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Seasonal variations in the population structure and depth distribution of *Calanus sinicus* in northern Kagoshima Bay

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Abstract: We investigated seasonal variations of population structure and depth distribution of *Calanus sinicus* in northern Kagoshima Bay. Animals were abundant during April to June when chlorophyll *a* concentrations were high. Young copepodites or adults appeared over the seasons, however, copepodite stage 5 dominated when the population abundance was the lowest during August 2009 and July 2010. We found little indication of seasonal vertical migration since all developmental stages occurred above 50 m and below 100 m throughout the year. RNA:DNA ratios revealed no significant decline throughout the year. These results suggest that *Calanus sinicus* reproduces throughout the year and does not experience a deep-water dormancy period in northern Kagoshima Bay.

Keywords : *Calanus sinicus*, population structure, depth distribution, nucleic acids

Introduction

Calanoid copepods are among the most diverse groups of crustacean zooplankton (more than 2000 species; RAZOULS *et al*., 2005–2013), contributing significantly to marine biomass (VERITY and SMETACEK, 1996) with greater than half of the global abundance of zooplankton (LONGHURST, 1985). These copepods integrate carbon flows from lower trophic levels of both the classical and microbial food webs because they consume phytoplankton, heterotrophic microzooplankton, and sinking detrital particles (DAGG, 1993; GIFFORD, 1993; KOBARI *et al*., 2003). They represent a major food resource for epi- and mesopelagic fishes (BRODEUR *et al*., 1999; MOKU *et al*., 2000; YAMAMURA *et al*., 2002), marine mammals (KAWAMURA, 1982), and seabirds (RUSSELL *et al*., 1999). Thus, an understanding of trophodynamics in marine ecosystems demands careful study of the population dynamics of calanoid copepods.

Calanus sinicus Brodsky (1965) is widely distributed around coastal areas in East Asia (e.g., HULSEMANN, 1994) where it contributes the biomass of zooplankton communities (UYE and SHIMIZU, 1997; UYE *et al*., 1999). However, the life history of *Calanus sinicus* is quite variable. For example, reproduction occurs throughout the year in the Inland Sea of Japan (HUANG *et al*., 1992,

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1993a, b), whereas stage 5 copepodites (C5) are found in abundance at depth in Sagami Bay (NONOMURA *et al*., 2008) and the Yellow Sea (PU *et al*., 2004a, b), indicating a dormant life history phase. In the Yellow Sea, they produce dormant stock residing at depth during the food-scarce summer and increase their abundance during the food-rich seasons (PU et al., 2004a, b; ZHANG *et al*. 2007). UYE (2000) reviewed that this species prospered in the shelf ecosystem with not only suitable ambient temperature and food supply for reproduction and development but also enough depth for diel vertical migration and egg hatching before sink to bottom. However, there is little information on the life cycle prospering in the region with suitable temperature, food availability and depth.

Kagoshima Bay is a large semi-enclosed embayment located at the southernmost part of Kyushu, Japan. This embayment is characterized by deep (deeper than 200 m) bathymetry. The deep and semi-enclosed nature of the embayment in the northern area promotes deep-water hypoxia during autumn to winter period. Although we know that *C. sinicus* occurs in the northern area of Kagoshima Bay (NOZAWA and SAISYO, 1980), there is little knowledge of seasonal variations in their population structure and depth distribution. We expect the specific life cycle with continuous reproduction and without dormancy in the northern area of Kagoshima Bay due to 1) the sufficient depth for vertical migration and egg hatching, 2) the limited water exchange in the semi-enclosed embayment and 3) the suitable temperatures and food supply for growth and egg production (KOBARI *et al*. 2002, 2009).

The objective of this study was to clarify a year-round reproduction and dormant stock of *C. sinicus* in the northern area of Kagoshima Bay. Our hypothesis is that the population's life cycle is adapted to the high temperature, high food availability and deep water column, and is characterized by year-round reproduction with no dormant stock. We tested our hypothesis by monitoring seasonal changes in population structure and depth distribution with monthly sampling. In addition, we measured nucleic acids contents of the C5s in order to evaluate the potential for dormancy similar to other subarctic Pacific copepod groups (KOBARI *et al*., 2013).

