Feeding and reproduction including diapausing egg production as cold-water adaptations for overwintering of *Acartia steueri* (Copepoda, Calanoida) in Okkirai Bay, Sanriku, northern Japan

Yuichiro Yamada *, Wataru Sato, Atsushi Kobiyama & Takehiko Ogata

School of Marine Biosciences, Kitasato University, 1–15–1 Kitasato, Minami–ku, Sagamihara, 252–0373, Japan

Received 3 December 2019; Accepted 24 May 2020  Responsible Editor: Shinji Shimode
doi: 10.3800/pbr.15.269

**Abstract:** Feeding experiments with a natural microplankton assemblage and egg production experiments with the neritic copepod *Acartia steueri* were conducted simultaneously in an inlet on the Sanriku coast during autumn. Diapausing egg production status of *A. steueri* was also investigated. Further, *A. steueri* dominated during September to October and then gradually decreased until December, before disappearing after January. The dominant microplankton in natural waters during the study period were dinoflagellates, followed by centric and pennate diatoms and oligotrich ciliates. *A. steueri* fed only on dinoflagellates and oligotrich ciliates at rates of 87.0–309 and 17.7–71.5 cells ind⁻¹ d⁻¹, respectively. Egg production rate of *A. steueri* ranged from 1.6 to 15.0 eggs female⁻¹ d⁻¹ and significantly increased as ingestion rates of dinoflagellates and oligotrich ciliates increased. The copepod began producing diapausing eggs in October, and by late December, 86% of the eggs produced were diapausing. The population’s egg production rate was highest in September (18,961 eggs m⁻³ d⁻¹) and gradually decreased through the end of December. These dietary and diapausing egg production seasons of *A. steueri* in the Sanriku area are significantly different from the results of previous studies in the temperate zone, where the copepod mainly fed on diatoms and produced diapausing eggs during spring. *A. steueri* can alter its feeding habits and the timing of diapausing egg production in response to changes in habitat. The flexibility of this species to environmental change has likely allowed expansion of its geographical distribution.

**Key words:** copepod, *Acartia steueri*, feeding habits, egg production, diapausing egg

**Introduction**

In estuarine, coastal and inlet waters, geographic and seasonal variations of environmental conditions, such as water temperature, salinity, and chlorophyll *a* concentration, are substantial. Zooplankton that inhabit these waters are usually small, with opportunistic lifestyles that allow them to survive unfavorable conditions and exploit favorable ones (Mauchline 1998). Thus, seasonal occurrence, food habits, and reproductive patterns of coastal zooplankton vary greatly depending on habitat, even for a single species.

Among coastal zooplankton, copepods in the genus *Acartia* are widely distributed in estuarine and inlet waters worldwide (Mauchline 1998, and citations therein). *Acartia steueri* Smirnov, 1936 appears in such waters along the coast of the western North Pacific from the subarctic northern Japan Sea (Brotsky 1948, Kos 1958) to subtropical Okinawa (Nishida 1985). This species is often the most dominant copepod in coastal and inlet waters, and its numbers may exceed 10,000 individuals m⁻³, (Onoue et al. 2006, Yamada et al. 2012). Seasonal succession of this species varies depending on habitat. In temperate waters, its abundance increases from winter to spring then drops from summer to autumn (Jung et al. 2004, Onoue et al. 2006).

Conversely, in subarctic coastal waters, abundance increases from summer to autumn and drops dramatically from winter to spring (Uye 1983). Yamada et al. (2012)
reported that the abundance of *A. steueri* in Okkirai Bay in northern Japan peaked from September to October, then numbers for all developmental stages decreased sharply. Abundance was extremely low from January to June. Seasonal changes in abundance of copepods are primarily due to changes in recruitment of new individuals, i.e., the ability of females to produce viable eggs. Further, female egg production is influenced mainly by the availability of food (Mauchline 1998). Therefore, the relationship between food availability and female egg production of *A. steueri* varies by habitat. However, no information is currently available on the feeding habits of this copepod or the relationship between its feeding habits and egg production.

Another important factor that influences the seasonal abundance of copepods is the production of diapaus ing eggs. Several species of neritic copepods produce diapausing eggs that survive periods when environmental conditions are incompatible with their habitat requirements (Marcus 1996, Holm et al. 2018 and citations therein). *A. steueri* produces diapausing eggs with spines on the surface that differ from subitaneous eggs with a smooth surface (Uye 1983, Onoue et al. 2004). The number of diapausing eggs produced increases from spring to summer in temperate waters (Jung et al. 2004, Onoue et al. 2006), while in subarctic coastal areas, the number of diapausing eggs in bottom sediments increases in autumn (Uye 1983). However, no study has experimentally confirmed when and how many diapausing eggs are produced by adult *A. steueri* females.

In this study, the causes of geographical differences in seasonal succession were examined through responses in reproduction of *A. steueri* to environmental changes in an inlet on the Sanriku coast. Feeding on natural microplankton assemblages and egg production were assessed simultaneously during autumn when diapausing egg production in *A. steueri* is most active. The study aimed to clarify the relationship between feeding habits and egg production as well as elucidate features of this production. Geographical differences in strategies for maintaining dense populations of *A. steueri* are discussed.

