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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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.

Keywords: Copepods, Egg production, Gut pigment, Life cycle, OECOS

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 5 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, 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. 3 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 the estimate development time of any stage. High-frequency sampling (1 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.

Materials and methods

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).

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

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

112 volume, made with a wide-bore pipette) of both 0.33 and 0.10 mm mesh net samples.
113 Then we compared the abundance based on 0.10 and 0.33 mm mesh nets and
114 summarized the ratio of abundance between them (Table 1). Ratios for most of the late
115 copepodid stages were near 1 (no differences between the two nets), while some early
116 copepodid stages showed substantially greater factors (0.10 mm> 0.33 mm mesh). For
117 the stages showing a high factor (>5: nauplii of *E. bungii*, C1, C2 and C3 of *M. pacifica*,
118 Table 1), we used the abundance data based on 0.10 mm mesh nets. For the other late
119 copepodid stages, we used the abundance data based on 0.33 mm mesh nets.

120 For *E. bungii*, the naupliar stages were easily distinguished from the other
121 species. Its naupliar stages were identified and enumerated based on the descriptions
122 of Johnson (1937). Also for *E. bungii*, gonad maturation stages of C6F were
123 enumerated (gonad maturation stages I-VII) based on the description by Miller et al.
124 (1984). Since the gonad developmental stage V (two full rows of ova that appear
125 ready for spawning) of Miller et al. (1984) could be separated into two (two full rows of
126 ova and three or more rows of ova), we enumerated them separately as gonad
127 developmental stages V-1 and V-2, respectively.

128 *Egg production and hatching*

129 Fresh adult females of *E. bungii*, *M. pacifica* and *M. okhotensis* collected with the ring
130 net at night were used for on-board egg production experiments. Three liters of
131 surface seawater were collected with a bucket, filtered with a GF/F filter and well
132 aerated by shaking in 500 ml bottles. Fresh adult females were transferred into plastic
133 chambers (ca. 300 ml) with 0.33-mm mesh bottoms (to prevent egg cannibalism by
134 females) inside the 500 ml bottles. In each chamber, three (*E. bungii*) or ten (*M.*

135 *pacifica*) adult females were added. The bottles with adult females were held for one
136 day in an incubator set at 3˚C (integrated mean temperature for 0-100 m during April,
137 Fig. 1c). After 24h, females in the chamber were removed. The remaining seawater
138 was examined under a stereomicroscope to seek eggs. When found they were counted
139 and transferred into 5-mL multi-well plates filled with chilled, filtered seawater. Eggs
140 in the multi-well were also incubated at 3˚C and checked daily for hatching. Egg
141 incubation lasted ca. 1 week.

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

150 *Gut pigment*

151 Using fresh specimens collected both day and night, pigments contained in the guts of
152 late copepodid stages of dominant copepods were examined. Portions of the samples
153 collected with the ring net were poured into a 1-l pitcher, and 10% v/v soda (saturated
154 CO_2 in water) was added to prevent gut evacuation and decomposition of pigment.
155 Fresh samples were examined under a stereomicroscope and C6F of *E. bungii*, *M.*
156 *pacifica* and *M. okhotensis* and C5 of *N. cristatus*, *N. flemingeri* and *N. plumchrus* were
157 sorted out. Batches of two (*N. cristatus* C5) to five (*M. pacifica* C6F) specimens were

158 immersed in N,N-dimethylformamide, stored in dark, cold conditions overnight to
159 extract chlorophyll and phaeopigments. After extraction of pigment, chlorophyll and
160 phaeopigments were measured with a Turner Designs fluorometer. Chlorophyll and
161 phaeopigment amounts were summed and expressed as ng pigment ind.$^{-1}$ (cf. Mackas
162 and Bohrer, 1976).

