



Population structure, egg production and gut content pigment of large grazing copepods during the spring phytoplankton bloom in the Oyashio region

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ARTICLE INFO

Article history:

Received 15 March 2010

Accepted 15 March 2010

Available online 27 March 2010

Topical issue on "Ecosystem Processes during the Oyashio Spring Bloom." The issue is compiled and guest-edited by the North Pacific Marine Science Organization (PICES).

Keywords:

Copepods

Egg production

Gut pigment

Life cycle

OECOS

ABSTRACT

As a basis for analyzing development of six large grazing copepods (*Eucalanus bungii*, *Metridia pacifica*, *M. okhotensis*, *Neocalanus cristatus*, *N. flemingeri* and *N. plumchrus*) in the Oyashio region, quasi-daily twin-NORPAC net (0.33 and 0.10 mm mesh) hauls were taken through the upper 150 m and 500 m at a station southwest of Hokkaido before (9–14 March) and after (6–30 April) the onset of the phytoplankton bloom in 2007. Based on additional fresh specimens collected from 0–150 m, egg production of *E. bungii*, *M. pacifica* and *M. okhotensis*, and gut pigments of late copepodid stages in each species were evaluated. Total zooplankton biomass was greater from 10 April onward by a factor of 2- to 8-fold the previous levels. This increase of the 0–150 m biomass was caused by development of *Neocalanus* spp. copepodids and upward migration of resting *E. bungii*. Egg production of *E. bungii* peaked on 18 April, while abundance of its nauplii and C1 peaked on 20 and 25 April, respectively. Sex ratio and C6-female gonad maturation index of *E. bungii* showed new recruitment to C6 during 20–30 April, likely derived from a population that over-wintered as C3 or C4. Egg production and hatchability of *M. pacifica* and *M. okhotensis* were highly variable and no temporal trend was detected. Comparison with field abundance data for *Metridia* spp. suggests that our estimates of egg production and hatchability are too low, despite care with experimental conditions. All the *Neocalanus* species utilize the bloom as energy for juvenile growth. *Neocalanus cristatus* developed from C2 through C4, and stage duration of C3 was estimated to be 24 days. *Neocalanus flemingeri* also developed from C1 through C3, and stage durations of C1 and C2 were estimated to be 7–9 days. *Neocalanus plumchrus* occurred in small numbers from mid-April onward. The stage duration estimates for *Neocalanus* spp. are similar to those reported from the high-nutrition southeastern Bering Sea shelf. Gut pigment variation clearly showed nocturnal feeding by *Metridia* spp., while no diel changes in gut pigment were recognized for *E. bungii* or *Neocalanus* spp. The diel changes in gut pigment of *Metridia* spp. were related to their diel vertical migrations. The calendar of sequential responses of copepods to the phytoplankton bloom is summarized.

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1. Introduction

Life cycles of large- and medium-sized, grazing copepods (*Neocalanus cristatus*, *Neocalanus flemingeri*, *Neocalanus plumchrus*, *Eucalanus bungii* and *Metridia pacifica*) have been studied extensively at Station P in the eastern subarctic Pacific by U.S. scientists during the 1980s (Miller et al., 1984; Batchelder, 1985; Miller and Clemons, 1988).

In the western subarctic Pacific, Japanese scientists have pursued intensive studies on life cycles of dominant mesozooplankton in the Oyashio region since 1996. A time series of

mesozooplankton data at Site H (areas covered between 41°30'–42°30'N and 145°00'–146°00'E) in the Oyashio region was developed by sampling at monthly intervals over one full year by vertical hauls with closing nets through five discrete strata between the surface and 2000 m. Whenever possible, some short-term reference stations were set in the central and eastern subarctic Pacific, Bering Sea, Okhotsk Sea, and Japan Sea during the program, and the results were compared with those from Site H. As a result, information about life cycles (spawning season, development pattern, generation time, longevity, etc.) has accumulated rapidly on a number of copepod species in the Oyashio region: i.e. *N. cristatus*, *N. flemingeri*, *N. plumchrus* (Kobari and Ikeda, 1999, 2001a, 2001b), *E. bungii* (Shoden et al., 2005), *Metridia okhotensis*, *M. pacifica* (Padmavati et al., 2004), *Gaidius variabilis*, *Heterorhabdus tanneri*,

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Paraeuchaeta elongata, *Paraeuchaeta birostrata*, *Paraeuchaeta rubra*, *Pleuromamma scutullata* (Yamaguchi and Ikeda, 2000a, 2000b, 2001) and *Oncaea grossa*, *Oncaea parila*, *Triconia borealis*, *Triconia canadensis* (Nishibe and Ikeda, 2007). There are differences in copepod phenology between the Gulf of Alaska and the Oyashio: *E. bungii* had a 1-year life cycle at Site H in contrast to its 2-year life cycle at Station P (Shoden et al., 2005), and *M. pacifica* completed two generations at Site H in contrast to three at Station P (Padmavati et al., 2004).

Low-frequency sampling (e.g., one sample per month), mentioned above, was sufficient to show the overall picture of mesozooplankton life cycles for species with generation lengths of one year or more. However, precise calculation of population production of these mesozooplankton requires high-frequency data on their developmental sequences. For example, all large to medium-sized copepods achieve rapid development from early to late copepodid stages during the phytoplankton bloom that typically lasts ca. three months (April–June). As seen in our low-frequency sampling, abundance peaks of 3 to 4 copepodid stages occurred simultaneously, that is, in the sample results for the same month, making it difficult to estimate the development time of any stage. High-frequency sampling (one sample per day) during OECOS was intended to solve that problem.

The OECOS project (Oceanic Ecodynamics Comparison in the Subarctic Pacific), sponsored by PICES, aims to evaluate detailed dynamics of physical, chemical and biological oceanography in the Alaskan Gyre and Oyashio region (Miller and Ikeda, 2006). From the results of the OECOS study, we can characterize short-term changes in mesozooplankton biomass, population structure, egg production and gut pigment of large- to medium-sized grazing copepods during the spring phytoplankton bloom of 2007 in the Oyashio region. We interpret the data in terms of the species-specific sequential responses of copepods to the phytoplankton bloom.

2. Materials and methods

2.1. Field sampling

During cruises of T/S *Oshoro-Maru* (March) and R/V *Hakuho-Maru* (April), 22 tows of twin-NORPAC nets (0.10 and 0.33 mm mesh, 45 cm diameter; Motoda, 1957) with flowmeters were made from 0–150 and 0–500 m at Station A-5 (42°00'N, 145°15'E) during 9–14 March and 6–30 April 2007 (Fig. 1A, B). Sampling was done in daytime (08:00–10:00 local time) and nighttime (19:00–21:00 local time), and the whole samples were immediately preserved in 5% borax-buffered formalin. To collect fresh, live specimens, 22 tows of a ring net (0.33 mm mesh, 80 cm diameter) with a large bottle for its cod end were made from 0–150 m during day and night on the same date. Using live specimens, temporal changes in egg production, gut pigment and individual mass were examined (methods below). CTD casts were also made three times per day. Water samples for chlorophyll-*a* were collected from 0, 5, 10, 20, 30, 40, 50, 75 and 100 m, then filtered through Whatman GF/F filters, and measured fluorometrically after extraction with dimethyl-formamide (Suzuki and Ishimaru, 1990).

2.2. Total zooplankton biomass

In the land laboratory, zooplankton samples from the 0.33 mm mesh NORPAC net (0–150 m and 0–500 m) were split with a Motoda splitting device (Motoda, 1959), and a one-half aliquot

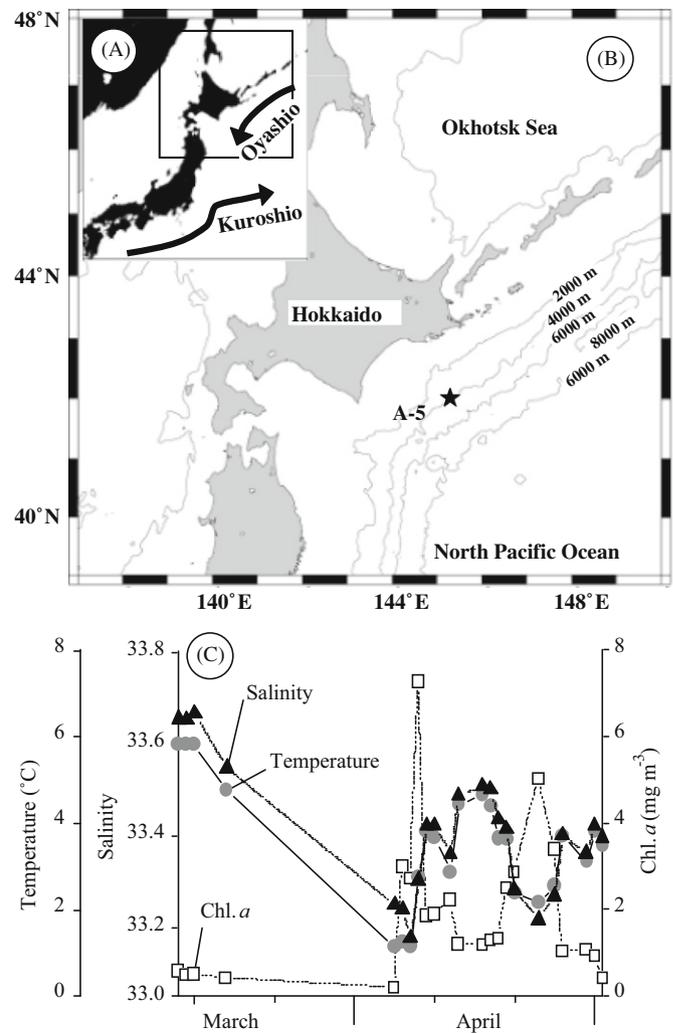


Fig. 1. Location of the Oyashio region in the western subarctic Pacific (A), and of sampling station A-5 in the Oyashio region (B), and temporal changes in integrated mean temperature, salinity and chlorophyll *a* in the upper 100 m depths during 9 March to 30 April 2007 (C). Depth contours are shown in (B).

was used for measurement of wet mass by microbalance (Mettler PM4000, precision 0.01 g).