Materials and Methods

Oceanographic observations and zooplankton sampling

Oceanographic observations and zooplankton sampling in Kagoshima Bay were carried out on a monthly basis, between April 2009 to December 2010 at S16 (31° 39.9′ N, 130° 41.9′ E: 140–m deep; Fig. 1). All sampling was carried out during research cruises of the T/S Nansei-Maru. Temperature and salinity profiles down to 135 m were carried out with CTD system (Seabird SBE–9). Water samples for chlorophyll *a* and dissolved oxygen measurements were collected from 8 depths (10, 20, 30, 50, 75, 100, 125 and 135 m) with a CTD-CMS system and from the sea surface with a plastic bucket. These samples were filtered through a Whatman GF/F filter (0.7–μm nominal pore size) under vacuum pressure <20kPa. Chlorophyll pigments were immediately extracted from material retained on filter papers by direct immersion into *N, N*-dimethylformamide (DMF) at –5℃ in darkness for more than 24 hours (SUZUKI and ISHIMARU, 1990). Chlorophyll *a* concentration was measured with a fluorometer (Turner Designs Co., TD–700) by the non-acidified fluorometric method (WELSCHMEYER, 1994). Dissolved oxygen was determined by the Winkler titration method.

Zooplankton collections and identification

Zooplankton samples for enumeration were col-

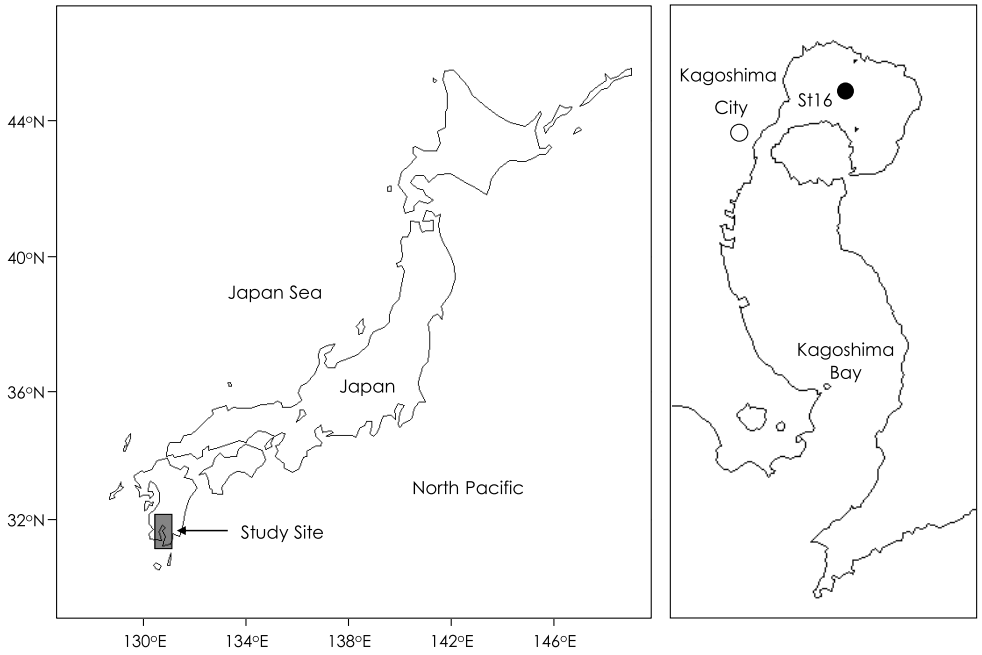

Fig. 1. Location of oceanographic observations and zooplankton sampling in northern area of Kagoshima Bay (S16: 31° 40.2'N, 130° 41.6'E).

lected with a Vertical Multiple Plankton Sampler (0.25-m^2 mouth square, 0.1-mm mesh size; TERAZAKI and TOMATSU, 1997) hauled at 1 m sec^{-1} during the day time (09:00–11:00) for each cruise. The following discrete depth intervals were sampled on the upcast: 0–50, 50–100 and 100–135 m. Subsamples for enumeration were preserved in a 5% borax-buffered formaldehyde seawater solution. Individual *C. sinicus* were identified by stage for copepodites (C1–C5) and by sex (i.e., male or female) for adults (C6). All animals were examined under a stereo dissecting microscope (Nikon SMZ1200).