### Materials & Methods

#### Feeding and egg production experiments

Field sampling for feeding and egg production evaluation occurred six times from 17 September, 2015, to 14 January, 2016, and seven times from 13 September, 2016, to 19 January, 2017. All samples were collected at the same station in Okkirai Bay (Fig. 1). To determine the abundance of mature *A. steueri* females (with a fully elongated genital segment), zooplankton samples were collected via vertical hauls of a net (mouth opening 30 cm, length 80 cm, mesh size 100 µm) from near the bottom to the surface. Plankton in the hauls were preserved in 5% buffered formalin-seawater immediately after collection. The amount of water filtered was measured with a flow meter (Rigo-sha Co., Ltd.) at the mouth of the net. Samples for feeding and egg production evaluation were collected by towing a 200 µm mesh net (mouth opening 30 cm, length 80 cm). Each time the net was retrieved, all contents were transferred to 1 L plastic bottles filled with surface seawater and transported to a land laboratory within 30 minutes. Seawater for *A. steueri* feeding and egg production experiments was collected from 3 m below the surface using a 6 L Van Dorn water sampler. Water was filtered through a 200 µm mesh to remove grazers, such as copepods, before being gently transferred into a 10 L carboy (Nalgene). A 100 ml aliquot of screened seawater was filtered using a GF/F filter to analyze *in situ* chlorophyll *a* concentration (Welschmeyer 1994). Vertical profiles of temperature were determined using a CTD system (ASTD102, JFE Advantech Co., Ltd.).

Feeding and egg production experiments were initiated within two hours after sample collection. A total of 40–50 mature *A. steueri* females were separated from the mixture of fresh zooplankton samples using a stereomicroscope. Copepods were kept in a plastic Petri dish filled with the filtered seawater until the start of the experiments. Screened seawater was added to ten 300 ml translucent polypropylene bottles (As One Co., Ltd.). A 300 ml sample was also taken from the filtered seawater and immediately fixed with acid Lugol’s solution (final concentration of 2%) to determine initial prey abundance. Five mature female *A. steueri* were added to each of seven bottles as experimental groups, and the remaining three bottles without copepods served as the controls. Bottles were placed at the three-meter depth from which copepod samples were taken and incubated *in situ* for 24 h. Except for wave action, the bottles were not stirred. However, calm waves in the bay provided gentle mixing that prevented microplankton sedimentation in bottles during *in situ* incubations. At the end of the incubation, the contents of each bottle were fixed with acid Lugol’s solution at a final concentration of 2% for enumeration of remaining prey organisms and eggs produced. When copepods are spawned in containers, cannibalism by females of spawned eggs is potentially a major source of error in the estimation of EPR (Runge & Roff 2000, and citations therein). However, eggs were not sepa-
Feeding and egg production characters of Acartia steueri

Field water temperatures and abundance of A. steueri

Maximum water temperatures were recorded on 17 September (20.2°C) in 2015 and 13 September (20.6°C) in 2016. Temperatures decreased with time, reaching lowest levels on 14 January (10.0°C) in 2016 and 19 January (9.2°C) in 2017 (Fig. 2). Chlorophyll a concentrations varied from 0.75 (5 November) to 0.97 µg L⁻¹ (2 December) in 2015 and from 0.72 (20 December) to 1.26 µg L⁻¹ (12 October) in 2016 (Fig. 2).

The abundance of adult female A. steueri increased from 1,267 ind. m⁻³ in September to 3,282 ind. m⁻³ in October, before decreasing substantially in mid-November (688 ind. m⁻³) in 2015 (Fig. 3). In 2016, A. steueri was most abundant in mid-September (2,317 ind. m⁻³) and decreased as time passed until late December (28 ind. m⁻³) (Fig. 3). A. steueri was not observed in January in 2016 or 2017.

Temporal changes of microplankton community structure

Because A. steueri was not present in January in 2016 or 2017, feeding experiments for A. steueri were conducted five times from 2 September to 2 December in 2015, and six times from 2 September to 20 December in 2016. Microplankton community structure (cell density of each microplankton taxa in the initial sample) was analyzed during these periods.

Dinoflagellates were most numerous in microplankton assemblages during the study period (Fig. 4), accounting for 42.6%–69.3% of total microplankton abundance. In 2015, the abundance of dinoflagellates was highest in September (11.4 cells ml⁻¹) then decreased with time until November (3.8 cells ml⁻¹). In 2016, dinoflagellates increased from 4.45 cells ml⁻¹ (13 September) to 13.1 cells ml⁻¹ (12 October) falling to 4.1 cells ml⁻¹ (20 December).

Pennate and centric diatoms were the second or third most dominant groups throughout the study period. In 2015, the diatom abundance decreased in September and...
October and peaked in December. In 2016, diatoms peaked in October, then fell with time until December (Fig. 4). Oligotrich ciliates were the second-most dominant taxa after diatoms and, depending on the period, were sometimes more abundant than diatoms (October and November 2015 and September 2016), however, their abundance was less than 2 cells ml\(^{-1}\) in other months. Tintinnids and silicoflagellates were rare, and abundance varied from 0 to 0.26 cells ml\(^{-1}\) (Fig. 4).