2.3. Population structure

Copepodids C1–C6F/M of *E. bungii*, *M. pacifica*, *M. okhotensis*, *N. cristatus*, *N. flemingeri* and *N. plumchrus* were enumerated in the samples collected with 0–500 m NORPAC nets. Prior to analysis, we examined subsamples (1/10–1/100 of total volume, made with a wide-bore pipette) of both 0.33 and 0.10 mm mesh net samples. Then we compared the abundance based on 0.10 and 0.33 mm mesh nets and summarized the ratio of abundance between them (Table 1). Ratios for most of the late copepodid stages were near 1 (no differences between the two nets), while some early copepodid stages showed substantially greater factors (0.10 mm > 0.33 mm mesh). For the stages showing a high factor (> 5: nauplii of *E. bungii*, C1, C2 and C3 of *M. pacifica*, Table 1), we used the abundance data based on 0.10-mm mesh nets. For the other late copepodid stages, we used the abundance data based on 0.33-mm mesh nets.

For *E. bungii*, the naupliar stages were easily distinguished from the other species. Its naupliar stages were identified and enumerated based on the descriptions of Johnson (1937). Also for

Table 1

Comparison in abundance which collected with 0.10 (XX13) and 0.33 (GG54) mm mesh of Twin NORPAC net during spring phytoplankton bloom in the Oyashio region.

Species	Stage						
	Nauplii	C1	C2	C3	C4	C5	C6
<i>Eucalanus bungii</i>	<u>79.4</u>	1.2	0.7	1.2	1.2	0.8	1.4
<i>Metridia pacifica</i>		<u>43.9</u>	<u>11.3</u>	<u>5.2</u>	1.6	1.6	1.7
<i>Neocalanus cristatus</i>		0.9	3.6	1.9	1.5	1.0	
<i>Neocalanus flemingeri</i>		1.2	1.0	1.0	0.6	0.9	0.4
<i>Neocalanus plumchrus</i>		1.0	0.8	0.6	1.5	0.7	

Values are means of ratio of XX13:GG54 (both abundance in ind. m⁻³). Stages substantially greater abundance was observed for XX13 were shown with underlines.

E. bungii, gonad maturation stages of C6F were enumerated (gonad maturation stages I–VII) based on the description by Miller et al. (1984). Since the gonad developmental stage V (two full rows of ova that appear ready for spawning) of Miller et al. (1984) could be separated into two (two full rows of ova and three or more rows of ova), we enumerated them separately as gonad developmental stages V-1 and V-2, respectively.

2.4. Egg production and hatching

Fresh adult females of *E. bungii*, *M. pacifica* and *M. okhotensis* collected with the ring net at night were used for on-board egg production experiments. Three liters of surface seawater were collected with a bucket, filtered with a GF/F filter and well aerated by shaking in 500-ml bottles. Fresh adult females were transferred into plastic chambers (ca. 300 ml) with 0.33-mm mesh bottoms (to prevent egg cannibalism by females) inside the 500-ml bottles. In each chamber, three (*E. bungii*) or 10 (*M. pacifica*) adult females were added. The bottles with adult females were held for one day in an incubator set at 3 °C (integrated mean temperature for 0–100 m during April, Fig. 1C). After 24 h, females in the chamber were removed. The remaining seawater was examined under a stereomicroscope to seek eggs. When found they were counted and transferred into 5-mL multi-well plates filled with chilled, filtered seawater. Eggs in the multi-well were also incubated at 3 °C and checked daily for hatching. Egg incubation lasted ca. one week.

Egg production rates were expressed as eggs female⁻¹ day⁻¹ and, multiplying these values with female abundance (ind. m⁻³), as eggs m⁻³ day⁻¹. Two types of egg morphology were recognized: eggs with solid (normal) and eggs with thin (abnormal) outer membranes. We counted them separately, presenting the percentage of normal eggs in the total. Hatchability of eggs (%) was calculated for the normal eggs. The total recruitment of nauplii to the population (ind. m⁻³ day⁻¹) was calculated by multiplying egg production (eggs m⁻³ day⁻¹) by the proportion of normal eggs and proportion hatching.

2.5. Gut pigment

Using fresh specimens collected both day and night, pigments contained in the guts of late copepodid stages of dominant copepods were examined. Portions of the samples collected with the ring net were poured into a 1-L pitcher, and 10% v/v soda (saturated CO₂ in water) was added to prevent gut evacuation and decomposition of pigment. Fresh samples were examined under a stereomicroscope and C6F of *E. bungii*, *M. pacifica* and *M. okhotensis* and C5 of *N. cristatus*, *N. flemingeri* and *N. plumchrus* were sorted out. Batches of two (*N. cristatus* C5) to five (*M. pacifica* C6F) specimens were immersed in N,N-dimethylformamide, stored in dark, cold condi-

tions overnight to extract chlorophyll and phaeopigments. After extraction of pigment, chlorophyll and phaeopigments were measured with a Turner Designs fluorometer. Chlorophyll and phaeopigment amounts were summed and expressed as ng pigment ind.⁻¹ (cf. Mackas and Bohrer, 1976).

2.6. Individual mass

To evaluate temporal changes in individual body composition, C6F of *M. pacifica* and C5 of *N. cristatus* were sorted from the daytime live samples. Specimens were incubated in filtered seawater for three hours to evacuate their gut contents, rinsed with distilled water, transferred into pre-weighed aluminum pans and stored in a freezer at –80 °C. In the land laboratory, wet masses (WM) were determined ($\pm 1 \mu\text{g}$) with a microbalance (Mettler Toledo MT5), then samples were freeze-dried and dry masses (DM) were determined. Dried specimens were incinerated at 480 °C for 5 h, and ash weight was determined. From these data, water content (WATER, %WM) and ash-free dry mass (AFDM, %DM) were estimated using the following equations:

$$\text{WATER} = 100(\text{WM} - \text{DM})/\text{WM} \quad \text{and} \quad \text{AFDM} = 100(\text{DM} - \text{Ash})/\text{DM}$$

3. Results

3.1. Hydrography

Through the study period, integrated mean temperature and salinity in the 0–100 m stratum varied between 1.5–6.0 °C and 33.16–33.65, respectively (Fig. 1C). Temperature and salinity varied in parallel. They were high in March, then had two minima on 7 and 22 April. Standing stock of chlorophyll-*a* varied between 0.2 and 7.6 mg m⁻³. It was low in March, and then had two maxima on 7 and 22 April. This temporal change pattern in chlorophyll-*a* was inverse to those of temperature and salinity. Temporal changes of temperature and salinity were governed by the mixing ratio of two water masses: one was low-temperature and less saline Coastal Oyashio Water, which carried high chlorophyll-*a*. The other was high-temperature and more saline modified Kuroshio Water, which carried lower chlorophyll-*a*. Details of temporal changes in these two water masses during the study period are reported by Kono and Sato (2010).

3.2. Zooplankton biomass

Zooplankton wet mass was low during 8–14 March, increased by 10 April, and remained high during 10–30 April (Fig. 2A, B). This increase in zooplankton mass from March to April was ca. 8-fold in the upper 150 m and ca. 2-fold for the upper 500 m. This can only happen if the stock of the 0–500 m layer mostly becomes concentrated in the 0–150 m layer, while the overall abundance only doubles. Day vs. night differences were only evident for 0–150 m in March, but were not observed for 0–500 m throughout the study period (Fig. 2A, B). Depth distributions of zooplankton mass changed drastically around 10 April. Before that most (means $\pm 1\text{sd}$: $92 \pm 3\%$ for day and $82 \pm 5\%$ for night) of the biomass was seen in the 150–500 m stratum. After 10 April, zooplankton biomass in the shallower 0–150 m depth was greater than from 150–500 m (mean $\pm 1\text{sd}$: $59 \pm 10\%$ for day and $65 \pm 17\%$ for night) (Fig. 2C, D).