Fresh zooplankton samples for biochemical analyses were collected with vertical net hauls (0.5 m sec^{-1}) from near bottom to sea surface using a modified North Pacific Standard net (MOTODA, 1957: diameter 45 cm, mesh size 0.335 mm) equipped with a large cod end (2L). On deck, individual specimens were quickly identified to species and developmental stage under a dissecting microscope. *C. sinicus* C5s picked for biochemical analyses were placed in 2 mL vials, stored at –80℃, and transported back to land for laboratory analyses of nucleic acids contents.

Nucleic acids analyses

We measured RNA and DNA with the microplate fluorescent assay (MFA) developed by WAGNER *et al.* (1998). The MFA assay is a modification of the sequential fluorometric method of BENTLE *et al.* (1981), in which DNA and RNA in a single sample are determined sequentially by the addition of DNase and RNase using Ethidium Bromide as fluorescent dye (see CALDARONE *et al.*, 2006 for details). WAGNER *et al.* (1998) modified the sequential fluorometric method to the MFA with 96-well microtiter plates by adopting a sarcosyl extraction technique and eliminating the DNase step, thus allowing application of the assay

to small samples (single copepods) without extended working time.

Each specimen was thawed and then homogenized by vigorous shaking with 5 zirconia beads in 200 µL of a 1% sarcosyl extraction buffer. Samples were shaken for 5 minutes at room temperature on a vortex mixer equipped with a multiple-vial head. Following the initial homogenization, samples were then diluted with Tris buffer to reduce the sarcosyl concentration to 0.1%, and then shaken for an additional 10 minutes. Finally, sampled were centrifuged (15000 g) for 10 min at 4℃ to separate insoluble copepod remains.

In each run, 75 µL aliquots of sample supernatant and 8 replicates from 0.01 to 0.5 µg mL^{-1} DNA (calf-thymus, Sigma-Aldrich D4522) and from 0.02 to 3 µg mL^{-1} RNA (bakers-yeast, Sigma-Aldrich R7125) were transferred to 96-well microplates (Nunc). Zero concentrations of the standard solutions were also treated as reagent blanks (containing all chemicals but no copepod homogenate). Here, we used RiboGreen (Invitrogen R11491) as a fluorescent dye instead of Ethidium Bromide (used by WAGNER *et al.* 1998) because of its greater nucleic acid sensitivity and efficacy (GOROKHOWA and KYLE, 2002). We added 100 µL of 0.2% RiboGreen to each well, and the plates were then kept at room temperature for 5 minutes. The fluorescence of RiboGreen was then scanned using a microplate reader (Perkin-Elmer, ARVO MX1420) with 485 nm (excitation) and 535 nm emission) filters (First scan). The RNase solution (10 µL) was then added to each well. The microplate was kept at room temperature for another 20 minutes and RiboGreen fluorescence was scanned (Second scan). The concentrations of RNA (µg mL^{-1}) were calculated as the differences between first (DNA + RNA) and second (DNA) scans, and the standard curve of RNA versus the fluorescence established from the first scan of the same plate. DNA concentrations (µg mL^{-1}) were computed from the second scan and the standard curve of DNA versus fluorescence established from the second scan (RNase treated) of the same plate.

Results

Environmental variables

Sea surface temperature ranged from 16.3 to 29.0℃ (Fig. 2). The thermocline and halocline were developed above 30 m during May to August 2009 and during May to October 2010. Surface waters during the summer of 2010 were much less saline than for the same period in 2009. Over the course of the study period, seasonal variations in temperature (16.1 to 18.3℃) below