**Feeding of A. steueri**

In the feeding experiments, significant differences in cell density between the control and experimental groups were observed only for dinoflagellates and oligotrich ciliates at the end of the field incubation (Mann–Whitney U test, Table 1). Therefore, clearance and ingestion rates were calculated only for these organisms (Fig. 5). Clearance rates varied between 27.7 and 64.4 ml ind\(^{-1}\) d\(^{-1}\) and 20.3 and 77.0 ml ind\(^{-1}\) d\(^{-1}\) for dinoflagellates and oligotrich ciliates, respectively. Ingestion rates ranged from 87.0 to 309 cells ind\(^{-1}\) d\(^{-1}\) and 17.7 to 71.5 cells ind\(^{-1}\) d\(^{-1}\) for dinoflagellates and oligotrich ciliates, respectively. Ingestion rates were positively correlated with initial cell densities (both \(p<0.01, r^2=0.87\) and 0.80, respectively, Fig. 6). Total carbon ingestion ranged from 0.40 \(\mu\)g C ind\(^{-1}\) d\(^{-1}\) (12 November, 2015) to 1.09 \(\mu\)g C ind\(^{-1}\) d\(^{-1}\) (17 September, 2015) (Fig. 7). Percentage of carbon ingestion associated with oligotrich ciliates varied between 16.4% (5 November, 2015) to 39.7% (29 September, 2016) (Fig. 7).

**Egg production of A. steueri**

Smooth and spiny eggs produced by adult A. steueri females were incubated at in situ water temperature in November 2008. All smooth eggs hatched within two days, whereas spiny eggs did not hatch for at least 30 days.

---

**Fig. 4.** Changes in cell density of each microplankton taxon in the initial sample collected from 3 m depth in Okkirai Bay during September to December, 2015 and 2016.

**Fig. 5.** Acartia steueri. Changes in clearance rates (upper) and ingestion rates (lower) of each microplankton taxon.

---

**Table 1.** Comparisons of cell densities of six prey organisms between control and experimental bottles (mean±SD, cells ml\(^{-1}\)) after 24 h incubation by Mann–Whitney U test \(^*p<0.05, \text{ns: no significant}\). The cell sizes (major axis; mean±SD, \(\mu\)m) were indicated under each taxon.

| Date | Pennate diatoms (45.6±10.3 \(\mu\)m) | Centric diatoms (24.4±10.1 \(\mu\)m) | Dinoflagellates (35.4±8.7 \(\mu\)m) | Oligotrich ciliates (44.9±7.3 \(\mu\)m) | Tintinnids (106.6±22.0 \(\mu\)m) | Silicoflagellates (25.2±3.5 \(\mu\)m) |
|------|----------------------------------|-----------------------------------|---------------------------------|-----------------------------------|------------------------|-------------------------|
|      | control | experimental | control | experimental | control | experimental | control | experimental | control | experimental | control | experimental |
| 2015 |         |               |         |               |         |               |         |               |         |               |         |               |
| 17 Sep. | 1.7±0.7 | 2.2±0.5 ns | 7.7±2.2 | 8.4±1.3 ns | 5.8±0.4 | 2.1±0.3* | 1.3±0.1 | 0.3±0.1* | 0.2±0.0 | 0.2±0.1 ns | 0.1±0.1 | 0.1±0.0 ns |
| 1 Oct. | 1.2±0.2 | 1.1±0.3 ns | 1.0±0.3 | 1.5±0.6 ns | 4.9±1.0 | 2.3±0.3* | 0.9±0.1 | 0.5±0.1* | 0.3±0.2 | 0.3±0.1 ns | 0.3±0.0 | 0.3±0.0 ns |
| 5 Nov. | 1.2±0.1 | 1.1±0.1 ns | 1.1±0.2 | 1.1±0.1 ns | 6.6±0.4 | 3.3±0.5* | 0.8±0.2 | 0.5±0.1* | 0.0±0.0 | 0.0±0.0 ns | 0.6±0.1 | 0.5±0.1 ns |
| 12 Nov. | 3.1±0.4 | 3.0±0.3 ns | 3.1±0.5 | 3.7±0.2 ns | 2.9±0.3 | 1.5±0.2* | 0.7±0.1 | 0.4±0.1* | 0.0±0.0 | 0.0±0.0 ns | 0.3±0.0 | 0.4±0.1 ns |
| 2 Dec. | 3.3±0.1 | 3.4±0.1 ns | 2.9±0.1 | 3.0±0.2 ns | 5.4±0.2 | 2.9±0.3* | 1.1±0.1 | 0.8±0.1* | 0.0±0.0 | 0.0±0.0 ns | 0.1±0.0 | 0.1±0.0 ns |
| 2016 |         |               |         |               |         |               |         |               |         |               |         |               |
| 13 Sep. | 0.3±0.1 | 0.3±0.1 ns | 1.6±0.2 | 1.8±0.3 ns | 3.2±0.8 | 1.2±0.3* | 0.8±0.1 | 0.3±0.1* | 0.1±0.1 | 0.1±0.0 ns | 0.0±0.0 | 0.0±0.0 ns |
| 29 Sep. | 0.1±0.1 | 0.1±0.1 ns | 0.9±0.8 | 0.7±0.3 ns | 3.9±0.7 | 1.4±0.2* | 1.7±0.1 | 0.6±0.1* | 0.2±0.1 | 0.2±0.0 ns | 0.3±0.1 | 0.2±0.0 ns |
| 12 Oct. | 2.8±0.6 | 2.6±0.2 ns | 5.5±0.4 | 5.7±0.3 ns | 11.9±0.8 | 7.3±0.8* | 0.8±0.1 | 0.4±0.1* | 0.1±0.0 | 0.0±0.0 ns | 0.2±0.0 | 0.2±0.0 ns |
| 17 Nov. | 1.5±0.2 | 1.5±0.2 ns | 2.9±0.2 | 3.0±0.2 ns | 7.5±0.7 | 4.7±0.5* | 1.3±0.1 | 0.7±0.1* | 0.2±0.0 | 0.1±0.0 ns | 0.3±0.0 | 0.2±0.1 ns |
| 1 Dec. | 1.6±0.2 | 1.6±0.2 ns | 2.0±0.2 | 2.0±0.2 ns | 3.4±0.3 | 1.5±0.3* | 0.7±0.0 | 0.4±0.1* | 0.1±0.0 | 0.1±0.0 ns | 0.1±0.0 | 0.1±0.0 ns |
| 20 Dec. | 0.3±0.0 | 0.3±0.1 ns | 0.9±0.1 | 1.0±0.1 ns | 3.5±0.2 | 2.2±0.3* | 0.7±0.0 | 0.4±0.1* | 0.1±0.0 | 0.1±0.0 ns | 0.3±0.1 | 0.2±0.0 ns |
Feeding and egg production characters of *Acartia steueri* (Takemoto & Yamada, unpublished data). Thus, smooth and spiny eggs produced by adult *A. steueri* females were defined as subitaneous and diapausing eggs, respectively.