3.3. Population structure

Eucalanus bungii - In March 2007 the stock was composed of C3–C6 females and C6males (C6F/M), but no nauplii or C1–C2

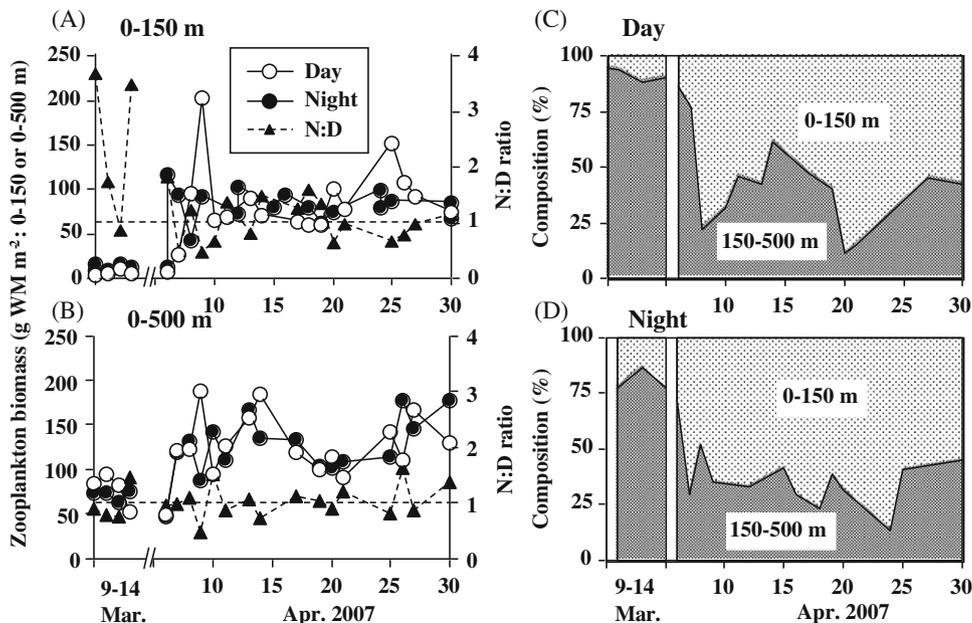


Fig. 2. Temporal changes in zooplankton wet biomass in the 0–150 m (A) and 0–500 m (B) water column in the Oyashio region both day and night during 9–14 March and 6–30 April 2007. Night:day (N:D) ratios are shown, and horizontal dashed lines indicate the position of N:D=1. Temporal changes in depth composition of zooplankton biomass in the 0–150 m and 150–500 m during day (C) and night (D) during the same period.

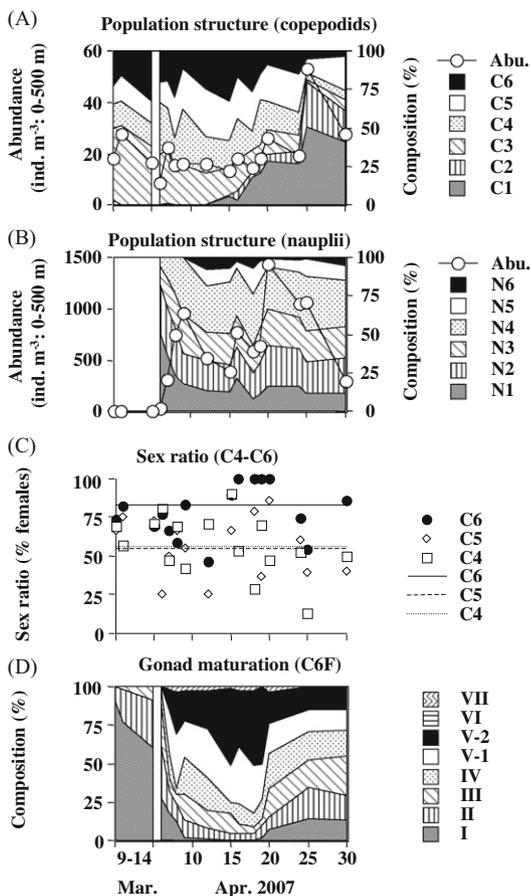


Fig. 3. *Eucalanus bungii*. Temporal changes in abundance (circles) and composition of copepodid stages (A), of naupliar stages (B), sex ratio (C4–C6) (C) and gonad maturation phases of C6F (d) in the Oyashio region during 9–14 March and 6–30 April 2007. Horizontal lines in (C) indicate means of each copepodid stage. For gonad maturation of C6F (D), eight categories were applied.

were collected (Fig. 3A, C). The dominant stage was C3 comprising 38–51% of the copepodid population. First copepodids (C1) were first observed on 15 April. Total copepodid abundance increased gradually after that and peaked on 25 April, when the C1 and C2 dominated (50% and 29% of the copepodids) (Fig. 3A).

Nauplii of *E. bungii* were observed after 6 April with only N1–N4 present during 6–9 April (Fig. 3B). Naupliar stage composition was stable after 12 April, and N4 was the dominant stage ($31 \pm 2\%$; mean \pm 1sd during 12–30 April). The peak of naupliar abundance (1440 ind. m^{-3}) was observed on 20 April, 5 days before the peak of copepodid abundance (Fig. 3B).

The sex ratio of *E. bungii* was near 1:1 for C4 (females were $57 \pm 20\%$ of the population) and for C5 ($56 \pm 19\%$) (Fig. 3C). The average ratio was skewed to females in C6 ($79 \pm 17\%$). Sex ratio of C6 *E. bungii* showed short-term changes: some males (ca. 25% of adults) occurred during March–12 April, while only females were collected during 15–20 April. Then males occurred again, about 25% of adults, during 24–30 April (Fig. 3C).

Gonads of C6F *E. bungii* were mostly in immature stages I–II during March (Fig. 3D). The dominance of stage I decreased to 61% of C6F by 14 March. On 6 April, the proportion of spawning females (stage V-1 and V-2) was small, but their frequency then increased within one or two days. Actively spawning females were a majority ($30 \pm 6\%$ V-1 and $34 \pm 13\%$ V-2) during 8–18 April, and then after 20 April the proportion of stages I–IV increased again (57–72% of C6F) (Fig. 3D).

Metridia spp.—The population structure of *M. pacifica* was dominated by C6 on 9–10 March, after which dominance of C6 declined and abundance of C1 increased (Fig. 4A). Abundance of *M. pacifica* on 6–7 April was extremely low ($9\text{--}16 \text{ ind. m}^{-3}$). After that abundance of *M. pacifica* was stable, varying between 51 and 91 ind. m^{-3} (mean \pm 1sd: $72 \pm 14 \text{ ind. m}^{-3}$), and their population structure was also stable through April: C1 dominated ($49 \pm 11\%$) followed by C2 ($14 \pm 7\%$), C3 ($16 \pm 6\%$), C4 ($6 \pm 3\%$), C5 ($6 \pm 3\%$) and C6 ($9 \pm 4\%$) (Fig. 4A).

Abundance of *M. okhotensis* ($3\text{--}30 \text{ ind. m}^{-3}$) was lower than that of *M. pacifica* (Fig. 4B). After 20 April, their abundance peaked

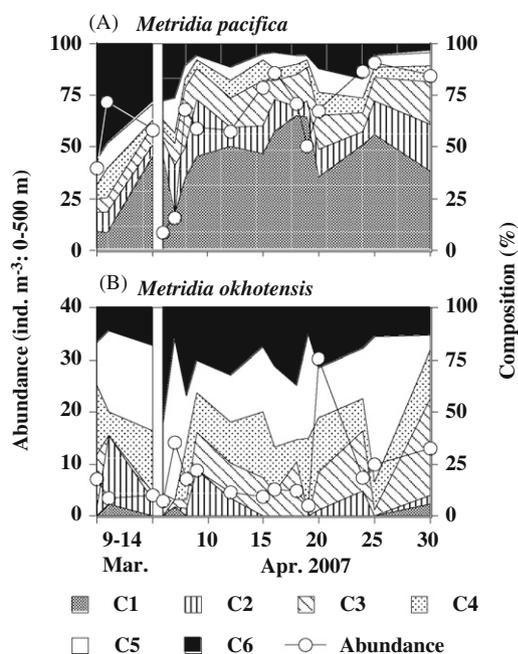


Fig. 4. *Metridia pacifica* (A) and *M. okhotensis* (B). Temporal changes in abundance (circles) and composition of copepodid stages in the Oyashio region during 9–14 March and 6–30 April 2007.

and was slightly greater than before. Its population structure was dominated by C5 ($34 \pm 19\%$), followed by C6 ($24 \pm 13\%$) and C4 ($21 \pm 10\%$); thus these late copepodid stages composed 79% of the population and showed no strong temporal variation (Fig. 4B).

Neocalanus spp.—Abundance of *N. cristatus* was greater in March than in April (Fig. 5A). Their abundance can be expressed by a regression: $Y=7.9-0.065X$, where Y is abundance (ind. m^{-3}) and X is Julian day starting from 1 March ($r^2=0.25$, $p<0.05$). Population structure of *N. cristatus* was dominated by C1–C3 in March, by C3 on 6–7 April and by C4 and C5 on 20–30 April. Thus, dominant stages shifted from early copepodid stages to late copepodid stages.