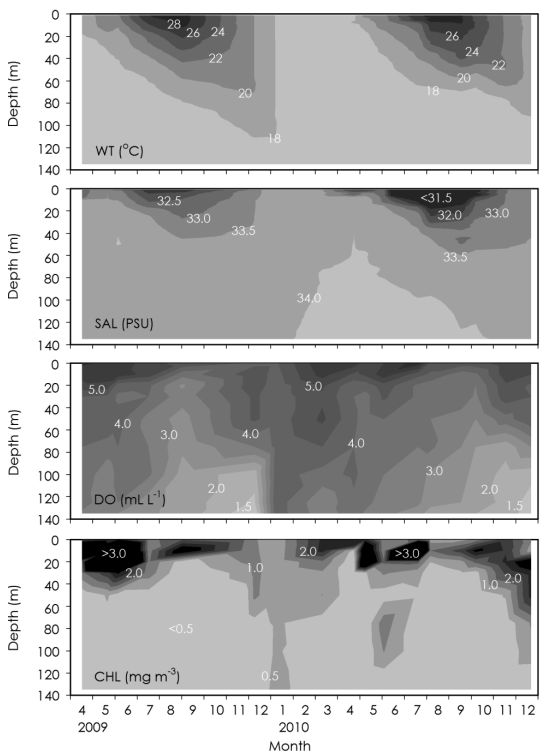

Fig. 2. Seasonal changes in vertical profiles of temperature (WT: ℃), salinity (SAL: PSU), dissolved oxygen (DO: mL L^{-1}) and phytoplankton biomass (CHL: mg chlorophyll *a* m^{-3}) at S16 in Kagoshima Bay.

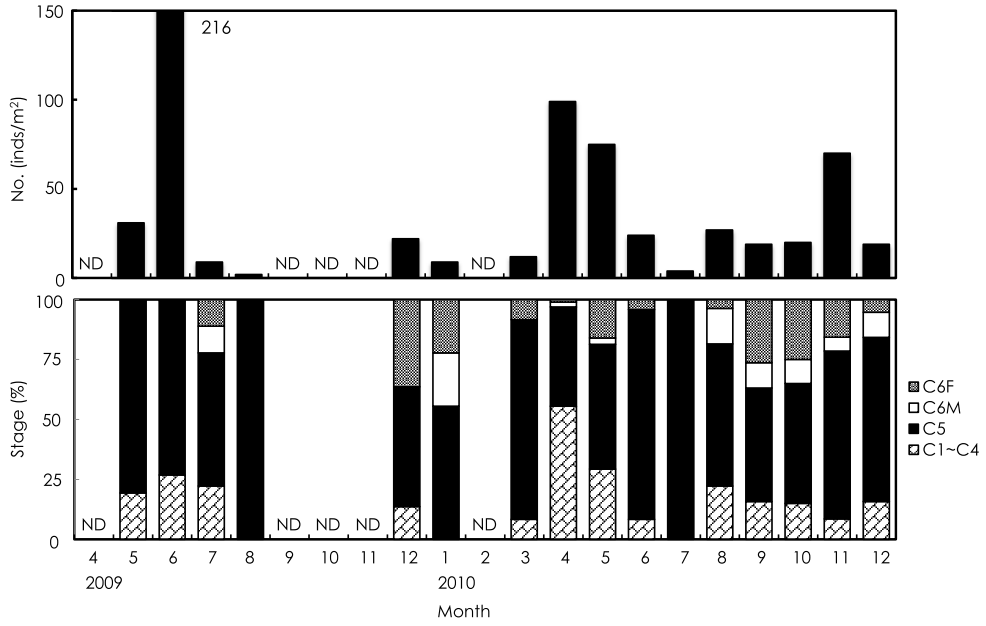

Fig. 3. Seasonal changes in abundance (individuals m^{-2}) and stage composition (%) of *Calanus sinicus* in the water column from 135 m to sea surface at S16 in Kagoshima Bay. ND: No data.

100 m were less pronounced than variation of salinity (33.9 to 34.1PSU) in the same depths. The dissolved oxygen concentrations above 50 m were greater than 3.0 mL L^{-1} during the entire study period. However, the dissolved oxygen concentration below 100 m gradually declined after July 2009 and 2010 and reached to 1.1 mL L^{-1} near the base of the water column in December 2009. Chlorophyll *a* concentrations were greater than 5 mg m^{-3} above 30 m during April to July in both years, but were 0.5 to 1.0 mg m^{-3} throughout the water column during December 2009 to January 2010.

