

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

内湾性かいあし類 *Acartia steueri* Smirnov (Calanoida: Acartiidae) の
エネルギー蓄積と飢餓耐性

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SYNOPSIS

内湾生態系は、潮汐、陸水の流入、複雑な海底地形、人間活動の影響を直接受け、絶えず生物種の移入・消失による生態系の構造変化や、生物生産の変動が起きている。内湾域の生物多様性は、沿岸域や外洋域と比較して低く、内湾生態系において再生産を行って優占する生物種は限られている。内湾域の植物プランクトン量は、短期間のうちに急激に減少し、その後低濃度餌料環境が形成され、内湾性かいあし類は頻繁に餌不足に曝される。内湾性かいあし類は、このような変動の激しい餌環境に対して生理的な耐性メカニズムを持つことで適応し、個体群を維持しているものと考えられる。本研究は、飢餓に対する内湾性かいあし類の生理的な耐性メカニズムを明らかにするために、西部北太平洋内湾に広く優占するかいあし類 *Acartia steueri* Smirnov を対象種とし、(1)本種のエネルギー蓄積特性と、(2)飢餓状態における生物学的応答を研究した。エネルギー蓄積特性については、飽食条件および飢餓条件でそれぞれ 10 日間培養した *A. steueri* メス成体 1 個体あたりの前体部長、乾燥重量、生化学組成を測定した。くわえて、飽食条件のメス成体、卵、糞粒のアミノ酸量、アミノ酸の窒素同位体比を測定してアミノ酸のエネルギー収支を調べた。内湾、沿岸、外洋性かいあし類約 50 種の体長一体重の関係は、1つの回帰式で表されたが、本研究の *A. steueri* はこの回帰式から大きく乖離し、体長に比して、極端に重量が高いことが明らかとなった。60°C、24 h における本種の乾燥重量は、80°C、100°C、120°C の高温条件下で乾燥させると顕著に減少したことから、たんぱく質分子を覆う結合水が含まれていると示唆された。脂肪酸量、アミノ酸量、アミノ酸の窒素同位体比からは、卵・糞粒生産および代謝に利用した後の余剰分のアミノ酸を体内に蓄積し、脂肪酸を主とする他の生化学組成へ常時生合成することで、短期間のエネルギー蓄積を可能にすることが示唆された。飢餓状態における生物学的応答は、生残率、卵生産速度、糞粒生産速度、呼吸速度を測定した。生残率、卵生産速度、糞粒生産速度を調べるための、20 日間における飢餓実験は、2 日もしくは 5 日間、高濃度の餌料条件に曝した後、個体維持に必要な最低限の濃度条件と飢餓条件に曝す、計 4 条件を設定した。高濃度条件後の飢餓環境下で、*A. steueri* は 15 日間卵生産を継続し、18 日以上生存した。*A. steueri* は、内湾性種の中で長い飢餓耐性日数を示し、飢餓環境下で卵を生産し続ける期間が長く、現場環境下における極めて低濃度の餌料環境下でも代謝・卵生産を継続し、次の好適な餌料環境が訪れるまで個体群を繋ぐことができる。飢餓状態における呼吸速度は、高濃度条件よりも 1.5–3 分の 1、低い値を示したことから、本種は飢餓状態では呼吸量を低く保ち、エネルギーを卵生産に分配することで、長期間卵生産を継続することが明らかとなった。本研究は、*A. steueri* の飢餓に対する生理的な耐性メカニズムとして (1)遊離アミノ酸を利用した、短期間の速やかなエネルギー蓄積能を有すること、(2)飢餓状態における代謝や卵生産へのエネルギー分配を変化させることを解明した。このような本種の飢餓耐性は、変動が絶えず起こる、生物多様性の低い内湾生態系で個体群を維持するために必要不可欠な生存戦略であると言える。

Keywords: energy reserves, metabolic change, egg production, dry weight, amino acid, fatty acid