Abundance of *N. flemingeri* was low in March and increased through April (Fig. 5B). Population structure of *N. flemingeri* passed through successive stages: a peak of C1 was observed on 8 April, of C2 on 18 April and of C3 on 25 April. The proportion of C5 and C6 remained extremely low for *N. flemingeri* through the end of April (Fig. 5B).

Abundance of *N. plumchrus* was extremely low in March (Fig. 5C). It was present in April, but its abundance was consistently lower than that of *N. flemingeri*. The population structure of *N. plumchrus* during 15–30 April was dominated by C1 ($30 \pm 11\%$), C2 ($33 \pm 12\%$) and C3 ($17 \pm 7\%$) (Fig. 5C).

3.4. Egg production

Egg production of *E. bungii* was zero in March and remained low (<50 eggs $\text{female}^{-1} \text{day}^{-1}$) during 6–9 April (Fig. 6A). Abnormal eggs with a weak membrane structure were also observed. Both normal and abnormal egg production rates increased during 6–12 April; after that production of abnormal eggs was rare (Fig. 6A). In early April, both the proportion of normal eggs and the hatchability of normal eggs were low. Those increased during 6–16 April, after which normal eggs were $93 \pm 6\%$ of egg production and hatchability was $83 \pm 5\%$ of normal eggs (Fig. 6A). Recruitment of nauplii $\text{female}^{-1} \text{d}^{-1}$ was

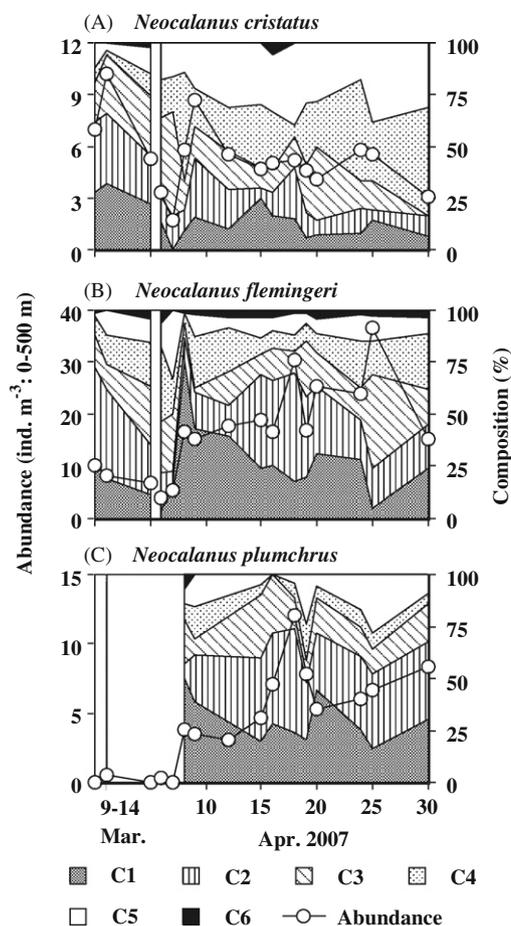


Fig. 5. *Neocalanus cristatus* (A), *N. flemingeri* (B) and *N. plumchrus* (C). Temporal changes in abundance (circles) and composition of copepodid stages in the Oyashio region during 9–14 March and 6–30 April 2007.

low during 6–9 April, and then much higher during 11–30 April (mean $\pm 1\text{sd}$: 88 ± 22 $\text{ind. female}^{-1} \text{d}^{-1}$). Total recruitment of nauplii to the water column *E. bungii* population was also greater during 11–30 April (206 ± 124 $\text{ind. m}^{-3} \text{day}^{-1}$), and peaked on 18 April (447 $\text{ind. m}^{-3} \text{day}^{-1}$) (Fig. 6A).

Egg production of *M. pacifica* fluctuated greatly through the study period (Fig. 6B). It also produced abnormal eggs with weak membranes, more of them (76%) than normal eggs (24% of total egg production). Hatchability of normal eggs also fluctuated around a mean of 67%. Temporal variability of percentage of normal eggs and hatchability of normal eggs were correlated (Fig. 6B). Individual recruitment peaked on 30 April with 18 $\text{ind. female}^{-1} \text{day}^{-1}$, and population recruitment also peaked then (37 $\text{ind. m}^{-3} \text{day}^{-1}$).

Egg production of *M. okhotensis* was observed from 17 April onward (Fig. 6C). Their percentage of normal eggs was $88 \pm 13\%$, but hatchability fluctuated. Their individual recruitment peaked on 20 April at 7.3 $\text{ind. female}^{-1} \text{day}^{-1}$, and population recruitment peaked in same period at 22 $\text{ind. m}^{-3} \text{day}^{-1}$ (Fig. 6C).

3.5. Gut pigment

Diel changes in gut content pigment of late copepodid stages separated into two types: (1) *E. bungii* C6F, *N. cristatus* C5 and *N. flemingeri* C5 showed low gut pigment in March and no day-night differences in gut pigment during April (except for *N. cristatus* C5 during 6–10 April) (Fig. 7A, D, E). (2) *M. pacifica*

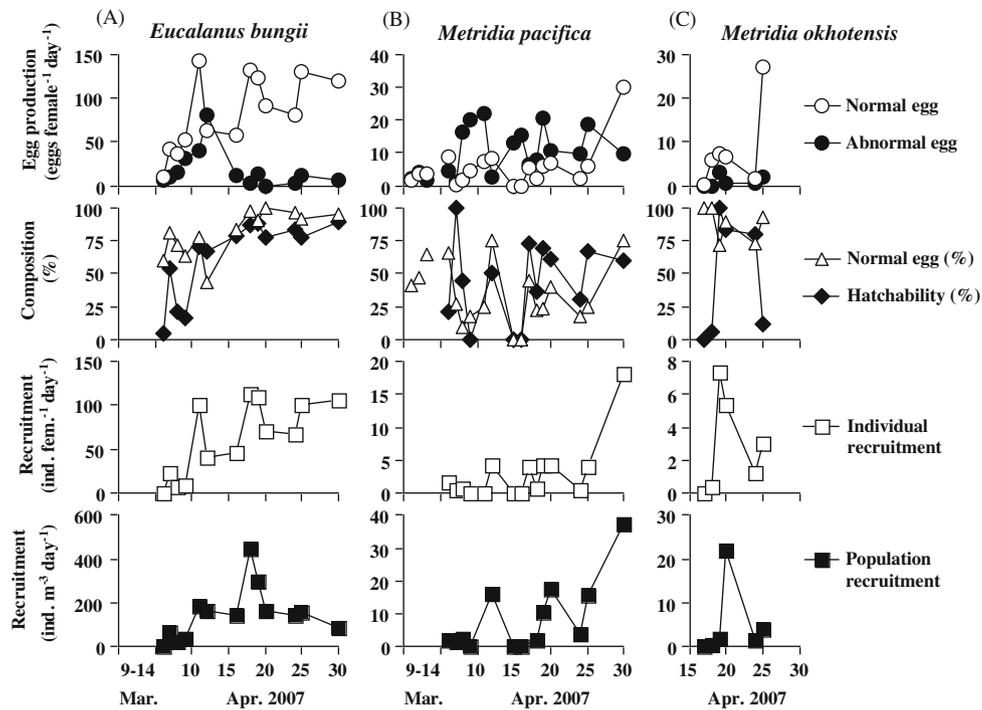


Fig. 6. *Eucalanus bungii* (A), *Metridia pacifica* (B) and *M. okhotensis* (C). Time series of egg production rate estimates (upper panels), proportion of normal eggs and hatchability (second panels), individual recruitment (ind. female⁻¹ day⁻¹) (third panels) and population recruitment (ind. m⁻³ day⁻¹) (bottom panels) in the Oyashio region during 9–14 March and 6–30 April 2007.

