Subitaneous egg	Diapusing egg	Fecal pellet	Total egg	Nauplius	Subitaneous egg	Diapusing egg	Fecal pellet	Total egg	Nauplius	Subitaneous egg	Diapusing egg	Fecal pellet
1	0	0	0	0	15	0	1	1	0	2	0	0	0	0	3	0	0	0	0	7	0	1	1	0	13
2	0	3	3	0	7	0	0	0	0	6	0	0	0	0	3	0	5	5	0	8	4	4	8	0	3
3	0	6	6	0	18	0	0	0	0	6	0	1	1	0	6	0	3	3	0	11	0	0	0	0	7
4	0	0	0	0	2	0	1	1	0	1	0	0	0	0	6	0	0	0	0	3					
5	0	0	0	0	4	0	3	3	0	4	0	0	0	0	0	0	0	0	0	1					
6	0	0	0	0	6	0	0	0	0	4	0	0	0	0	2	0	0	0	0	3					
7	0	0	0	0	6						0	0	0	0	0	0	0	0	0	0					
8	0	0	0	0	9						0	0	0	0	6	0	0	0	0	1					
9	0	1	1	0	21											0	0	0	0	1					
10	0	0	0	0	16											0	0	0	0	7					
11	0	2	2	0	17											0	0	0	0	3					
12	0	0	0	0	20											0	0	0	0	4					
13	0	0	0	0	2											0	0	0	0	6					
14	0	0	0	0	4											0	0	0	0	6					
15	0	0	0	0	8													0							
16	1	0	1	0	4													0							
17	0	1	1	0	9													0							
18	0	0	0	0	4													0							
19	0	0	0	0	3													0							
20																		0							

B	6					7					8					9					10					
Day	Total egg	Nauplius	Subitaneous egg	Diapusing egg	Fecal pellet	Total egg	Nauplius	Subitaneous egg	Diapusing egg	Fecal pellet	Total egg	Nauplius	Subitaneous egg	Diapusing egg	Fecal pellet	Total egg	Nauplius	Subitaneous egg	Diapusing egg	Fecal pellet	Total egg	Nauplius	Subitaneous egg	Diapusing egg	Fecal pellet	Survival number
1	0	0	0	0	13	1	2	3	0	13	0	1	1	0	8	0	4	4	1	26	0	0	0	0	4	10
2	0	3	3	0	14	0	0	0	0	5	0	0	0	0	4	0	0	0	0	1	0	0	0	0	3	10
3	0	1	1	0	17	0	0	0	0	4	0	0	0	0	6	0	0	0	0	2	0	0	0	0	1	10
4	0	0	0	0	3	0	0	0	0	0	0	0	0	0	5						0	0	0	0	1	8
5	0	0	0	0	5	0	0	0	0	2	0	0	0	0	1						0	0	0	0	2	8
6	0	0	0	0	11	0	0	0	0	2	0	0	0	0	1						0	0	0	0	2	8
7	0	0	0	0	6						0	0	0	0	0						0	0	0	0	3	6
8	0	0	0	0	4						0	0	0	0	1						0	0	0	0	5	6
9	0	0	0	0	3																0	0	0	0	5	4
10	0	0	0	0	7																0	0	0	0	3	4
11	0	0	0	0	23																0	0	0	0	5	4
12	0	0	0	0	27																0	0	0	0	4	4
13	0	0	0	0	2																0	0	0	0	1	4
14	0	0	0	0	2																0	0	0	0	2	4
15	0	0	0	0	3																0	0	0	0	0	3
16	0	0	0	0	7																					2
17	0	0	0	0	2																					2
18	0	0	0	0	3																					2
19	0	0	0	0	1																					2
20	0	0	0	0	0																					1

Appendix 10 Survival rate, egg production and fecal pellet production of condition C: high food for 5 days consecutively transferred to the low food concentration. in Chapter IV