In 2015, egg production rate (EPR), including both subitaneous and diapausing eggs, was highest on 17 September (15.0 eggs female$^{-1}$ d$^{-1}$), decreased to 2.2 eggs female$^{-1}$ d$^{-1}$ on 1 October, and then varied from 1.3 to 2.8 eggs female$^{-1}$ d$^{-1}$ until 2 December (Fig. 8). In 2016, the EPR gradually increased from September and reached the maximum on 12 October (7.8 eggs female$^{-1}$ d$^{-1}$). It subsequently decreased and varied from 1.6 to 2.6 eggs female$^{-1}$ d$^{-1}$ from November to December (Fig. 8). In 2015, diapausing eggs were first confirmed on 1 October and represented 43.4% of total egg production on 5 November (Fig. 8). However, in 2016, diapausing egg production was not observed between September and November, occurring only in December (Fig. 8). A significant positive correlation was observed between EPR and the sum of the carbon ingestion rates of dinoflagellates and oligotrich ciliates ($r^2=0.79$, $p<0.01$, Fig. 9).

In 2015, population EPR, determined by multiplying the number of adult females in the field with the EPR per adult female, decreased significantly from September to October (18,961 to 7,720 eggs m$^{-3}$ d$^{-1}$) and further decreased from early to mid-November (7,329 to 885 eggs m$^{-3}$ d$^{-1}$) (Fig. 10). In 2016, population EPR increased greatly from mid to late September (9,201 to 15,827 eggs m$^{-3}$ d$^{-1}$), maintained a high value until mid-October (14,615 eggs m$^{-3}$ d$^{-1}$), then...
Discussion

Feeding habits

In the Sanriku coastal area, four Acartia copepods appear alternately. A. longiremis (Lilljeborg, 1853) and A. omorii Bradford, 1976 dominate from March to May, and A. hudsonica Pinhey, 1926 is abundant from May to September. In contrast, A. steueri abundance increases during the summer with a peak in September, then decreases until December or January (Yamada et al. 2012). This seasonal cycle is consistent with the cycle in Onagawa Bay, located in northern Japan (Uye 1983). In temperate waters, A. steueri is most abundant during May to June in Sagami Bay, central Japan (Onoue et al. 2006) and June to July in Ilkwang Bay, on the southeastern coast of Korea (Kang & Kang 2005). In the above waters the temperature at which A. steueri is most abundant is approximately 20°C. Therefore, the optimum water temperature for A. steueri is about 20°C, regardless of the habitat. The dominant microplankton taxa during the period when A. steueri was abundant in Okkirai Bay was dinoflagellates (Fig. 4), which were the primary food source in the microplankton assemblage (Fig. 5). For a particulate feeding copepod, taking advantage of the dominant microplankton in the water column is the most efficient method for obtaining food. Turner & Tester (1989) investigated the feeding habits of A. tonsa and concluded that species of phytoplankton were primarily consumed in proportion to their abundance (Mauchline 1998). However, the cell density of oligotrich ciliates was lower than the densities of dinoflagellates and even diatoms (Fig. 4). Furthermore, A. steueri actively fed on oligotrich ciliates, with high clearance rates (Fig. 5). High ingestion and clearance rates for oligotrich ciliates may be owing to the higher motility of these prey. Unlike non-motile prey, oligotrich ciliates more frequently encounter copepods. Additionally, motile prey should be more easily detected by predators because their swimming generates a hydrodynamic signal (Jonsson & Tiselius 1990, Putland & Iverson 2007). Oligotrich ciliates are nutritionally advantageous prey because they account for up to nearly 40% of the total carbon uptake (Fig. 7). Abundance of oligotrich ciliates in the study area was highest in autumn (Kobayashi 2011). Therefore, along with dinoflagellates, oligotrich ciliates play an important role for meeting the food demands of A. steueri during periods of abundance in late summer to autumn.