Population structure

Peak abundance values for *C. sinicus* in the water column were measured in June 2009 (216 individuals m^{-2}), April 2010 (99 individuals m^{-2}) and in November 2010 (70 individuals m^{-2}) (Fig. 3), when phytoplankton biomass above 50 m was high. *C. sinicus* C5s were typically the most abundant stage and were present throughout the study period. The population was composed entirely of C5s in August 2009 and July 2010 when the water column abundance of *C. sinicus* was the lowest (< 10 individuals m^{-2}). Young copepodites from C1 to C4 composed less than 30% of the population throughout the study period, except in April 2010 (~50% of the total abundance). Adult males and females were sampled during the most months, however, their seasonal pattern was not clear.

Depth distribution

We generally measured a high abundance of *C. sinicus* both above 50 m and below 100 m, excepted for November 2010 when the relative abundance was the greatest in the 50–100 m layer (Fig. 4). Over the course the year, *C. sinicus* was abundant above 50 m during June to July and

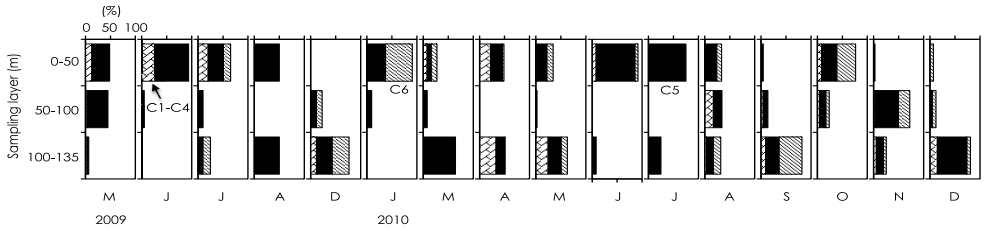

Fig. 4. Seasonal changes in relative depth distributions of *Calanus sinicus* developmental stages at S16 in Kagoshima Bay. C1–C4: Copepodite stage 1 to 4. C5: Copepodite stage 5. C6: Copepodite stage 6 (adult male and female). Note that some months are missing due to no sampling.

below 100 m in December but seasonal migration was not apparent for the population. Moreover, we noted no clear seasonal pattern when we considered each developmental stage separately. Young copepodites (C1 to C4) appeared abundantly in the layers above 50 m as well as below 100 m. Adults and C5s were abundant both above 50 m and/or below 100 m, except for November 2010 when they appeared abundantly in the 50–100 m layer.

Nucleic acids ratio

Individual DNA and RNA contents both decreased during August to October and then increased from November to December (Fig. 5). The RNA:DNA ratio was relatively stable during the summer with values increasing toward December.

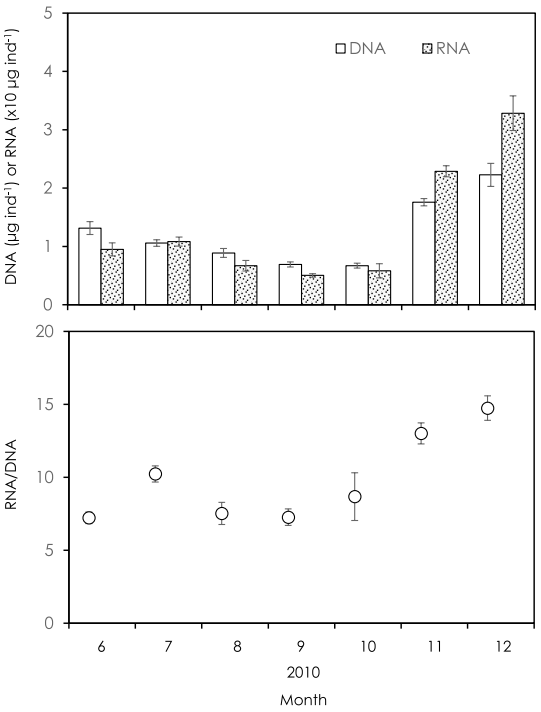

Fig. 5. Seasonal changes in individual RNA and DNA content (μg individual^{-1}) and RNA:DNA ratios of copepodite stage 5 for *Calanus sinicus* at S16 in Kagoshima Bay. Error bars represent ±1SE.