C	1					2					3					4					5				
Day	Total egg	Nauplius	Subitaneous egg	Diapusing egg	Fecal pellet	Total egg	Nauplius	Subitaneous egg	Diapusing egg	Fecal pellet	Total egg	Nauplius	Subitaneous egg	Diapusing egg	Fecal pellet	Total egg	Nauplius	Subitaneous egg	Diapusing egg	Fecal pellet	Total egg	Nauplius	Subitaneous egg	Diapusing egg	Fecal pellet
1	0	3	3	0	13	0	0	0	0	19	0	1	1	0	20	0	4	4	0	41	0	1	1	0	20
2	0	2	2	0	16	0	8	8	0	16	0	7	7	0	16	0	10	10	0	16	0	0	0	0	13
3	0	5	5	0	34	1	16	17	0	33	0	4	4	0	23	0	6	6	0	15	0	0	0	0	21
4	0	25	25	0	19	0	33	33	0	20	0	10	10	0	21						0	0	0	0	23
5	8	23	31	0	34	0	32	32	0	36	3	8	11	0	42						3	6	9	0	36
6	15	23	38	0	40	1	14	15	0	23	2	5	7	0	20						1	0	1	0	22
7	9	9	18	0	27	1	23	24	0	30	3	7	10	0	20						3	0	3	0	16
8	10	13	23	0	42	6	20	26	0	51	1	8	9	0	31						0	2	2	0	15
9	4	22	26	0	29	2	11	13	0	31	0	6	6	0	14						0	4	4	0	10
10	7	8	15	0	51	4	10	14	0		0	3	3	0	20						0	2	2	0	30
11	3	16	19	0	25	1	9	10	0	25	1	5	6	0	22						1	2	3	0	19
12	2	3	5	0	26	3	3	6	0	22	0	1	1	0	26						0	0	0	0	36
13	0	0	0	0	0	1	12	13	0	23											1	0	1	0	15
14						2	7	9	0	15											0	0	0	0	17
15						1	2	3	0	24											1	0	1	0	9
16						0	4	4	0	9											1	0	1	0	8
17						1	3	4	0	6											0	0	0	0	2
18						0	7	7	0	9											0	1	1	0	4
19						1	5	6	0	3											0	0	0	0	10
20						0	4	4	0	6											1	0	1	0	4

C	6					7					8					9					10					
Day	Total egg	Nauplius	Subitaneous egg	Diapusing egg	Fecal pellet	Total egg	Nauplius	Subitaneous egg	Diapusing egg	Fecal pellet	Total egg	Nauplius	Subitaneous egg	Diapusing egg	Fecal pellet	Total egg	Nauplius	Subitaneous egg	Diapusing egg	Fecal pellet	Total egg	Nauplius	Subitaneous egg	Diapusing egg	Fecal pellet	Survival number
1	0	2	2	0	21	0	7	7	0	40	0	0	0	0	26	0	4	4	0	20	0	0	0	0	26	
2	0	0	0	0	5	0	1	1	0	8	0	7	7	0	13	0	10	10	0	20	0	6	6	0	23	10
3	0	0	0	0	30	0	1	1	0	14	0	6	6	0	13	0	9	9	0	20	0	14	14	0	36	10
4	0	3	3	0	22	0	10	10	0	17	0	32	32	0	17	0	24	24	0	10	0	16	16	0	22	10
5	1	1	2	0	56	0	3	3	0	28	0	11	11	0	30	0	33	33	0	33	1	18	19	0	33	9
6	2	4	6	0	15	2	4	6	0	8	0	14	14	0	14	0	22	22	0	24	0	20	20	0	28	9
7	0	3	3	0	28						0	11	11	0	22	0	12	12	0	19	0	10	10	0	21	9
8	0	1	1	0	2						0	8	8	0	24	0	11	11	0	45	0	9	9	0	35	8
9	0	1	1	0	6						0	12	12	0	22	0	13	13	0	35	0	15	15	0	24	8
10	0	1	1	0	20						0	10	10	0	28	2	1	3	0	45	0	0	0	0	42	8
11	2	1	3	0	23						2	8	10	0	31	0	16	16	0	35	0	11	11	0	33	8
12	0	0	0	0	19						4	1	5	0	40	2	2	4	0	34	1	1	2	0	42	8
13	1	0	1	0	4						7	0	7	0	14	0	11	11	0	27	0	0	0	0	22	8
14	0	0	0	0	1						4	1	5	0	16	0	5	5	0	3	0	7	7	0	12	7
15	0	0	0	0	2						3	2	5	0	14	1	2	3	0	9	0	3	3	0	8	6
16	0	0	0	0	8						5	1	6	0	7	1	0	1	0	8	0	8	8	0	8	6
17	0	1	1	0	2						2	3	5	0	7	0	2	2	0	7	0	5	5	0	2	6
18	0	0	0	0	6						3	0	3	0	6	0	2	2	0	4	0	1	1	0	3	6
19	0	0	0	0	5						5	1	6	0	13	0	0	0	0	7	0	0	0	0	4	6
20	0	0	0	0	6						1	1	2	0	15	0	1	1	0	10	0	0	0	0	6	6

Appendix 11 Survival rate, egg production and fecal pellet production of condition C: high food for 5 days consecutively transferred to the starvation condition in Chapter IV