No information is available on the feeding behaviors of A. steueri on microplankton assemblages in temperate waters, however, abundances of this copepod were the highest when chlorophyll a concentration in the water column peaked in Sagami Bay (Onoue et al. 2006) and Ilkwang Bay (Kang & Kang 2005). High chlorophyll a concentrations in these areas is due to an increase in diatoms (Onoue et al. 2006, Kang & Kang 2005), and diatoms are considered the main diet of the species in these areas.

In the present study, A. steueri did not feed on diatoms and instead focused its feeding behavior on dinoflagellates and oligotrich ciliates. This behavior is entirely different from its feeding habits in the above two temperate waters. Several cases exist in which copepod diet changes depending on environment. For example, A. tonsa usually feeds primarily on ciliates, but when diatoms are abundant, the feeding rate on diatoms increases (Saiz & Kiorboe 1995, Kiorboe et al. 1996). Further, Yang et al. (2010) reported that microzooplankton account for >70% of total carbon ingestion by A. hongi throughout the year, except during the winter diatom blooming periods, when about 60% of carbon intake is from phytoplankton in Gyeonggi Bay, Yellow Sea. A. steueri may also can alter its feeding habits in response to habitat changes. For the Sanriku coast, three Acartia copepods (A. longiremis, A. omorii, and A. hudsonica) predominate in spring when chlorophyll a concentrations are high (1.88 to 2.13 µg L⁻¹, Yamada et al. 2012). Diatoms dominate phytoplankton assemblages during these periods (Tachibana et al. 2017), causing high chlorophyll a concentrations. Therefore, these three Acartia species likely feed primarily on diatoms in the spring. A. steueri is thought to separate its diet by feeding primarily on dinoflagellates and ciliates. As a result, its population increases in autumn, when other Acartia copepods are not abundant.

Egg production

Population growth is the result of increased recruitment of new individuals, in this case, increased egg production. In the present study, the EPR of A. steueri increased as feeding rates on dinoflagellates and oligotrich ciliates increased (Fig. 9). These results are consistent with Kleppel (1991), where the egg production of A. tonsa was positively correlated with ingestion rates of dinoflagellates and ciliates. Dutz & Peters (2008) reported that A. clausi obtains 86% of daily organic carbon consumption from oligotrich ciliates and the remainder from thecate dinoflagellates.

However, several studies suggest that active consumption of diatoms increases egg production in A. steueri. Jung et al. (2004) showed that the EPR of this species in Ilkwang Bay, on the southeastern coast of Korea, is positively correlated with the chlorophyll a concentration in the field. Because high chlorophyll a levels are owing to diatom growth (Kang & Kang 2005), high EPR of this copepod is associated with active feeding on diatoms. Additionally, Onoue et al. (2006) reported that peak periods of chlorophyll a concentration and EPR of A. steueri are almost coincidental in Sagami Bay. A. steueri likely feeds on microplankton primarily in proportion to their abundance in the water column, and produces eggs efficiently by immediate use of ingested organic carbon. In contrast,
several studies report negative effects on copepod egg production when fed on diatoms, due to a lack of sufficient nutritional content (Ban et al. 1997, Paffenhöfer et al. 2005, and citations therein). However, for *A. steueri*, there were no apparent differences in EPRs among habitats (Table 2), suggesting that EPR may not be affected by variations in food type.

**Timing of diapausing egg production**

*A. steueri* begins producing diapausing eggs from the end of summer to the beginning of autumn. This period coincides with the time when abundance reaches its peak and starts to decline. The abundance of this copepod is extremely low between winter and the following summer, which generally coincides with the time when abundance reaches its peak. This period is the main diapausing egg production period of this copepod, was higher in 2016 than the same period in 2015 (Iwate Fisheries Technology Center 2016), and may have delayed the onset of diapausing egg production.

Photoperiod is also an important factor in the production of copepod diapausing eggs (Holm et al. 2018, and citations therein). Uye (1983) reared *A. clausi* under a variety of temperature and photoperiodic conditions. The study showed that numbers of diapausing eggs produced changed more when the photoperiod was altered under the same temperature conditions than when water temperature was altered under the same photoperiod conditions. Chinnery & Williams (2003) investigated the effect of temperature and photoperiod on the production of diapausing eggs by *A. bifinosa* and suggested that photoperiod is the primary proximate cue for induction of diapausing egg production. In contrast, Avery (2005) examined the influence of temperature and photoperiod using *A. hudsonica* and found that temperature was the primary driver for production of diapausing eggs. In the present study, temperature and photoperiodic conditions could not be set arbitrarily because bottles containing copepods were placed *in situ*. Seasonal changes in daylength are the same in all years, but the onset of diapausing egg production was later in 2016 than the same period in 2015 (Iwate Fisheries Technology Center 2016). Therefore, temperature variation, rather than photoperiod variation, may be the primary driver for production of diapausing eggs in Okkirai Bay. Water temperature between September to October in 2016 was approximately 0.5–1°C warmer than the same period in 2015 (Iwate Fisheries Technology Center 2016), and may have delayed the onset of diapausing eggs production.