Discussion

While *C. sinicus* reproduction takes place throughout the year in the Inland Sea of Japan, the population follows a clear seasonal pattern with high abundance during June to August and low during September to March (Huang *et al*., 1992, 1993a, b). Studies by Huang et al. (Huang *et al*., 1992, 1993a, b) found no evidence of a dormant stock in the Inland Sea of Japan and concluded that the population was able to produce three generations a year because ambient temperatures and food availability were suitable. However, Uye (2000) has pointed out that *C. sinicus* prospers in shelf waters with enough depth to perform full-scale vertical migration and to avoid embedding at bottom before egg hatch-

ing. On the other hand, a different life history was found for the Sagami Bay (NONOMURA *et al*., 2008) and Yellow Sea (PU *et al*., 2004a, b; ZHANG *et al*., 2007) populations. While all developmental stages were abundant above 100 m during May in Sagami Bay, C5s were found in the layers below 200 m (NONOMURA *et al*., 2008). Such a deep appearance of C5 was also evident for the *C. sinicus* population in Yellow Sea (PU *et al*., 2004a, b; ZHANG *et al*., 2007). While all development stages including nauplii occurred in the water column during August, C5s were concentrated at depth in the central part of Yellow Sea, indicating a dormant stock. These latter studies suggest that the Yellow Sea population of *C. sinicus* produces dormant stock during the summer when food availability is low and temperatures are high because their reproductive and development timings fit the more favorable seasons (PU *et al*., 2004a, b; ZHANG *et al*., 2007). These findings indicate that a variety of the life histories for *C. sinicus* is characterized by presence or absence of dormancy. Also, they seem to result from the combined effects of food availability, thermal regime, and deep bathymetry.

Population abundance was high during April to June and low during July to August in the northern area of Kagoshima Bay. The seasonal changes in abundance corresponded to those in chlorophyll *a* concentrations in the surface layer. A similar seasonal pattern was observed for the *C. sinicus* population in the Kii Channel of the Inland Sea of Japan (HUANG *et al*., 1993a). Young copepodites and adults were present during most of the study period and year-round reproduction was also evident for the populations in the Inland Sea of Japan and the neighboring waters. In the present study, seasonal migration was not clear for all development stages. While only C5 was found in summer when the population abundance was the lowest, they appeared in both surface and bottom layers. These results suggest that *C. sinicus* conducts a year-round reproduction and does not produce a dormant stock in the northern area of Kagoshima Bay. However, the timing of greatest abundance was earlier in northern area of Kagoshima Bay (April to June) compared to that of the Inland Sea of Japan (June to August) and the regional variations may be due to differences in seasonal temperature and phytoplankton biomass patterns.

Laboratory studies on the growth and development of the congeneric species, *Calanus pacificus* (VIDAL, 1980a, b, c, d), demonstrated that growth rate varied with temperature only under food-saturated conditions. Similar interactive effects of food availability and temperature have not been demonstrated for *C. sinicus* growth and development rates. However, egg production rates have been demonstrated to increase logistically with phytoplankton biomass (UYE, 2000) while both growth and development rates appear to be temperature-dependent (UYE, 1988). Sea surface temperatures greater than 20℃ were evident during July in the Inland Sea of Japan (HUANG *et al*., 1993a) and from May through December in the northern area of Kagoshima Bay. High phytoplankton concentrations were measured during August and September in the Inland Sea of Japan (>5 mg m^{-3}: HUANG *et al*., 1993a) and during May and June in the present study (>10 mg m^{-3}). Thus, it is appears that *C. sinicus* abundance increases during the spring when thermal and food (i.e., phytoplankton biomass) conditions are optimal for development and growth. This optimal period occurs earlier in the northern area of Kagoshima Bay relative to the Inland Sea of Japan.

In the northern area of Kagoshima Bay, year-to-year variations were found for the environmental conditions such as ambient temperature, salinity and chlorophyll *a* from spring to summer.