163 *Individual mass*

164 To evaluate temporal changes in individual body composition, C6F of *M. pacifica* and
165 C5 of *N. cristatus* were sorted from the daytime live samples. Specimens were
166 incubated in filtered seawater for three hours to evacuate their gut contents, rinsed with
167 distilled water, transferred into pre-weighed aluminum pans and stored in a freezer at
168 –80˚C. In the land laboratory, wet masses (WM) were determined ($\pm$1 µg) with a
169 microbalance (Mettler Toledo MT5), then samples were freeze-dried and dry masses
170 (DM) were determined. Dried specimens were incinerated at 480˚C for 5 h, and ash
171 weight was determined. From these data, water content (WATER, %WM) and ash-free
172 dry mass (AFDM, %DM) were estimated using the following equations:

173 $$\text{WATER} = 100(\text{WM}-\text{DM})/\text{WW} \text{ and } \text{AFDM} = 100(\text{DM}-\text{Ash})/\text{DM}.$$

174 **Results**

175 *Hydrography*

176 Through the study period, integrated mean temperature and salinity in the 0-100 m
177 stratum varied between 1.5-6.0˚C and 33.16-33.65, respectively (Fig. 1c).
178 Temperature and salinity varied in parallel. They were high in March, then had two
179 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 (this issue).

Zooplankton biomass

Zooplankton wet mass was low during 8-14 March, increased by 10 April, and then 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 ±1sd: 92 ±3% for day and 82 ±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 ±1sd: 59 ±10% for day and 65 ±17% for night) (Fig. 2c, d).

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 were collected (Fig. 3a, b). 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 ±2%: mean ±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 ±20% of the population) and for C5 (56 ±19%) (Fig. 3c). The average ratio was skewed to females in C6 (79 ±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 ±6% V-1 and 34 ±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 (9-16 ind. m^{-3}). After that abundance of *M. pacifica* was stable, varying between 51 and 91 ind. m^{-3} (mean ±1sd: 72 ±14 ind. m^{-3}), and their population structure was also stable through April: C1 dominated (49 ±11%) followed by C2 (14 ±7%), C3 (16 ±6%), C4 (6 ±3%), C5 (6 ±3%) and C6 (9 ±4%) (Fig. 4a).

Abundance of *M. okhotensis* (3-30 ind. m^{-3}) was lower than that of *M. pacifica* (Fig. 4b). After 20 April, their abundance peaked and was slightly greater than before. Its population structure was dominated by C5 (34 ±19%), followed by C6 (24 ±13%) and C4 (21 ±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

248 present in April, but its abundance was consistently lower than that of *N. flemingeri*.
249 The population structure of *N. plumchrus* during 15-30 April was dominated by C1 (30
250 ±11%), C2 (33 ±12%) and C3 (17 ±7%) (Fig. 5c).

251 *Egg production*

252 Egg production of *E. bungii* was zero in March and remained low (<50 eggs female^{-1}
253 day^{-1}) during 6-9 April (Fig. 6a). Abnormal eggs with a weak membrane structure
254 were also observed. Both normal and abnormal egg production rates increased during
255 6-12 April; after that production of abnormal eggs was rare (Fig. 6a). In early April,
256 both the proportion of normal eggs and the hatchability of normal eggs were low.
257 Those increased during 6-16 April, after which normal eggs were 93 ±6% of egg
258 production and hatchability was 83 ±5% of normal eggs (Fig. 6a). Recruitment of
259 nauplii female^{-1} d^{-1} was low during 6-9 April, and then much higher during 11-30 April
260 (mean ±1sd: 88 ±22 ind. female^{-1} d^{-1}). Total recruitment of nauplii to the water
261 column *E. bungii* population was also greater during 11-30 April (206 ±124 ind. m^{-3}
262 day^{-1}), and peaked on 18 April (447 ind. m^{-3} day^{-1}) (Fig. 6a).

263 Egg production of *M. pacifica* fluctuated greatly through the study period (Fig.
264 6b). It also produced abnormal eggs with weak membranes, more of them (76%) than
265 normal eggs (24% of total egg production). Hatchability of normal eggs also
266 fluctuated around a mean of 67%. Temporal variability of percentage of normal eggs
267 and hatchability of normal eggs were correlated (Fig. 6b). Individual recruitment
268 peaked on 30 April with 18 ind. female^{-1} day^{-1}, and population recruitment also peaked
269 then (37 ind. m^{-3} day^{-1}).

270 Egg production of *M. okhotensis* was observed from 17 April onward (Fig. 6c).

271 Their percentage of normal eggs was 88 ±13%, but hatchability fluctuated. Their
272 individual recruitment peaked on 20 April at 7.3 ind. female^{-1} day^{-1}, and population
273 recruitment peaked in same period at 22 ind. m^{-3} day^{-1} (Fig. 6c).