In addition to water temperature and photoperiod, diapausing egg production is triggered by various factors, e.g., abrupt salinity changes, crowding, oxygen deficiency and low food availability (Holm et al. 2018, and citations therein). In this study area, the cell density of dinoflagellates during October to November, which is the main diapausing egg production period of this copepod, was higher in 2016

### Table 2. Comparisons of the egg production rates (EPR) of *Acartia steueri* in different waters.

| Area                        | Temperature (°C) | EPR (eggs female⁻¹ d⁻¹) | Reference     |
|-----------------------------|------------------|--------------------------|---------------|
| Ilkwang Bay, SE Korea       | 11.0–25.4        | 5.4–12.5                 | Kang & Kang (1998) |
| Ilkwang Bay, SE Korea       | 11.5–25.6        | 3.9–10.1                 | Jung et al. (2004) |
| Sagami Bay, Central Japan   | 12.6–26.8        | 1.3–11.9                 | Onoue et al. (2006) |
| Okkirai Bay, NE Japan       | 11.9–20.6        | 1.3–15.0                 | This study     |

### Table 3. Water temperature, day length* and ratio of diapausing eggs to total egg production of *A. steueri* females on each experimental day.

| Date    | Temperature (°C) | Day length (h)* | % of diapausing eggs |
|---------|------------------|-----------------|----------------------|
| 2015    |                  |                 |                      |
| 17 Sep. | 20.5             | 12.4            | 0                    |
| 1 Oct.  | 19.3             | 11.8            | 5.2                  |
| 5 Nov.  | 16.4             | 10.4            | 43.4                 |
| 12 Nov. | 15.9             | 10.1            | 4.4                  |
| 2 Dec.  | 13.8             | 9.6             | 19.4                 |
| 2016    |                  |                 |                      |
| 13 Sep. | 20.6             | 12.6            | 0                    |
| 29 Sep. | 20.2             | 11.9            | 0                    |
| 12 Oct. | 19.4             | 11.3            | 0                    |
| 17 Nov. | 15.0             | 9.9             | 0                    |
| 1 Dec.  | 14.2             | 9.6             | 30.9                 |
| 20 Dec. | 11.9             | 9.4             | 86.2                 |

*calculated by: https://eco.mtk.nao.ac.jp/koyomi/dni/*
(8.6–13.1 cells ml\(^{-1}\)) than in 2015 (3.8–7.2 cells ml\(^{-1}\)) (Fig. 4). Considering that the most important food items of this copepod are dinoflagellates, it is possible that higher food availability in addition to the higher water temperature delayed the production of diapausing eggs in this copepod.

Seven species of copepods are known to change the timing of diapausing egg production depending on water temperature (Holm et al. 2018). Four of these species are *Acartia* copepods, including *A. steueri*. Species are distributed widely, from the Baltic Sea (Vittasalo 1992) to Jiaozhou Bay, China (Zhong & Xiao 1992) for *A. bifolosa*; from southern Norway (Naess 1996) to the Seto Inland Sea, Japan (Kasahara et al. 1975) for *A. clausi*; from the estuary of the Amur River (Brodsky 1948) to the southwest of India (Wellershous 1969) for *A. pacifica*; and the northern Japan Sea (Brodsky 1948) to Okinawa, Japan (Nishida 1985) for *A. steueri*. These *Acartia* copepods also show a wide distribution across latitudes and, thus, inhabit a wide range of water temperatures. The ability of *Acartia* to produce diapausing eggs in response to both low and high water temperatures has likely been a factor in expanding their geographic distribution.

The timing of onset of diapausing egg production of *A. steueri* differed between subarctic (this study, Uye 1983) and temperate waters (Onoue et al. 2006), although it coincided with the time when the population abundance of the species turns from peak to decline. This production pattern is confirmed in other *Acartia* copepods (Castro-Longoria & Williams 1999). Accumulation of diapausing eggs on the seafloor is maximized by matching the beginning of egg production with peak abundance. The observation that the number of diapausing eggs of *A. steueri* in the bottom mud of Onagawa Bay is highest at peak abundance is consistent with this conclusion (Uye 1983). Further, *A. bifolosa* and *A. tonsa* in the Baltic Sea show the highest number of diapausing eggs on the seafloor at the time of peak adult abundance (Katajisto et al. 1998). The coincidence of the onset of diapausing egg production with peak abundance is a major advantage for copepods for maintaining dense populations at the margins of their range. *A. steueri* is known to live in enclosed bays (Ueda 2001) and it is assumed that the influence of populations from outside of such closed waters would be minimal (Onoue et al. 2006). In Okkirai Bay, the distribution of *A. steueri* is limited to the shallower shore, and it does not occur in the center part and/or the mouth of the bay (depth ca. 60 and 90 m, respectively, Yamada unpublished data). Okkirai Bay is geographically open in shape, however, it is suggested that the population of this copepod in the inner part of the bay is isolated from neighboring waters. Therefore, the presence of diapausing eggs in the sediments may be an important source of recruits for maintaining the population, especially during winter.

Among neritic *Acartia* copepods, *A. tonsa* also has a wide geographical distribution and different life history strategies including diapausing egg production processes, in each habitat (Caudill & Bucklin 2004, and citations therein). Molecular techniques revealed that *A. tonsa* has experienced geographical isolation resulting in the divergence of multiple, distinct cryptic lineages (Caudill & Bucklin 2004, Chen & Hare 2011, Costa et al. 2014, Figueroa et al. 2020). The results of these studies indicate the possible presence of cryptic species also in *A. steueri*, and molecular biological methods are considered to be extremely useful for elucidating the diversity of life histories in this copepod.