INTRODUCTION

Environmental factors in embayment areas change abruptly due to irregular inflows of freshwater, tidal movements, complex submarine topography, and seasonal changes. Resultantly, chlorophyll a concentrations fluctuate widely, from 10 to 50 $\mu\text{g L}^{-1}$ in the inner part of San Francisco Bay, 3.0 to 47 $\mu\text{g L}^{-1}$ in Chesapeake Bay, and from 5.0 to 73 $\mu\text{g L}^{-1}$ in Narragansett Bay (Durbin & Durbin 1981, Cole et al. 1986, Ray et al. 1989). Fluctuations in chlorophyll a concentrations in the spring or autumn blooms are the largest annual fluctuations. In Otsuchi Bay, Japan, diatom blooms occur several times during the spring, and are induced by local wind stress (Furuya et al. 1993). Blooms with chlorophyll a concentrations of 10 to 15 $\mu\text{g L}^{-1}$ last from several days to 10 days, followed by a non-bloom period of 1 to 2 $\mu\text{g L}^{-1}$ that lasts several days to 3 weeks and is accompanied by an increase in other organisms, such as flagellates and ciliates (Tsuda et al. 1994). Intensive grazing by copepods has also been observed just

after blooms. The feeding environment of copepods in embayment areas has been thought to be diluted and highly heterogeneous both temporally and spatially (e.g., Agboola et al. 2013). Copepods exposed to fluctuations in chlorophyll a concentrations suffer from a food-limited environment for a period of days to weeks (Niehoff & Hirche 2000). Thus, the abrupt decreases in food concentrations or starvation is one of the most probable obstacles to the survival or reproduction of herbivorous copepods. Copepods might have a mechanism of physiological tolerance to starvation periods to maintain their population embayments.

Energy flows obtained by feeding in adult female copepods have been reported by Ikeda & Motoda (1978). After feeding, adult females eliminate 30% of consumed food as fecal pellets and assimilate 70% into their body, of which 30% and 40% are distributed to metabolism and egg production, respectively. It has been thought that the rate of energy distribution in adult female copepods changes depending on the food conditions. Under high

food availability, such as during a spring bloom, it is assumed that adult females accumulate excess energy above levels used in metabolism and egg production. On the other hand, when there is little or no food, such as during abrupt declines post-bloom, the rates of energy distribution for assimilation, metabolism, and egg production are thought to change. Therefore, two hypotheses were developed as potential survival strategies against starvation: (1) embayment copepods can accumulate energy into their body under high food conditions, and (2) they can change their energy distributions to metabolism and reproduction under starvation scenarios.

Genus *Acartia*, which is well studied because of its promise in live feeds in marine larviculture, is adapted to sudden unfavorable environmental conditions in the embayment area (Hansen et al. 2016). An 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 (Kos 1958, Nishida 1985). This species is one of the most dominant embayment copepods, playing a key role as an essential food source for larvae of commercially important fishes.

In this study, we firstly investigated the chemical composition of *A. steueri* to elucidate the characteristics of energy accumulation (Study 1). Secondly, we investigated the rates of survival, respiration, and egg production of *A. steueri* under starvation scenarios to reveal physiological responses induced by food stress (Study 2). Finally, we discussed the metabolic specificity of *A. steueri* in embayment areas.

MATERIALS AND METHODS

Study 1. Characteristics of energy accumulation of Acartia steueri

Chemical composition of *A. steueri*

To investigate the function of energy accumulation, we measured prosome lengths and dry weights of *Acartia steueri* under three different conditions: *in situ*, high-food concentrations, and starvation. Sampling was conducted at a fixed station of Manazuru Port (St. A; 35°09'49"N, 139°10'33"E, maximum depth: about 6 m) located on the northwestern coast of Sagami Bay, Japan from May to July 2017, and April, July, and November 2018. Plankton samples were collected by gently towing a plankton net obliquely from 5 m depth to the surface. Live adult female *A. steueri* were randomly sorted from the plankton samples under a dissecting microscope. Adult females were firstly incubated at high-food (2.0 µgC mL⁻¹; Berggreen et al. 1988) concentrations for 5 days. Subsequently, they were exposed to two food conditions for 10 days each: high-food concentrations and starvation (<0.22 µm filtered sea water). The diatom *Thalassiosira weissflogii* (64.4 pgC cell⁻¹) was used for dietary algae. At the end of the experiments, the dry weight, carbon weight, nitrogen weight, elemental composition, and the amounts of free amino acids, proteins, lipids, and carbohydrates were measured. The weight of *A. steueri* dried at 60°C for 24 h as conventional drying protocol was out of a length-weight relationship of marine planktonic copepods (Fig. 1). To clarify the diremption of the dry weight of *A. steueri*,

weights dried at 80°C, 100°C and 120°C for 12 h and 24 h, respectively were measured after dried at 60°C for 24 h.