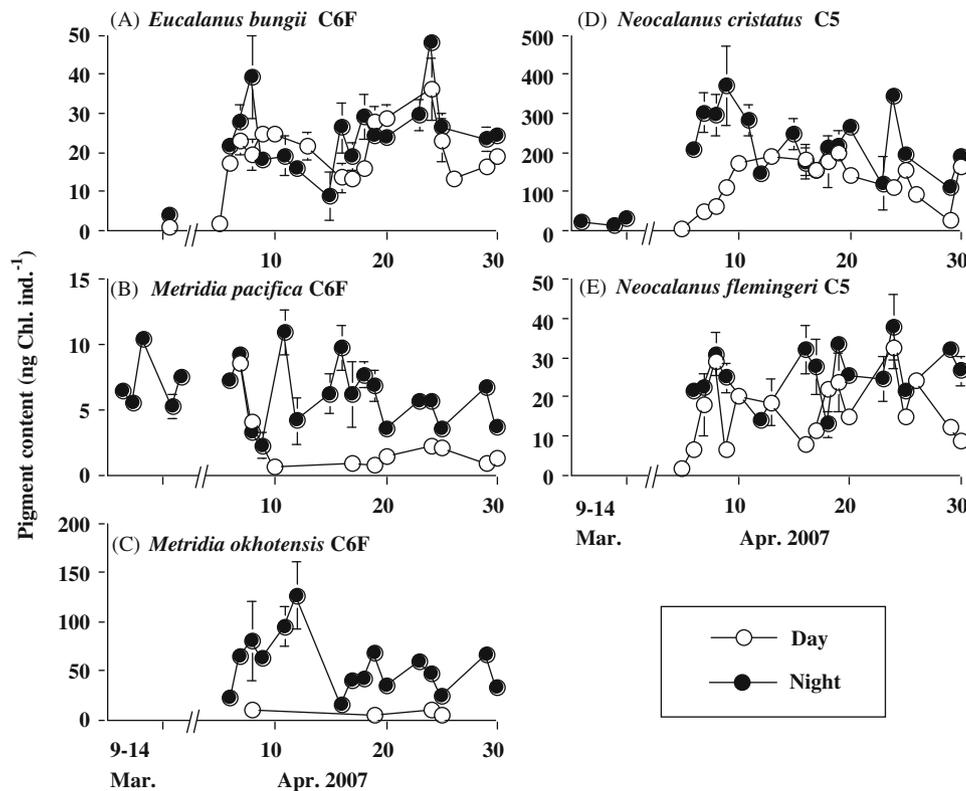


Fig. 7. *Eucalanus bungii* C6F (A), *Metridia pacifica* C6F (B), *M. okhotensis* C6F (C), *Neocalanus cristatus* C5 (D) and *N. flemingeri* C5 (E). Temporal changes in gut pigment content of individuals collected in the upper 150 m during day (open circles) and night (solid circles) in the Oyashio region during 9–14 March and 6–30 April 2007. Vertical bars indicate standard deviations.

C6F and *M. okhotensis* C6F showed significantly higher gut pigment at night than in daylight ($p < 0.01$, U-test) and had similar gut pigment levels at night in March and April (Fig. 7B, C).

Mean gut pigment varied with species, highest for *N. cristatus* C5 (133–197 ng Chl. ind.⁻¹) and lowest for *M. pacifica* C6F (2.3–6.2 ng Chl. ind.⁻¹), a function of their body sizes (Table 2).

Table 2

Day and night comparison on gut pigment of late copepodid stages of dominant copepods in the Oyashio region during 9 March–30 April 2007. Values are mean \pm 1sd.

Species (stage)	Gut pigment (ng Chl. ind. ⁻¹)		U-test
	Day	Night	
<i>Eucalanus bungii</i> (C6F)	20.8 \pm 6.4	25.0 \pm 8.9	ns
<i>Metridia pacifica</i> (C6F)	2.3 \pm 2.4	6.2 \pm 2.5	**
<i>Metridia okhotensis</i> (C6F)	6.7 \pm 3.2	55.1 \pm 29.2	**
<i>Neocalanus cristatus</i> (C5)	133.6 \pm 61.3	197.5 \pm 66.1	ns
<i>Neocalanus flemingeri</i> (C5)	15.7 \pm 8.5	25.9 \pm 6.9	ns

** $p < 0.01$, ns: not significant. Details of data see Fig. 7.

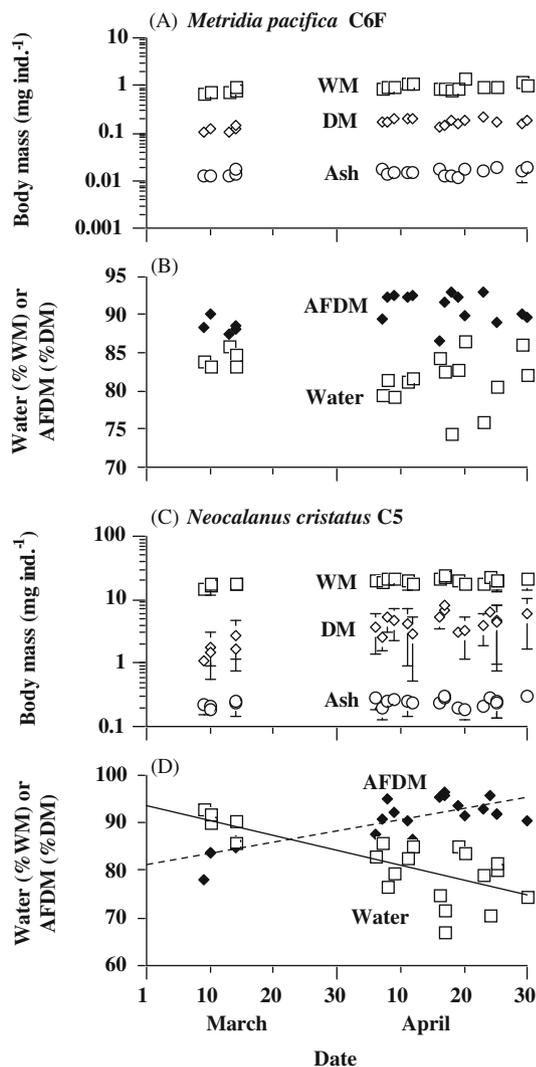


Fig. 8. *Metridia pacifica* C6F (A, B) and *Neocalanus cristatus* C5 (C, D). Temporal changes in individual body mass (WM, DM and Ash) of specimens collected during daytime in the upper 150 m (A, C) and their water content (%WM) and AFDM content (%DM) (B, D) in the Oyashio region during 9–14 March and 6–30 April 2007. Vertical bars indicate standard deviations. For body composition, a significant relationship with time was recognized only for *N. cristatus* (D).

3.6. Individual mass and body composition

The WM, DM and ash of *M. pacifica* C6F were 0.94 ± 0.17 , 0.16 ± 0.03 and 0.015 ± 0.002 mg ind.⁻¹, respectively (means \pm 1sd)

Table 3

Correlation coefficient between date (julian day) and individual body masses (wet mass [WM], dry mass [DM], ash and ash-free dry mass [AFDM]) or body composition (water and AFDM) of *Metridia pacifica* C6F and *Neocalanus cristatus* C5 in the Oyashio region during March–April 2007.

Species (stage)	Body masses				Chemical contents	
	WM	DM	Ash	AFDM	Water	AFDM
<i>Metridia pacifica</i> (C6F)	0.60**	0.69**	0.42 ^{ns}	0.68**	-0.30 ^{ns}	0.43 ^{ns}
<i>Neocalanus cristatus</i> (C5)	0.64**	0.72***	0.37 ^{ns}	0.72***	-0.73***	0.80***

ns: not significant.

* $p < 0.05$. ** $p < 0.01$. *** $p < 0.001$.

(Fig. 8A). Its water and AFDM contents were $82.1 \pm 3.2\%$ of WM and $90.3 \pm 2.0\%$ of DM, respectively (Fig. 8B). Regressions of compositional components vs. date showed that water and AFDM of *M. pacifica* were uncorrelated with date (Table 3).

The WM, DM and ash of *N. cristatus* C5 were 20.0 ± 2.3 , 3.99 ± 1.89 and 0.30 ± 0.04 mg ind.⁻¹, respectively (mean \pm 1sd) (Fig. 8C). Its Water and AFDM contents averaged $81.5 \pm 7.1\%$ of WM and $90.1 \pm 4.9\%$ of DM, respectively (Fig. 8D). Regressions vs. date showed that AFDM content of *N. cristatus* C5 was significantly increasing with date, with water content decreasing with date (Table 3).

4. Discussion

4.1. Zooplankton biomass

Total zooplankton biomass in the upper 500 m showed little diel variability, while that in the 0–150 m layer was greater at night during March 2007 (Fig. 2A, B). The magnitude of this diel change in biomass was a factor of ca. 2.5 ($N > D$), which corresponds to the values Steinberg et al. (2008) reported from Station K2 in the western subarctic Pacific. According to Goldblatt et al. (1999), diel changes in zooplankton biomass were present at Station P in summer, while not present during winter-spring. Thus, the presence (or not) of diel changes in zooplankton biomass in the upper waters seems to vary with season and location. No diel changes of total zooplankton biomass above 500 m implies that the magnitude of DVM of mesozooplankton in this region is less than 500 m, which corresponds to the results of Yamaguchi et al. (2004).

Comparing March to April, we observed several temporal changes in total zooplankton biomass: (i) total zooplankton stocks increased after 10 April by a factor of 8 for 0–150 m and 2-fold for 0–500 m, (ii) diel changes in zooplankton biomass ceased after 10 April (Fig. 2A), and (iii) more than half of the zooplankton biomass was in the upper 150 m after 10 April (Fig. 2C, D). All of these might be caused by two factors: surface stocks of *Neocalanus* species developed into larger, late copepodid stages and upward migration of resting stocks of *E. bungii* and *M. okhotensis* occurred then (Yamaguchi et al., 2010). Recently, Ikeda et al. (2008) summarized seasonal changes in biomass of large and medium-sized copepods in two depth strata (0–250 and 250–2000 m) at Site H in the Oyashio region. In the 250–2000 m layer, peak biomass of these copepods occurs in early September, decreasing gradually until April of the next year, and then increases again until August–October (cf. Fig. 3 of Ikeda et al., 2008). Thus, the period of the present study (late March to early May) corresponds to the season when most of the dominant copepods are concentrated in the near-surface layer, and biomass in the deep

layer is the lowest in the year. The new results confirm the spring portion of that pattern (Fig. 2C, D).