274 *Gut pigment*

275 Diel changes in gut content pigment of late copepodid stages separated into two types:
276 (1) *E. bungii* C6F, *N. cristatus* C5 and *N. flemingeri* C5 showed low gut pigment in
277 March and no day-night differences in gut pigment during April (except for *N. cristatus*
278 C5 during 6-10 April) (Fig. 7a, d, e). (2) *M. pacifica* C6F and *M. okhotensis* C6F
279 showed significantly higher gut pigment at night than in daylight (p<0.01, U-test) and
280 had similar gut pigment levels at night in March and April (Fig. 7b, c). Mean gut
281 pigment varied with species, highest for *N. cristatus* C5 (133-197 ng Chl. ind.$^{-1}$) and
282 lowest for *M. pacifica* C6F (2.3-6.2 ng Chl. ind.$^{-1}$), a function of their body sizes (Table
283 2).

284 *Individual mass and body composition*

285 The WM, DM and ash of *M. pacifica* C6F were 0.94 ±0.17, 0.16 ±0.03 and 0.015
286 ±0.002 mg ind.$^{-1}$, respectively (means ±1sd) (Fig. 8a). Its water and AFDM contents
287 were 82.1 ±3.2% of WM and 90.3 ±2.0% of DM, respectively (Fig. 8b). Regressions
288 of compositional components vs. date showed that water and AFDM of *M. pacifica*
289 were uncorrelated with date (Table 3).

290 The WM, DM and ash of *N. cristatus* C5 were 20.0±2.3, 3.99±1.89 and
291 0.30±0.04 mg ind.$^{-1}$, respectively (mean ±1sd) (Fig. 8c). Its Water and AFDM contents
292 averaged 81.5±7.1% of WM and 90.1±4.9% of DM, respectively (Fig. 8d).

293 Regressions vs. date showed that AFDM content of *N. cristatus* C5 was significantly
294 increasing with date, with water content decreasing with date (Table 3).

295 **Discussion**

296 **Zooplankton biomass**

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

308 Comparing March to April, we observed several temporal changes in total
309 zooplankton biomass: (i) total zooplankton stocks increased after 10 April by a factor of
310 8 for 0-150 m and 2-fold for 0-500 m, (ii) diel changes in zooplankton biomass ceased
311 after 10 April (Fig. 2a), and (iii) more than half of the zooplankton biomass was in the
312 upper 150 m after 10 April (Fig. 2c, d). All of these might be caused by two factors:
313 surface stocks of *Neocalanus* species developed into larger, late copepodid stages and
314 upward migration of resting stocks of *E. bungii* and *M. okhotensis* occurred then
315 (Yamaguchi et al., this issue). Recently, Ikeda et al. (2008) summarized seasonal

316 changes in biomass of large and medium-sized copepods in two depth strata (0-250 and
317 250-2000 m) at Site H in the Oyashio region. In the 250-2000 m layer, peak biomass
318 of these copepods occurs in early September, decreasing gradually until April of the
319 next year, and then increases again until August-October (cf. Fig. 3 of Ikeda et al., 2008).
320 Thus, the period of the present study (late March to early May) corresponds to the
321 season when most of the dominant copepods are concentrated in the near-surface layer,
322 and biomass in the deep layer is the lowest in the year. The new results confirm the
323 spring portion of that pattern (Fig. 2c, d).

324 **Population structure**

325 *Eucalanus bungii*

326 The life cycle of *E. bungii* in the Oyashio region was studied by Tsuda et al. (2004) and
327 Shoden et al. (2005). According to Tsuda et al. (2004), life cycle timing of *E. bungii* is
328 two months ahead of the annual schedule in the Alaskan Gyre, and this earlier timing is
329 likely related to the timing and magnitude of primary production. Overwintering
330 stages in the Oyashio are C3-C6F, and the resting C5 molt to C6 males and females in
331 February and March, respectively (Shoden et al., 2005). Dominance of C3-C6 and no
332 occurrence of nauplii or C1-C2 during March in this study (Fig. 3a) correspond well to
333 the previously reported life cycle schema. Sex ratio was nearly equal (F:M= 1:1) for
334 C4 and C5 and ca. 3:1 for C6 during March to 10 April (Fig. 3c). This skewed sex
335 ratio in C6 may reflect the shorter longevity of C6M, since they remain at 250-500 m
336 without feeding (Miller et al., 1984; Shoden et al., 2005). Mating perhaps occurs at
337 depth, slightly ahead of the phytoplankton bloom, and only mated C6F ascend to the
338 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. 3a). 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.