Stable nitrogen isotopic composition of *A. steueri*

Fifty adult females collected on October 2014 were sorted and incubated under high-food concentrations for 10 days in 500 mL beakers with <0.22 µm filtered sea water. Fatty acids, amino acids, and stable nitrogen isotopic composition were measured to construct the energy flow of amino acids and fatty acids in *A. steueri* under high-food conditions.

Study 2. Physiological responses of A. steueri to starvation

Respiration rate during starvation

Sampling was conducted at Manazuru Port following the protocols of Study 1 in June, July 2016, and July 2017. Adult females were first exposed to high-food (2.0 µgC mL⁻¹; Berggreen et al. 1988) concentration for 5 days and then transferred to two food conditions for 10 days: high-food concentration and starvation (<0.22 µm filtered sea water). Two to three individuals were placed into a gas-tight experimental bottle (3 mL) filled with filtered sea water. Four gas-tight glass bottles were situated in a water bath placed in an incubator to maintain constant temperature. Respiration rates under each condition were measured by a fiber-optic oxygen meter at 20°C for 12-18 h under dark conditions.

Survival rate, egg production rate, and fecal pellet production rate during starvation

Sampling was conducted at Manazuru Port following the protocols of Study 1 in April 2014. Incubation experiments were conducted to examine the production rates of eggs and fecal pellets. Four experimental conditions were designed with two different high-food concentration periods followed by two low-food concentration periods. Adult females were first exposed to high-food conditions (2.0 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 without food. The low-food condition was set as the concentration required for survival (Berggreen et al. 1988). Ten adult females were reared for each condition. 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 mL near-ambient temperature and natural light cycles. The number of surviving females, eggs, and fecal pellets were counted every 24 hours.

RESULTS AND DISCUSSION

Study 1. Characteristics of energy accumulation of A. steueri

Measurements of dry weights compared to prosome length of *Acartia steueri* raised under high-food conditions for 15 days were much higher than those of other *Acartia* copepods. Average dry weight values of *A. steueri* were more than three times higher than those of the other *Acartia* copepods. Although length-weight relationships of embayment, neritic and oceanic marine planktonic copepods are represented by a regression line (Fig. 1), *A. steueri* study was beyond this relationship

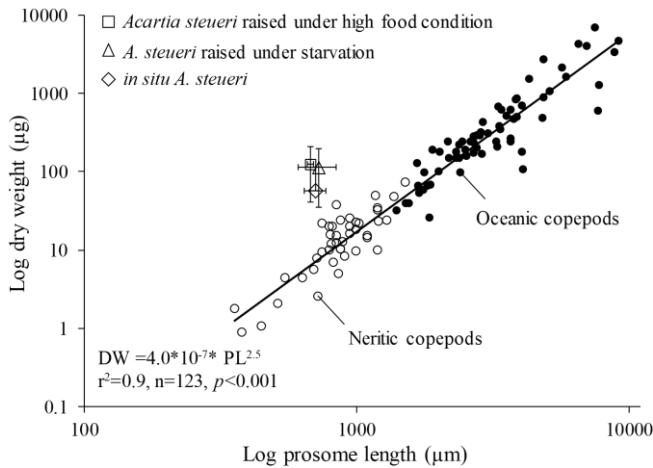


Fig. 1 Log-transformed dry weight and log-transformed prosome length relationship for neritic and oceanic copepods (modified from Hirahara & Toda 2018). Open and closed circles represent neritic and oceanic copepods, respectively. Diamond symbol represents *in situ* *Acartia steueri* in May to July 2017. 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.

because of their heavy dry weight. The weights dried at all higher temperatures (80°C, 100°C and 120°C for 12 h and 24 h) decreased notably compared with those using conventional drying conditions at 60°C for 24 h. It is possible that water content such as bound water covering the surface of protein molecules was contained in the body of *A. steueri* dried at 60°C for 24 h. Dry weights even under the higher-temperature conditions were much higher than those of other neritic copepods. Oil droplets such as the oil sac well-known to overwintering oceanic copepods were observed in the body cavity of *A. steueri* exposed to high food concentrations for 10 days, whereas no oil droplet was found when raised under starvation conditions for 10 days.