**Conclusion**

Characteristic feeding habits, reproduction, and timing of diapausing egg production by *A. steueri* along the Sanriku coastal area are significantly different from those of copepods in temperate waters reported previously. This copepod is considered to possess the ability to change its feeding habits, physiological responses to nutritional conditions, and diapausing egg production timing depending on the habitat. Therefore, this species’ physiological flexibility to environmental changes, and expected genetic variability and the presence of cryptic species may explain its wide latitudinal distribution from subarctic to subtropical waters.

**Acknowledgements**

We are grateful to the staff of the Sanriku Education and Research Center for Marine Biosciences, Kitasato University for helping with field sampling and experiments. Part of this study was supported by the research program “Tohoku Ecosystem-Associated Marine Science” from the Ministry of Education, Culture, Sports, Science and Technology in Japan.

**References**

Avery D (2005) Induction of embryonic dormancy in the calanoid copepod *Acartia hudsonica*: Heritability and phenotypic plasticity in two geographically separated populations. J Exp Mar Biol Ecol 314: 215–225.

Ban S, Burns C, Castel J, Chaudron Y, Christou E, Escribano R, Umani SF, Gasparini S, Ruiz FG, Hoffmeyer M, et al. (1997) The paradox of diatom-copepod interactions. Mar Ecol Prog Ser 157: 287–293.

Brodsky KA (1948) Free-living copepod crustaceans (Copepoda) of the Sea of Japan. Izv Tikhookean Nauchno-issled Inst ryb Khoz Okeanogr 26: 3–130. (in Russian)

Castro-Longoria E, Williams, JA (1999) The production of sublittoral and diapause eggs: a reproductive strategy for *Acartia bifolosa* (Copepoda: Calanoida) in Southampton Water, UK. J Plankton Res 21: 65–84.

Caudill CC, Bucklin A (2004) Molecular phylogeography and evolutionary history of the estuarine copepod, *Acartia tonsa*, on the Northwest Atlantic coast. Hydrobiologia 511: 91–102.
Chen G, Hare MP (2011) Cryptic diversity and comparative phyl-ogeography of the estuarine copepod Acartia tonsa on the US Atlantic coast. Mol Ecol 20: 2425–2441.

Chinnery FE, Williams JA (2003) Photoperiod and temperature regulation of diapause egg production in Acartia biflosa from Southampton Water. Mar Ecol Prog Ser 263: 149–157.

Costa, KG, Filho LFSR, Costa, RM, Vaillinoto M, Schneider H, Sampaio I (2014) Genetic variability of Acartia tonsa (Crustacea: Copepoda) on the Brazilian coast. J Plankton Res 36: 1419–1422.

Dagg M (1977) Some effects of patchy food environments on copepods. Limnol Oceanogr 22: 99–107.

Dutz J, Peters J (2008) Importance and nutritional value of large ciliates for the reproduction of Acartia clausi during the post spring-bloom period in the North Sea. Aquat Microbial Ecol 50: 261–277.

Figueroa NJ, Figueroa DF, Hicks D (2020) Phylogeography of Acartia tonsa Dana, 1849 (Calanoida: Copepoda) and phylogenetic reconstruction of the genus Acartia Dana, 1846. Mar Biodivers 50: 1–20.

Frost BW (1972) Effects of size and concentration of food particles on the feeding behavior of the marine planktonic copepod Calanus pacificus. Limnol Oceanogr 17: 805–815.

Holm MW, Kiorboe T, Brun P, Licandro P, Almeda R, Hansen BW (2018) Resting eggs in free living marine and estuarine copepods. J Plankton Res 40: 2–15.

Holmborn T, Dahlgren K, Holeton C, Hogfors H, Gorokhova E (2009) Biochemical proxies for growth and metabolism in Acartia biflosa (Copepoda, Calanoida). Limnol Oceanogr Methods 7: 785–794.

Iwate Fisheries Technology Center (2016) Preliminary report of observation results in the Iwate Prefecture coastal area. Available at: https://www2.suigi.pref.iwate.jp/download/dl_k_research01 (accessed on 1 May 2020)

Jonsson PP, Tiselius P (1990) Feeding behaviour, prey detection and capture efficiency of the copepod Acartia tonsa feeding on planktonic ciliates. Mar Ecol Prog Ser 60: 35–44.

Jung Y, Kang H, Kang Y (2004) In situ egg production rate of the planktonic copepod Acartia steueri in Ilkwang Bay, southeastern coast of Korea. J Plankton Res 26: 1547–1553.

Kang HK, Kang YJ (1998) Egg production of the copepod Acartia steueri in Ilkwang Bay, southeastern coast of Korea. J Korean Fish Soc 31: 288–295. (in Korean with English abstract)

Kang HK, Kang YJ (2005) Production of Acartia steueri (Copepoda: Calanoida) in Ilkwang Bay, southeastern coast of Korea. J Oceanogr 61: 327–334.

Kasahara S, Uye S, Onbê T (1975) Calanoid copepod eggs in seabottom muds. II. Seasonal cycles of abundance in the populations of several species of copepods and their eggs in the Inland Sea of Japan. Mar Biol 31: 25–29.

Katajisto T, Viitasalo M, Koski M (1998) Seasonal occurrence and hatching of calanoid eggs in sediments of the northern Baltic Sea. Mar Ecol Prog Ser 163: 133–143.

Kiorboe T, Saiz E, Viitasalo M (1996) Prey switching behaviour in the planktonic copepod Acartia tonsa. Mar Ecol Prog Ser 143: 65–75.