4.2. Population structure

4.2.1. *Eucalanus bungii*

The life cycle of *E. bungii* in the Oyashio region was studied by Tsuda et al. (2004) and Shoden et al. (2005). According to Tsuda et al. (2004), life cycle timing of *E. bungii* is two months ahead of the annual schedule in the Alaskan Gyre, and this earlier timing is likely related to the timing and magnitude of primary production. Overwintering stages in the Oyashio are C3–C6F, and the resting C5 molt to C6 males and females in February and March, respectively (Shoden et al., 2005). Dominance of C3–C6 and no occurrence of nauplii or C1–C2 during March in this study (Fig. 3A) correspond well to the previously reported life cycle schema. Sex ratio was nearly equal (F:M=1:1) for C4 and C5 and ca. 3:1 for C6 during March to 10 April (Fig. 3C). This skewed sex ratio in C6 may reflect the shorter longevity of C6M, since they remain at 250–500 m without feeding (Miller et al., 1984; Shoden et al., 2005). Mating perhaps occurs at depth, slightly ahead of the phytoplankton bloom, and only mated C6F ascend to the surface layer in April for spawning (Shoden et al., 2005). Gonad maturation of C6F was a dominant process in March with immature ovarian stages I and II present. Ovaries matured rapidly after the phytoplankton bloom started around 7 April (Fig. 3D). A similarly rapid response to phytoplankton abundance has also been reported for *Eucalanus californicus*, which can be dormant as both adult females and C5s, with winter females responding relatively rapidly to elevated food and temperature conditions; they begin feeding and producing eggs within 2–3 days (Ohman et al., 1998). During 10–20 April, gonad maturation of C6F had advanced and most ovaries were in reproductive stages V-1 and V-2 (Fig. 3D), which corresponds to the previously reported reproductive timing of this species (Tsuda et al., 2004; Shoden et al., 2005).

Occurrence of *E. bungii* nauplii was observed from 7 April onward, although there was no clear developmental sequence detected for naupliar stage composition (Fig. 3B). This indicates that continuous recruitment of nauplii occurred through April. The peak abundance of nauplii occurred on 20 April (Fig. 3B), and that of C1 was on 25 April (Fig. 3B). At Station P in the Alaskan Gyre, nauplii peaked sharply in abundance on 19 July, C1 and C2 peaked on 1 August, and all had advanced to the third copepodid stage by September (Miller et al., 1984). Thus the development period in the Oyashio region as observed in this study was much earlier than that in the Alaskan Gyre.

The remarkable point of this study is that newly recruited C6 of *E. bungii* were observed during 20–30 April (Fig. 3C, D). C6M were expected to have short longevity, since they do not feed in the current year. The energy of C6M is accumulated in the previous year, and is then utilized to maintain them as a resting population of C5M in deep layers. They molt to C6M without feeding, produce spermatophores and mate in their second spring. When C5F molt to C6F, they migrate upward and feed on phytoplankton, obtaining energy for reproduction and maintenance of their population. Thus the complete absence of C6M during 16–20 April in this study (Fig. 3C) might have been caused by their death after a period of mating activity.

Then, C6M of *E. bungii* were observed again during 24–30 April, and the sex ratio was ca. 3:1 (F:M), similar to those in March–early April (Fig. 3C). In terms of C6F gonad maturation, dominance of reproductive stages V-1 and V-2 decreased suddenly on 20 April, and the proportion of immature gonad stages I–IV increased during 20–30 April (Fig. 3D). Dominance of immature ovaries in this period is anomalous, because a massive phytoplankton bloom

was occurring in the epipelagic layer at that time. This phenomenon is best explained as new recruitment to the C6F population in this period, as well as that of C6M. Since the first C6F population is composed by the population which overwinters as C5F (molting to C6F in March, Shoden et al., 2005), these newly recruited C6F are considered to have over-wintered as C3 and C4F. The over-wintering C3 and C4 likely migrated into shallower layers in early April (Yamaguchi et al., 2010), developed through C4–C5 during early–mid April, and then reached C6F during 20–30 April (Fig. 3C, D).

A similar two-phase reproductive mode of *E. bungii* is also reported for the populations in the Alaskan Gyre. According to Miller et al. (1984), *E. bungii* reproduced in the mixed layer in early May and in early July. The first event was a spawning by females that had previously spawned and then had returned to diapause. The second, heavier spawning (more females, more eggs per female) was by newly matured females from stocks that had over-wintered as C5. The flexibility of life cycle is considered to be a special characteristic of eucalanid copepods. Several authors have characterized such patterns as “bet-hedging” tactics: the young from a given mother having more than one chance to encounter sustaining conditions. Apparently, *E. bungii* in the subarctic Pacific is an “event-driven” species, capable of responding on a short-term basis to favorable environmental circumstances. Similar flexibility in life-cycle timing has been observed for *E. californicus* in the coastal NE Pacific (Ohman et al., 1998) and for *Rhincalanus gigas* in the Southern Ocean (Ward et al., 1997), which varies in generation length from 1 year to 2 years.

4.2.2. *Metridia pacifica*

Life cycles of *M. pacifica* and *M. okhotensis* in the Oyashio region were studied by Padmavati et al. (2004). Although recruitment of *M. pacifica* occurs throughout the year in the top 250 m of the water column, two pronounced generations have been observed. The first is characterized by rapid development during the spring phytoplankton bloom (generation length: 2–3 months), and the second by slow development (9–10 months) with over-wintering at C5 in deeper layers (up to 1000–2000 m) (Padmavati et al., 2004). Stage structure of *M. pacifica* in this study was dominated by C6 in March and by early copepodid stages in April (Fig. 4A), which corresponds well to previous life cycle studies in the Oyashio region (cf. Fig. 10A of Padmavati et al., 2004).

During March, pigment was present in the guts of *M. pacifica* (Fig. 7B), and there were both egg production (Fig. 6B) and active diel vertical migration (Yamaguchi et al., 2010). All of these indicate that *M. pacifica* was not in a resting phase. Since the rest period of *M. pacifica* is variable with location (cf. Hirakawa and Imamura, 1993), perhaps it should be termed quiescence not diapause. In the Oyashio region, the resting stages are predominantly C5M or C6F with immature ovaries (Padmavati et al., 2004). This resting stage structure implies that this species is ready to start reproduction once food is supplied. Much earlier occurrence of *M. pacifica* C1 (Fig. 4A) than of *E. bungii* (Fig. 3A) reflects this stage structure of *M. pacifica*.

4.2.3. *Metridia okhotensis*

In contrast to *M. pacifica*, the life cycle of *M. okhotensis* is estimated to be that C1 produced during one year over-winter and develop to C5 through the phytoplankton bloom of the next year. Those C5 over-winter, molt to C6 and reproduce during the phytoplankton bloom of the following year; thus, the generation length of *M. okhotensis* is estimated as 2 years (Padmavati et al., 2004). The over-wintering C5 emerge from diapause and start diel vertical migration in early April (Yamaguchi et al., 2010). They

feed, molt to C6F and then reproduce. Because of the time required for this final development, the start of their reproduction, as shown by our data, was later than that of *M. pacifica* (Fig. 6B, C).

4.2.4. *Neocalanus cristatus*

The life cycle of *N. cristatus* is reported to be similar for its entire distribution range in the subarctic Pacific (Tsuda et al., 2004). Reproduction occurs below 500 m during October to December, with a resultant peak of C1 in the near-surface layer during January to February. Populations develop through C5 by about June (Miller et al., 1984; Kobari and Ikeda, 1999; Tsuda et al., 2004). Development from C2 to C4 during March to April corresponds well to the life cycle results from the Oyashio region (Kobari and Ikeda, 1999; Tsuda et al., 2004). Since our OECOS study period (March–April) was at the annual minimum of resting stock below 250 m (cf. Fig. 3 of Ikeda et al., 2008), population analysis from 0–500 m might be useful to estimate developmental time of *Neocalanus* spp. in the epipelagic layer.

Following a procedure like the cumulative stage composition analyses of Miller and Nielsen (1988) and Miller (1993), stage developmental times were analyzed for *Neocalanus* spp. (Fig. 9). For *N. cristatus*, significant regressions were observed for shifting proportions of the stock between C1–C2, C2–C3, C3–C4 and C4–C5 ($p < 0.05$, Fig. 9A). Assuming the interval between half of the population entering one stage and half entering the next is the stage development time, we obtained *N. cristatus* data for C2 (–17 March), C3 (17 March–11 April) and C4 (11 April–) (Fig. 9A). Thus we estimate the duration of *N. cristatus* C3 is 24 days. This roughly corresponds to the stage duration reported from the southeastern Bering Sea shelf (20 days) (Vidal and Smith, 1986).