D	1					2					3					4					5				
Day	Total egg	Nauplius	Subitaneous egg	Diapusing egg	Fecal pellet	Total egg	Nauplius	Subitaneous egg	Diapusing egg	Fecal pellet	Total egg	Nauplius	Subitaneous egg	Diapusing egg	Fecal pellet	Total egg	Nauplius	Subitaneous egg	Diapusing egg	Fecal pellet	Total egg	Nauplius	Subitaneous egg	Diapusing egg	Fecal pellet
1	1	1	2	0	6	0	0	0	0	11	0	3	3	0	8	0	1	1	0	23	0	2	2	0	26
2	0	0	0	0	11	0	2	2	0	0	0	2	2	0	6	0	17	17	0	9	0	1	1	0	23
3	0	3	3	0	17	0	3	3	0	7	0	2	2	0	4	0	7	7	0	9	0	1	1	0	16
4	0	1	1	0	10	0	0	0	0	5	0	0	0	0	13	0	13	13	0	24	0	1	1	0	27
5	0	1	1	0	12	0	0	0	0	14	0	1	1	0	17	1	21	22	0	29	0	3	3	0	38
6	2	3	5	0	17	0	0	0	0	24	0	1	1	0	11	0	15	15	0	8	0	5	5	0	8
7	1	0	1	0	4	0	1	1	0	1	0	0	0	0	7	0	8	8	0	14	0	0	0	0	21
8	0	0	0	0	3	0	0	0	0	2	0	1	1	0	17	1	0	1	0	12	0	1	1	0	37
9	0	0	0	0	6	0	0	0	0	2	0	0	0	0	12	4	0	4	0	16	0	0	0	0	22
10	0	0	0	0	4	0	0	0	0	6	0	0	0	0	8	4	1	5	0	13	0	0	0	0	40
11	0	1	1	0	5			0			0	0	0	0	10	0	4	4	0	10	0	0	0	0	52
12	0	1	1	0	6			0			0	2	2	0	3	0	4	4	0	4	0	0	0	0	35
13	3	0	3	0	5			0			1	0	1	0	3	1	2	3	0	3	0	0	0	0	5
14	0	0	0	0	8			0			0	0	0	0	1	0	2	2	0	5	0	0	0	0	2
15	0	1	1	0	2			0					0			0	0	0	0	3	0	0	0	0	4
16	0	0	0	0	3			0					0			0	0	0	0	4	0	0	0	0	29
17	0	0	0	0	1			0					0			0	0	0	0	0	0	0	0	0	5
18	0	0	0	0	5			0					0								0	0	0	0	16
19	0	0	0	0	5			0					0					0			0	0	0	0	10
20	0	1	1	0	27			0					0					0			0	0	0	0	25

D	6					7					8					9					10					
Day	Total egg	Nauplius	Subitaneous egg	Diapusing egg	Fecal pellet	Total egg	Nauplius	Subitaneous egg	Diapusing egg	Fecal pellet	Total egg	Nauplius	Subitaneous egg	Diapusing egg	Fecal pellet	Total egg	Nauplius	Subitaneous egg	Diapusing egg	Fecal pellet	Total egg	Nauplius	Subitaneous egg	Diapusing egg	Fecal pellet	Survival number
1	0	0	0	0	12	0	11	11	0	22	0	6	6	0	34	0	1	1	0	14	0	5	5	0	19	
2	0	0	0	0	6	0	1	1	0	7	0	8	8	0	16	0	0	0	0	14	0	4	4	0	18	10
3	0	0	0	0	10	0	11	11	0	9	0	13	13	0	8	0	1	1	0	10	0	2	2	0	8	10
4	0	0	0	0	13	0	21	21	0	16	0	13	13	0	14	0	1	1	0	11	0	3	3	0	24	10
5	0	0	0	0	20	0	25	25	0	36	2	23	25	0	20	0	1	1	0	9	1	20	21	0	35	10
6	0	0	0	0	12	0	10	10	0	12	0	2	2	0	14	0	4	4	0	13	0	4	4	0	14	10
7	0	1	1	0	6	0	22	22	0	22	0	11	11	0	20	0	1	1	0	8	0	16	16	0	17	10
8	0	0	0	0	2	1	14	15	0	33	1	10	11	0	35	0	2	2	0	2	0	12	12	0	24	10
9			0			0	13	13	0	32	0	8	8	0	28			0			0	6	6	0	25	10
10			0			0	6	6	0	40	0	8	8	0	41			0			0	9	9	0	14	8
11			0			0	12	12	0	32	0	9	9	0	27			0			0	14	14	0	31	8
12			0			1	5	6	0	39	4	4	8	0	13			0			1	4	5	0	16	7
13			0			0	1	1	0	13	3	3	6	0	17			0			0	6	6	0	8	7
14			0			0	0	0	0	8	1	1	2	0	4			0			0	0	0	0	6	7
15			0			0	0	0	0	16	0	1	1	0	8			0			1	2	3	0	6	7
16			0			0	0	0	0	11	1	1	2	0	10			0			0	0	0	0	3	6
17			0					0			0	0	0	0	6			0			0	0	0	0	5	6
18			0					0			0	4	4	0	9			0			0	2	2	0	13	5
19			0					0			0	1	1	0	30			0			0	1	1	0	27	4
20			0					0			0	3	3	0	27			0			0	1	1	0	25	4