As seen in less abundance for *C. sinicus* under the low food availability in 2010, environmental conditions during spring to summer are likely important for their life history strategy. However, even if they increased their abundance during spring to early summer like in 2009, the population abundance was lowest during summer when the population was composed entirely of C5s. In the Yellow Sea and Sagami Bay, C5s found in deep layers are considered to be dormant (Pu *et al*., 2004a, b; Zhang *et al*., 2007, Nonomura *et al*., 2008). In the present study, however, we find no evidence for dormancy in the northern area of Kagoshima Bay. For marine calanoid copepods, dormancy has been identified by an empty and reduced gut (Miller *et al*. 1984) or by the shape and/or structure of diapause eggs (Ban, 1997). More recently, dormancy for copepods has also been identified biochemically by low aminoacyl-tRNA synthetase activities (Yebra *et al*., 2006) or low RNA:DNA ratios (Kobari *et al*., 2013). We did not measure a significant decline in RNA:DNA ratios for our study population. Based on the equation developed by Kobari *et al*. (Kobari *et al*., 2013), the RNA: DNA ratios measured for *C. sinicus* were much higher than ratios measured for "active" copepods (1.0 to 1.6). These findings suggest that no dormant stock is formed for the *C. sinicus* population in the northern area of Kagoshima Bay. Why then is the population abundance low and composed of C5s during the summer in the northern area of Kagoshima Bay? Summer phytoplankton biomass never dropped below 1 mg chlorophyll *a* m^{-3}, indicating that clutch size and spawning frequency were probably food-saturated (Uye, 2000). It is possible that hatching and development failures (i.e., embryonic and naupliar malformations) were enhanced with high summer surface temperatures (>25℃) as suggested by Zhang *et al*. (2007), resulting in elevated mortality for early life stages in Kagoshima Bay. On the other hand, copepod mortality increases with high thermal regimes since predation activity is enhanced (Huntley and Lopez, 1992; Hirst and Kiørboe, 2002). The naupliiar and young copepodite stages of *C. sinicus* are known to reside in surface layers throughout the day (Uye *et al*., 1990; Huang *et al*. 1992, 1993b). Uye (2000) pointed out that the C5 and adult stages can, however, avoid visual predators in the shelf waters where they are able to carry out a full-scale diel vertical migration. The water column depth at our study site was 140 m, deeper than Kii Channel where the full-scale diel vertical migration has been observed (Uye, 2000). Taking into account for the predominance of C5s even under the cold thermal regime without the growth inhibition (i.e., winter to spring), we suggest that the predominance of C5s occurs (especially in summer) as a result of high predation mortality on early life stages. However, how do they sustain their population with quite low abundance of the early life stages even in the year-round reproduction and equiproportional development (Uye, 1998)? Indeed, the abundances of C1 to C4 were lower than those in the previous studies (Uye *et al*., 1990; Huang *et al*. 1992, 1993b). Based on the results in the Yellow Sea (Pu *et al*., 2004b), *C. sinicus* exhibited different life history strategies to conduct summer dormancy at depth in the deepest area and to reproduce throughout the year in the neighboring shallow area. Since there are basin areas deeper than 200 m in northern and southern Kagoshima Bay, *C. sinicus* might be sustained by recruitment of dormant stock forming in the basin areas.

Uye (2000) pointed out that shelf waters might provide a suitable habitat for *C. sinicus* due to the ideal temperature, food supply and depth. These conditions of a sufficiently deep water column, the high thermal regime, and suitably high food supply appear to be met for the *C. sinicus* population

in the northern area of Kagoshima Bay. The importance of both ambient temperature and food supply in copepod life cycles has been well studied (HUANG *et al*., 1993a; PU *et al*., 2004a, b; ZHANG *et al*., 2007). Our study also emphasizes that water column depth plays an important role in calanoid copepod life cycles because of the potential limits imposed vertical migrants. It has been hypothesized that coastal populations of *C. sinicus* are supplied by estuarine circulation transporting populations flourishing in shelf waters (UYE, 2000). However, such circulation was limited in the northern area of Kagoshima Bay due to the presence of a narrow channel (TAKAHASHI, 1977). We suggest that *C. sinicus* can complete their life cycle in the northern area of Kagoshima Bay where food availability and depth are suitable, even in the high thermal regime.

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