Since most of the population of *N. cristatus* developed from C2 to C4 during the study period, their abundance regression ($Y = 7.9 - 0.065X$, where Y is abundance [ind. m^{-3}] and X is Julian day starting from 1 March, cf. Fig. 3A) may reflect mortality. If we assume that the regression is the effect of mortality, the mortality rate of *N. cristatus* is calculated as $0.82\% \text{ day}^{-1}$ ($= 0.065/7.9$). This

value corresponds to the lower mortality rate of pre-diapause copepodid stages reported in the upper layers in the eastern subarctic Pacific ($0.75\text{--}2.0\% \text{ day}^{-1}$; Miller, 1993; Mackas et al., 1998).

4.2.5. *Neocalanus flemingeri*

The life history of *N. flemingeri* varies with location, particularly in respect to the copepodid stage during diapause in deep layers. Rest as fertilized C6F with an immature ovary was originally reported for the population at Station P in the Alaskan Gyre (Miller and Clemons, 1988), while rest of C4 and of C6F with immature ovaries are reported for the population in the Japan Sea (Miller and Terazaki, 1989). Both resting stages (C4 and C6F with immature ovary) are confirmed to be present also in the Oyashio region (Tsuda et al., 1999; Kobari and Ikeda, 2001a). The body sizes of *N. flemingeri* C4 and C5 in the Oyashio region are reported to be bimodal, which has been suggested to result from differences in generation length (Tsuda et al., 1999) or from sexual dimorphism (Kobari and Ikeda, 2001a).

Throughout the study period, all the copepodid stages of *N. flemingeri* were observed, with an abundance peak of the total on 25 April (Fig. 5B). The proportional composition of the copepodid stages showed a clear sequence with time: C1 peaked on 9 April, C2 on 18 April and C3 on 25 April (Fig. 9B). If we assume that the intervals between peaks were stage development times, stage durations of C1 and C2 were ~ 9 days (9 April–18 April) and ~ 7 days (18 April–25 April), respectively. There are no other field developmental data for *N. flemingeri* with which to compare these results. We can, however, compare our results to published values for the similarly sized sibling species, *N. plumchrus*. At Station P in the Alaskan Gyre, stage duration of its C3 and C4 during May 1984 have been reported to be 24.0 and 24.8 days, respectively (Miller and Nielsen, 1988), while that of C3 during May 1988 was closer to 13.4 days (Miller, 1993). For more nutrition-rich regions, stage durations of each copepodid stage of *N. plumchrus* have been reported to be 8–16 days in the southeastern Bering Sea shelf (Vidal and Smith, 1986) and 12.4–14.1 days in the northern Gulf of Alaska (Liu and Hopcroft, 2006). The stage duration we observed, 7–9 days, corresponded to that in the southeastern Bering Sea. Since the chlorophyll-*a* observed during this study (maximum $34 \mu\text{g l}^{-1}$) was similar to that in the southeastern Bering Sea, copepodid development of *Neocalanus* spp. with replete nutrition appears to require just over a week in each stage. According to Dagg (1991), development of *N. plumchrus* from C1 to C5 requires 91 days in the Alaskan Gyre vs. only 46 days in the southeastern Bering Sea. In addition, the average body mass of a C5 *N. plumchrus* from the Bering Sea is more than twice that of an individual from Ocean Station P (Dagg, 1991). Thus the developmental rate of *Neocalanus* spp. varies with location, apparently governed by available nutrition.

4.2.6. *Neocalanus plumchrus*

The life cycle of *N. plumchrus* is known to vary in reproductive timing in different subarctic areas of the Pacific (cf. Miller and Clemons, 1988), while the season of development in the surface layer is similar among areas (Miller and Clemons, 1988; Tsuda et al., 1999; Kobari and Ikeda, 2001b). Throughout the region, surface occurrence of *N. plumchrus* is consistently much later than that of *N. flemingeri* (cf. Tsuda et al., 1999). Developmental periods of the two species (winter-spring for *N. flemingeri* and spring-summer for *N. plumchrus*) imply species-differences in lipid accumulation pattern; it starts in early copepodid stages of *N. flemingeri*, but only occurs in late copepodid stages of *N. plumchrus* (Tsuda et al., 2001). This temporal separation between the two species was again observed in the present

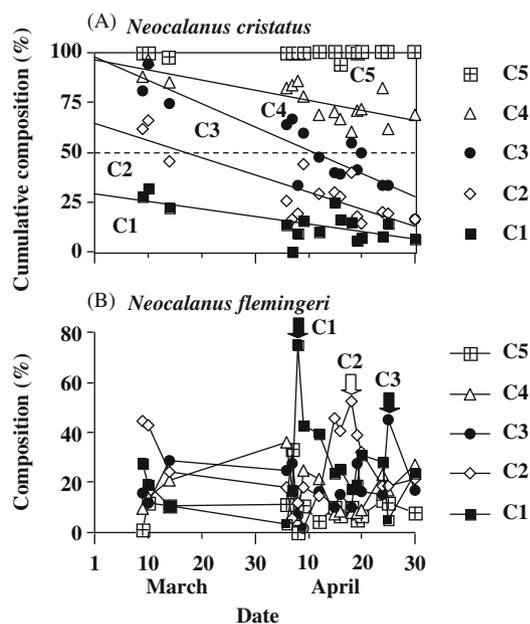


Fig. 9. *Neocalanus cristatus* (A) and *N. flemingeri* (B). Temporal changes in cumulative stage composition of *N. cristatus* (A) and proportions of each stage in *N. flemingeri* (B) in the Oyashio region during 9–14 March and 6–30 April 2007. Horizontal dashed line in (A) indicates position of 50% in each of younger and older stages, and arrows in (B) indicate peaks of each copepodid stage.

study. In March, *N. plumchrus* was extremely low in abundance from 0–500 m the water column, with small numbers occurring from 7 April onward (Fig. 5C). In the Oyashio region, abundance of *N. plumchrus* is reported to increase during May–June (Tsuda et al., 1999; Kobari and Ikeda, 2001b). Assuming this seasonality was repeated in 2007, the very small surface stocks of *N. plumchrus* in this study were expected.

4.3. Egg production

4.3.1. *Eucalanus bungii*

Egg production rate of *E. bungii* was in good agreement with their field population; that is, the general levels of copepodid and naupliar stage abundance (Fig. 3A, B) were similar to the water column rate of egg production (Fig. 6A). Egg production rate of *E. bungii* was reported as 129 ± 26 eggs female⁻¹ day⁻¹ (range: 88–170) during spring in the southeastern Bering Sea (Vidal and Smith, 1986). That corresponds to our present estimates for the Oyashio (Fig. 6A). The abnormal eggs of *E. bungii* were characterized by a weak outer membrane, no cell division and finally bursting into oil droplets. Abnormal eggs were observed mainly during the early phase of egg production (before 10 April) and were extremely rare from 15 April onward (Fig. 6A). We suggest that they are unfertilized eggs. The fractions of normal eggs and of successful hatching were low at the initiation of egg production (6 April), then rapidly improved from 15 April onward (Fig. 6A). This timing corresponds well to the proportional data for C6F gonad maturity stages (Fig. 3D).

The recruitment rate to the field *E. bungii* population (ind. m⁻³ day⁻¹: egg production × proportion of normal egg × hatchability × C6F abundance in the field) peaked on 18 April (bottom panel of Fig. 6A). Naupliar abundance in the field peaked on 20 April (Fig. 3B). Thus, timing of egg production corresponded well to the field abundance data for newly hatched young. Considering these facts, negative effects on copepod hatching success of the diatoms abundant during the spring bloom (the ‘paradox’ of diatom-copepod interaction, Ianora et al., 2003) could not have been particularly severe for *E. bungii* at A5 during our study. The fractions of abnormal eggs and the low hatching success of *E. bungii* improved through April during the massive diatom bloom (Fig. 6A). This also suggests that the initial egg abnormality and low hatchability were caused by spawning of immature ova or fertilization failures.

4.3.2. *Metridia pacifica*

Concerning egg production of *Metridia* spp., a discrepancy between high abundance and low egg production and hatchability rates has been documented (Halsband-Lenk, 2005; Hopcroft et al., 2005; Plourde and Joly, 2008). Suggested possible causes of the low egg production and hatchability of *Metridia* spp., at least in experiments, include egg cannibalism by C6F, insufficient size of incubation containers and damage to eggs when they sink through the egg separator mesh (Plourde and Joly, 2008). Egg production, fraction of normal eggs and hatchability of *M. pacifica* were highly variable in our experiments, and no constant trend was detected along the time course of the study (Fig. 6B). The peak of normal egg production in this study (30.1 eggs female⁻¹ day⁻¹) corresponds well to that observed by Hopcroft et al. (2005) (30 eggs female⁻¹ day⁻¹), suggesting that the incubation conditions of this study were not particularly deficient. The results of Hopcroft et al.’s (2005) were obtained with a specially designed incubation chamber (spawning tower).