363 Thus the complete absence of C6M during 16-20 April in this study (Fig. 3c) might
364 have been caused by their death after a period of mating activity.

365 Then, C6M of *E. bungii* were observed again during 24-30 April, and the sex
366 ratio was ca. 3:1 (F:M), similar to those in March-early April (Fig. 3c). In terms of
367 C6F gonad maturation, dominance of reproductive stages V-1 and V-2 decreased
368 suddenly on 20 April, and the proportion of immature gonad stages I-IV increased
369 during 20-30 April (Fig. 3d). Dominance of immature ovaries in this period is
370 anomalous, because a massive phytoplankton bloom was occurring in the epipelagic
371 layer at that time. This phenomenon is best explained as new recruitment to the C6F
372 population in this period, as well as that of C6M. Since the first C6F population is
373 composed by the population which overwinters as C5F (molting to C6F in March,
374 Shoden et al., 2005), these newly recruited C6F are considered to have over-wintered as
375 C3 and C4F. The over-wintering C3 and C4 likely migrated into shallower layers in
376 early April (Yamaguchi et al., this issue), developed through C4-C5 during early-mid
377 April, and then reached C6F during 20-30 April (Fig. 3c, d).

378 A similar two-phase reproductive mode of *E. bungii* is also reported for the
379 populations in the Alaskan Gyre. According to Miller et al. (1984), *E. bungii*
380 reproduced in the mixed layer in early May and in early July. The first event was a
381 spawning by females that had previously spawned and then had returned to diapause.
382 The second, heavier spawning (more females, more eggs per female) was by newly
383 matured females from stocks that had over-wintered as C5. The flexibility of life cycle
384 is considered to be a special characteristic of eucalanid copepods. Several authors
385 have characterized such patterns as “bet-hedging” tactics: the young from a given
386 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.

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., this issue). 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

410 reproduction once food is supplied. Much earlier occurrence of *M. pacifica* C1 (Fig.
411 4a) than of *E. bungii* (Fig. 3a) reflects this stage structure of *M. pacifica*.

412 *Metridia okhotensis*

413 In contrast to *M. pacifica*, the life cycle of *M. okhotensis* is estimated to be that C1
414 produced during one year over-winter and develop to C5 through the phytoplankton
415 bloom of the next year. Those C5 over-winter, molt to C6 and reproduce during the
416 phytoplankton bloom of the following year; thus, the generation length of *M. okhotensis*
417 is estimated as 2 years (Padmavati et al., 2004). The over-wintering C5 emerge from
418 diapause and start diel vertical migration in early April (Yamaguchi et al., this issue).
419 They feed, molt to C6F and then reproduce. Because of the time required for this final
420 development, the start of their reproduction, as shown by our data, was later than that of
421 *M. pacifica* (Fig. 6b, c).

422 *Neocalanus cristatus*

423 The life cycle of *N. cristatus* is reported to be similar for its entire distribution range in
424 the subarctic Pacific (Tsuda et al., 2004). Reproduction occurs below 500 m during
425 October to December, with a resultant peak of C1 in the near-surface layer during
426 January to February. Populations develop through C5 by about June (Miller et al.,
427 1984; Kobari and Ikeda, 1999; Tsuda et al., 2004). Development from C2 to C4 during
428 March to April corresponds well to the life cycle results from the Oyashio region
429 (Kobari and Ikeda, 1999; Tsuda et al., 2004). Since our OECOS study period
430 (March-April) was at the annual minimum of resting stock below 250 m (cf. Fig. 3 of
431 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% 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 (0.75-2.0% day^{-1}: Miller, 1993; Mackas et al., 1998).

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

455 are reported for the population in the Japan Sea (Miller and Terazaki, 1989). Both
456 resting stages (C4 and C6F with immature ovary) are confirmed to be present also in the
457 Oyashio region (Tsuda et al., 1999; Kobari and Ikeda, 2001a). The body sizes of *N.*
458 *flemingeri* C4 and C5 in the Oyashio region are reported to be bimodal, which has been
459 suggested to result from differences in generation length (Tsuda et al., 1999) or from
460 sexual dimorphism (Kobari and Ikeda, 2001a).