Kleppel GS (1991) Trophic interactions between copepods and microplankton: A question about the role of diatoms. Limnol Oceanogr 36: 172–178.

Kleppel GS (1992) Environmental regulation of feeding and egg production by Acartia tonsa off southern California. Mar Biol 112: 57–65.

Kleppel GS, Hazzard S (2000) Diet and egg production of the copepod Acartia tonsa in Florida Bay. II. Role of the nutritional environment. Mar Biol 137: 111–121.

Kobayashi T (2011) Analysis of microplankton food web structure on Sanriku Rias coast. MSc thesis. Kitasato University, Japan. (in Japanese)

Kos MS (1958) Some data on the coastal planktonic Copepoda from South-Kuril Bay. Dokl Akad Nauk SSSR 120: 191–192. (in Russian)

Mauchline J (1998) The biology of calanoid copepods. Adv Mar Biol 33: 1–710.

Marcus NH (1996) Ecological and evolutionary significance of resting eggs in marine copepods: Past, present, and future studies. Hydrobiologia 320: 141–152.

Menden-Deuer, S, Lessard EJ (2000) Carbon to volume relations of diñoflagellates, diatoms, and other protist plankton. Limnol Oceanogr 45: 569–579.

Nies T (1996) Benthic resting eggs of calanoid copepods in Norwegian enclosures used in mariculture: abundance, species composition and hatching. Hydrobiologia 320: 161–168.

Nishida S (1985) Pelagic copepods from Kabira Bay, Ishigaki Island, southwestern Japan, with the description of a new species of the genus Pseudodiaptomus. Publ Seto Mar Biol Lab 30: 125–144.

Onoue Y, Toda S, Ban S (2004) Morphological features and hatching patterns of eggs in Acartia steueri (Crustacea, Copepoda) from Sagami Bay, Japan. Hydrobiologia 511: 17–24.

Onoue Y, Shimode S, Toda T, Kikuchi T (2006) Reproductive strategy of Acartia steueri in Sagami Bay, Japan. Coastal Mar Sci 30: 353–359.

Paßlénhöfer GA, Ianora A, Miralto A, Turner JT, Kleppel GS, d’Alcala MR, Casotti R, Caldwell GS, Pohner G, Fontana A, et al. (2005) Colloquium on diatom-copepod interactions. Mar Ecol Prog Ser 286: 293–305.

Putland JN, Iverson RL (2007) Ecology of Acartia tonsa in Apalachee Bay, Florida, and implications of river water diversion. Mar Ecol Prog Ser 340: 173–187.

Runge JA, Roff JC (2000) The measurement of growth and reproductive rates. In: ICES Zooplankton Methodology Manual (eds Harris RP, Wiebe PH, Skjoldal HR, Huntley M) Academic Press, London, pp. 401–444.

Saiz E, Kiorboe T (1995) Predatory and suspension feeding of the copepod Acartia tonsa in turbulent environments. Mar Ecol Prog Ser 122: 147–158.

Tachibana A, Nishibe Y, Fukuda H, Kawanobe K, Tsuda A (2017) Phytoplankton community structure in Otsuchi Bay, northeastern Japan, after the 2011 off the Pacific coast of Tohoku Earthquake and tsunami. J Oceanogr 73: 55–65.

Turner JT, Tester PA (1989) Zooplankton feeding ecology: non-selective grazing by the copepods Acartia tonsa, Centropages velificatus De Oliveira, and Eucalanus pileatus Giesbrecht in the plume of the Mississippi River. J Exp Mar Biol Ecol 126: 21–43.

Ueda H (2001) Ecology of inlet copepods. Kaiyo Monthly Spec
Y. Yamada et al.

Vol 27: 96–104. (in Japanese)
Uye S (1983) Seasonal cycle in abundance of resting eggs of *Acartia steueri* Smirnov (Copepoda, Calanoida) in sea-bottom mud of Onagawa Bay, Japan. Crustaceana 44: 103–105.
Uye S (1985) Resting egg production as a life history strategy of marine planktonic copepods. Bull Mar Sci 37: 440–449.
Viitasalo M (1992) Calanoid resting eggs in the Baltic Sea: implications for the population-dynamics of *Acartia bifilosa* (Copepoda). Mar Biol 114: 397–405.
Wellershaus S (1969) On the taxonomy of planktonic Copepoda in the Cochin Backwater (a south Indian estuary). Veröffentlichungen des Instituts für Meeresforschung Bremerhaven 9: 245–286.
Welschmeyer NA (1994) Fluorometric analysis of chlorophyll *a* in the presence of chlorophyll *b* and pheopigments. Limnol Oceanogr 39: 1985–1992.
Yamada Y, Kobiyama A, Ogata T (2012) Seasonal succession of four *Acartia* copepods (Copepoda, Calanoida) in Okkirai Bay, Sanriku, northern Japan. Plankton Benthos Res 7: 188–194.
Yang, EJ, Ju SJ, Choi JK (2010) Feeding activity of the copepod *Acartia hongi* on phytoplankton and micro-zooplankton in Gyeonggi Bay, Yellow Sea. Estuar Coast Shelf Sci 88: 292–301.
Zhong X, Xiao Y (1992) Resting eggs of *Acartia bifilosa* Giesbrecht and *A. pacifica* Steuer in Jiaozhou Bay. Mar Sci 5: 55–59.