Lipid and protein content of *A. steueri* under high-food conditions was significantly higher than under starvation (Fig. 2. Student's *t*-test, $p < 0.05$). The ratio of free amino acids to total amino acids (the total value of protein and free amino acids) in *A. steueri* under either condition was 36-48% and was higher than that in other zooplankton species. Phytoplankton have been reported to have high contents of free amino acids (e.g., Fyhn et al. 1993). Redundant free amino acids are often used as an energy source and in the biosynthesis of lipids (Muto et al. 2002). Ikeda (1974) and Mayzaud (1976) reported that *Acartia* copepods utilized free amino acids, which consist mainly of nitrogen, as an energy source under the latter period of starvation. Therefore, free amino acids were considered to be a major component for metabolism for *A. steueri*.

Energy flow of an amino acid was obtained from the results of amino acids and stable nitrogen isotopic compositions. Excretion and assimilation consumed 11% and 89% of all amino acids, respectively. Of the 89% assimilated, 68%, 4%, and 17% was used for metabolism, egg production and body accumulation as amino acids pool, respectively. On the other hand, it was clarified by

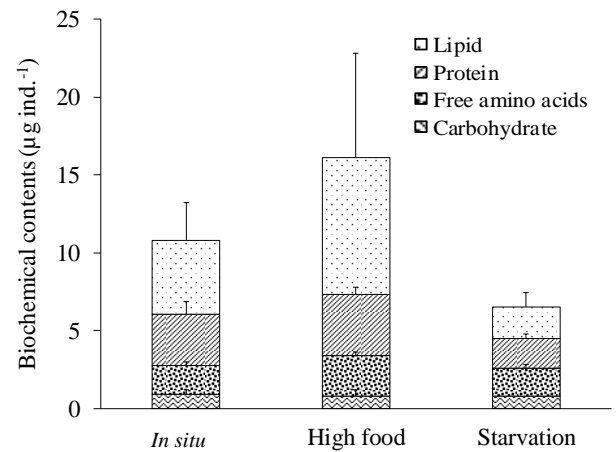


Fig. 2 Biochemical compositions (lipid, protein, free amino acids, and carbohydrate) of *Acartia steueri* under *in situ*, high food condition and starvation.

the fatty acid compositions of fecal pellets and eggs that 171% and 178% of fatty acids in food were used for excretion and egg production, respectively. The total amount of fatty acids used in egg and fecal pellet production exceeded fatty acid uptake by adult females. It is known that surplus amino acids are converted to fatty acids under saturated food conditions (Kimura & Negoro 2002). The present study suggests that *A. steueri* continually converts free amino acids to fatty acids for metabolism and egg production under adequate food conditions.

As for the results of elemental composition, phosphorus (P), sulfur (S), zinc (Zn) and bromine (Br) in *A. steueri* raised under high-food concentration were about twice as high as those found *in situ* in *A. steueri*. It is suggested that *A. steueri* accumulates composite high-density substances such as lipoproteins consisting of free amino acids, proteins and phospholipids, which explains the high dry weights reported.

Energy reserves play an important role in the life history of many copepods, providing energy for reproduction, metabolism under food scarcity, and ontogeny (Lee et al. 2006). Oceanic copepods accumulate large lipid stores to survive long starvation periods (Tsuda et al. 2001, Lee et al. 2006), whereas neritic copepods store little or no lipids and withstand only short periods of starvation (e.g., Mayzaud 1976, Lee et al. 2006). In this study, it was confirmed that *A. steueri* is able to accumulate energy into its body rapidly for a short period as shown by accumulated amino acids and the biosynthesis of fatty acids. *A. steueri* may utilize amino acids to maintain their metabolism during late-stage starvation after initially utilizing fatty acids at the beginning of starvation.

Study 2. Physiological responses of *A. steueri* to starvation

The respiration rate of *A. steueri* which were raised at high-food concentrations was $2.32 \pm 0.59 \text{ nmol O}_2 \text{ h}^{-1} \text{ ind.}^{-1}$. On the other hand, rates of *A. steueri* raised at starvation were $1.48 \pm 0.31 \text{ nmol O}_2 \text{ h}^{-1} \text{ ind.}^{-1}$. The respiration rate of

A. steueri raised at high-food concentrations was about two times higher than that of *A. steueri* raised at starvation. These results suggest that *A. steueri* regulates metabolism when the food concentration is low, explaining how *A. steueri* can accumulate energy rapidly.