Given the magnitude of population recruitment (bottom panel of Fig. 6B), based on the field abundance of copepodid stages (Fig. 4A), the experimental egg production and hatchability

estimates in this study are not great enough. Reproduction must be more successful in the ocean. In the present study, C6F incubations were done with plastic chambers (volume ca. 300 ml) with an attached 0.33 mm mesh on the bottom. Ten C6F were incubated for 24 hours in well-oxidized, filtered seawater at 3 °C in the dark. Thus the volume of the container per individual is 30 ml (=300/10). This volume was smaller than the 45 ml used by Plourde and Joly (2008), but larger than the 15 ml of Halsband-Lenk (2005) and Hopcroft et al. (2005). The container volume undoubtedly represents an additional factor explaining the large difference observed in clutch size and egg production rate between treatments with and without egg separation in Halsband-Lenk (2005) and Hopcroft et al. (2005). Plourde and Joly (2008) also suggest that freshly laid eggs could be damaged by sinking through the egg separator mesh, causing abnormal development and death. Thus, the strongly fluctuating egg production and hatchability data of *M. pacifica* in the present study are of questionable value. As an alternative explanation, since *M. pacifica* has several cohorts in one sampling period, there could be great differences in feeding history, gonad maturation and reproductive condition. These individual differences in reproductive condition may mask a clear synchronization of reproduction like that seen in *E. bungii* mentioned above.

4.3.3. *Metridia okhotensis*

Reproduction of *M. okhotensis* is thought to be carried out by adults that over-wintered as C5, and then molted to C6 at the onset of the spring phytoplankton bloom (Padmavati et al., 2004). Initiation of reproduction by *M. okhotensis* in 2007 (beginning 17 April, Fig. 6C) was later than that of *M. pacifica*. Diel vertical migration behavior of *M. okhotensis* is only observed in spring (Padmavati et al., 2004; Takahashi et al., 2008), and reproduction of this species is limited to spring only in the northern Gulf of Alaska (Hopcroft et al., 2005). This information suggests that *M. okhotensis* spends most of the year resting as C5, and previously resting C5 initiate DVM in spring (Takahashi et al., 2008; Yamaguchi et al., 2010). Feeding and then molting to C6 in spring, followed by reproduction is the pattern confirmed by the OECOS data set.

4.4. Gut pigment

Diel changes in gut pigment (greater at night) were only detected in the present study for *M. pacifica* and *M. okhotensis* (Table 2). Individuals of these relatively small copepods contain more phytoplankton pigment at night in March, than individuals of the much larger *Neocalanus* spp. or *E. bungii* (Fig. 7B). The diel cycling indicates that *M. pacifica* undertook a nocturnal ascent to feed, while the large *N. cristatus* and *E. bungii* did not show clear DVM (Yamaguchi et al., 2010) or strong diel feeding cycles (but see below for *N. cristatus*). Gut pigment in *Neocalanus* spp. is known to be determined by the availability of phytoplankton (cf. Dagg et al., 2006). For *Neocalanus* spp. and *E. bungii*, the lower gut pigment values in March than that in April in this study (Fig. 7A, D, E) are considered to have been mainly governed by the temporal changes in environmental chlorophyll *a* (greater in April, Fig. 1C). In contrast, nighttime gut pigment of *M. pacifica* did not vary between March and April (Fig. 7B). Since March was pre-bloom and April was during the massive bloom, similar gut pigment contents of *M. pacifica* between March and April are surprising. Their active DVM and swimming behavior are keys to the mechanism by which they apparently fed at the same rates in March and April, despite large differences in available phytoplankton. Bearing this in mind, in April when the food conditions were sufficient, *M. pacifica* may have met its food quota

with less activity than was required in March. This could have enabled the cessation of DVM behavior of both *M. pacifica* and *M. okhotensis* (except C6F) in late April (Yamaguchi et al., 2010). Terminating DVM under conditions of sufficient food was reported for summer populations of *Metridia* spp. in the Oyashio region by Hattori (1989).

Interestingly, diel changes in gut pigment were observed for *N. cristatus* before 10 April, while pigment was similar between day and night after 12 April (Fig. 7D). Since large *N. cristatus* C5 contain large amounts of gut pigment (cf. Table 2), coloration of their guts may be easily recognized by visual predators (cf. Tsuda et al., 1998). This could be why *N. cristatus* C5 sometimes feeds mainly at night in this region (Saito, 1996; Takahashi et al., 2008). During the phytoplankton bloom, there would have been greater attenuation of light intensity, which would make pigment-loaded copepods more difficult to see. Thus, there may have been little advantage in April for diel feeding cycles or daytime reduction in gut pigment of *E. bungii* or *Neocalanus* spp. toward the end of our study (Fig. 7A, D, E). Ingestion of highly concentrated food both day and night implies a doubling food intake each day. A plausible explanation for the similar life cycle patterns seen for *Neocalanus* spp. living at Site H and at Station P is that temperature and food abundance act oppositely to produce matched timing of the life cycle (Kobari and Ikeda, 1999, 2001a, 2001b). That is, the negative effect of lower temperature on the development of *Neocalanus* spp. at Site H is compensated by the positive effect of higher food concentrations in the same area.

4.4.1. Individual mass

There are previous reports of temporal changes of individual mass in subarctic copepods. For example, lipid changes in resting *N. plumchrus* were shown by Evanson et al., (2000) and Campbell et al. (2004), and lipid consumption is implied by energy requirement estimates for resting *N. cristatus* reported by Ikeda et al. (1990, 2004). All of these studies concern the fate of stored energy, and the sampling intervals were about one month. Thus no previous studies have been made on individual mass increment during a phytoplankton bloom. In body composition (water and AFDM content), there were no correlations with time for *M. pacifica*, while decreasing water content and increasing AFDM with time were observed for *N. cristatus* (Fig. 8B, D; Table 3). These differences in individual mass accumulation patterns may be related to their life cycle patterns. Thus multiple cohorts without resting by *M. pacifica* only require small amounts of accumulated lipids, while one-year generations with prolonged diapause require *N. cristatus* to accumulate large lipid stores.

5. Conclusion

There is increasing evidence that the patterns of zooplankton productivity are changing over time, probably in response to inter-decadal ocean climate variability (cf. Chiba et al., 2006). These changes include 2–3 fold shifts in total biomass, 30–60 day shifts in seasonal timing and 10–25% changes in average body length (Miller et al., 1992; Mackas et al., 1998; Tadokoro et al., 2005; Kobari et al., 2007). Bearing this in mind, the direct comparison of life cycle timing between locations is likely meaningless. However, it is useful to evaluate the sequence of copepod responses to phytoplankton blooms at a given location. This information is useful for predicting a future zooplankton community in terms of the matches or mismatches of each species with the seasonal events. Sequential responses of copepods to phytoplankton blooms are summarized in Fig. 10. Through this study, the reproductive and developmental timing of large copepods during the spring phytoplankton bloom was

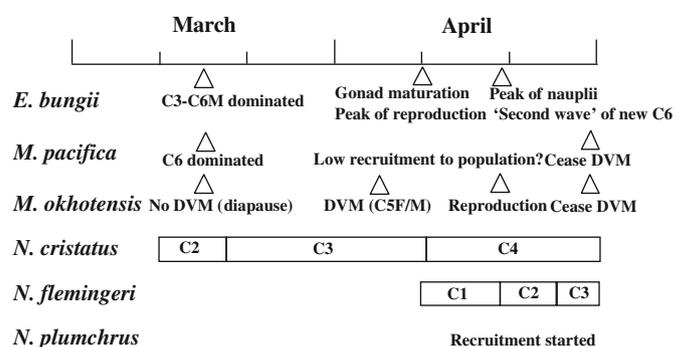


Fig. 10. Schematic diagram showing reproductive and developmental timing of large copepods (*Eucalanus bungii*, *Metridia pacifica*, *M. okhotensis*, *Neocalanus cristatus*, *N. flemingeri* and *N. plumchrus*) in the Oyashio region during the spring phytoplankton bloom in 2007.

evaluated. For *E. bungii*, domination by C3–C6F/M in March is considered to be the last phase of their diapause. In early April, their gonads matured rapidly, followed by a peak of reproduction. Peak naupliar abundance was observed in the middle of April. This reproduction was mainly governed by the specimens that over-wintered as C5 and C6. New recruitment of C6 that over-wintered as C3 and C4 started in the middle of April. For *M. pacifica*, the stock was mainly composed of C1, while egg production and hatchability were low. They cease DVM at the end of April, except for adult females (Yamaguchi et al., 2010). For *M. okhotensis*, no DVM was observed in March. C5F/M started DVM in early April, then ceased by the end of April, except for adult females, similarly to *M. pacifica*. No *Neocalanus* species undertook DVM, and a clear developmental sequence was observed for *N. cristatus* and *N. flemingeri*. In brief, *Neocalanus* species that utilize the spring bloom as energy for growth did not vary their vertical distribution during this period. In contrast, *E. bungii* and *Metridia* spp. that utilize the spring bloom as energy for reproduction can change their vertical distribution, DVM pattern, and gonad maturation drastically during the spring bloom. The results point out that the time-scales of phenology in these copepods can be stretched or compressed depending on conditions. Especially *E. bungii* but also *Neocalanus* spp. have the ability to adapt, as evidenced by the variability of life cycle timing across a wide range of North Pacific environments.

Acknowledgments

OECOS project was sponsored by PICES. We thank captains, officers and crews of T/S *Oshoro-Maru*, Hokkaido University and R/V *Hakuho-Maru*, JAMSTEC for their help during field sampling.

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