In the incubation experiment, *A. steueri* survived for 18 and 15 days under starvation, respectively. Individuals were able to maintain metabolic rates even under an environment in which the food concentration largely varies for a certain period without being negatively affected by the shortage of food. *A. steueri* continued to produce eggs for 14 days under starvation scenarios. The period of exposure to high-food concentrations at the beginning of the experiment influenced cumulative egg production under starvation conditions. The cumulative egg production by the females raised under high-food period for 5 days was not different between the following low food period and the starvation period on account of adequate energy accumulation. On the other hand, the cumulative egg production by females raised under the high-food period for 2 days showed differences between the following low food period and the starvation period on account of the inadequate energy accumulation.

Two embayment copepods, *Paracalanus parvus* and *A. tonsa* raised under adequate food conditions ceased egg production on day 3 and days 1-4 after exposure to starvation, respectively. All females of *P. parvus* and *A. tonsa* lived for 7 and 5-10 days during starvation, respectively (Checkley 1980, Finiguerra et al. 2013). Because the individuals of these species survived several days into starvation, they might have an ability to accumulate energy under adequate food conditions and utilize it for metabolism under starvation without immediately exhausting the stored energy. The remaining energy within their bodies was used only for metabolism to survive (Finiguerra et al. 2013). On the other hand, the oceanic copepods *Neocalanus plumchrus* and *N. flemingeri* can live for more than 1 year under starvation conditions. They conduct both metabolism and egg production for approximately 2 months in the deeper sea layers without feeding (Tsuda et al. 2001). The neritic copepods *Calanus finmarchicus* and *Pseudocalanus newmani* can live for more than 20 days under starvation (e.g., Tsuda 1994). *C. finmarchicus* females that had been fed, ceased egg production at the start of starvation but resumed egg production after 3 days and continued to produce after this point (Runge 1984). Because oceanic and neritic copepods have more storage lipids for energy than the embayment copepods, both can live and continue to produce eggs for a longer period than embayment copepods (Lee et al. 2006).

A. steueri showed a remarkable tolerance to the abrupt decrease in food concentrations and starvation compared to the other embayment species. 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 during the starvation period and survived for more than 18 days. *A. steueri* individuals raised under high-food concentrations for 5 days produced more eggs than those raised under high-food concentrations for 2 days even after exposure to low food

concentrations or starvation. *A. steueri* produced more than 50 eggs under starvation conditions. With the exception of *A. steueri*, other *Acartia* copepods had carbon weights from egg production (40.9 ± 7.00 ngC egg⁻¹; Kjørboe et al. 1985) under starvation that exceeds the carbon weight of the female body, suggesting that the carbon budget under starvation scenarios might not be balanced. On the other hand, carbon weights of egg production for *A. steueri* under starvation was about 20% of the carbon weight of the female body thanks to their rapid energy accumulation abilities. The reason why the rapid energy accumulation is because *A. steueri* can accumulate free amino acids at a high rate for a short period of high-food concentration and the free amino acids convert to the fatty acids.

It is thought that *A. steueri* can accumulate energy rapidly during short periods of sporadic, high food availability in the environment. These energy reserves can then be utilized for metabolism and egg production during subsequent periods of low food availabilities until they encounter favorable food conditions again. Thus, these survival strategies and reproductive responses to abrupt changes in food concentrations might support populations of *A. steueri* in the embayment area.

SUMMARY

In Study 1, the presence of the extraordinarily heavy embayment copepod *Acartia steueri* in Sagami Bay, Japan was revealed. *A. steueri* can accumulate free amino acids, which can be converted to fatty acids under high-food conditions. In Study 2, respiration rates of *A. steueri* under not only the high-food but also starvation conditions were relatively low compared to the other temperate copepods, and *A. steueri* was shown to regulate its metabolism under starvation. Individuals survived for 18 days and continued to produce eggs for 14 days under starvation conditions. This species also showed a remarkable starvation tolerance to abrupt decreases in food concentrations and starvation. Rapid energy accumulation during high food periods thanks to low metabolic rates contributed to egg and fecal pellet production under insufficient food conditions. In the field, *A. steueri* accumulates energy, including free amino acids and fatty acids, into their bodies during short-term, sporadic high food conditions; then, they are able to endure low food conditions by using the stored energy until they encounter favorable food conditions again. Thus, adaptability to abrupt changes in food concentrations or starvation might support the population of *A. steueri* in the embayment area.

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