461 Throughout the study period, all the copepodid stages of *N. flemingeri* were
462 observed, with an abundance peak of the total on 25 April (Fig. 5b). The proportional
463 composition of the copepodid stages showed a clear sequence with time: C1 peaked on
464 9 April, C2 on 18 April and C3 on 25 April (Fig. 9b). If we assume that the intervals
465 between peaks were stage development times, stage durations of C1 and C2 were ~9
466 days (9 April-18 April) and ~7 days (18 April-25 April), respectively. There are no
467 other field developmental data for *N. flemingeri* with which to compare these results.
468 We can, however, compare our results to published values for the similarly sized sibling
469 species, *N. plumchrus.* At Station P in the Alaskan Gyre, stage duration of its C3 and
470 C4 during May 1984 have been reported to be 24.0 and 24.8 days, respectively (Miller
471 and Nielsen, 1988), while that of C3 during May 1988 was closer to 13.4 days (Miller,
472 1993). For more nutrition-rich regions, stage durations of each copepodid stage of *N.*
473 *plumchrus* have been reported to be 8-16 days in the southeastern Bering Sea shelf
474 (Vidal and Smith, 1986) and 12.4-14.1 days in the northern Gulf of Alaska (Liu and
475 Hopcroft, 2006). The stage duration we observed, 7-9 days, corresponded to that in
476 the southeastern Bering Sea. Since the chlorophyll-*a* observed during this study
477 (maximum 34 μg l^{-1}) was similar to that in the southeastern Bering Sea, copepodid
478 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 size 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.

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 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.

501 **Egg production**

502 *Eucalanus bungii*

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

516 The recruitment rate to the field *E. bungii* population (ind. m^{-3} day^{-1}: egg
517 production x proportion of normal egg x hatchability x C6F abundance in the field)
518 peaked on 18 April (bottom panel of Fig. 6a). Naupliar abundance in the field peaked
519 on 20 April (Fig. 3b). Thus, timing of egg production corresponded well to the field
520 abundance data for newly hatched young. Considering these facts, negative effects on
521 copepod hatching success of the diatoms abundant during the spring bloom (the
522 'paradox' of diatom-copepod interaction, Ianora et al., 2003) could not have been
523 particularly severe for *E. bungii* at A5 during our study. The fractions of abnormal
524 eggs and the low hatching success of *E. bungii* improved through April during the

525 massive diatom bloom (Fig. 6a). This also suggests that the initial egg abnormality
526 and low hatchability were caused by spawning of immature ova or fertilization failures.

527 *Metridia pacifica*

528 Concerning egg production of *Metridia* spp., a discrepancy between high abundance
529 and low egg production and hatchability rates has been documented (Halsband-Lenk,
530 2005; Hopcroft et al., 2005; Plourde and Joly, 2008). Suggested possible causes of the
531 low egg production and hatchability of *Metridia* spp., at least in experiments, include
532 egg cannibalism by C6F, insufficient size of incubation containers and damage to eggs
533 when they sink through the egg separator mesh (Plourde and Joly, 2008). Egg
534 production, fraction of normal eggs and hatchability of *M. pacifica* were highly variable
535 in our experiments, and no constant trend was detected along the time course of the
536 study (Fig. 6b). The peak of normal egg production in this study (30.1 eggs female^{-1}
537 day^{-1}) corresponds well to that observed by Hopcroft et al. (2005) (30 eggs female^{-1}
538 day^{-1}), suggesting that the incubation conditions of this study were not particularly
539 deficient. The results of Hopcroft et al's (2005) were obtained with a specially
540 designed incubation chamber (spawning tower).

541 Given the magnitude of population recruitment (bottom panel of Fig. 6b),
542 based on the field abundance of copepodid stages (Fig. 4a), the experimental egg
543 production and hatchability estimates in this study are not great enough. Reproduction
544 must be more successful in the ocean. In the present study, C6F incubations were done
545 with plastic chambers (volume ca. 300 ml) with an attached 0.33 mm mesh on the
546 bottom. Ten C6F were incubated for 24 hours in well-oxidized, filtered seawater at
547 3˚C in the dark. Thus the volume of the container per individual is 30 ml (=300/10).

548 This volume was smaller than the 45 ml used by Plourde and Joly (2008), but larger
549 than the 15 ml of Halsband-Lenk (2005) and Hopcroft et al. (2005). The container
550 volume undoubtedly represents an additional factor explaining the large difference
551 observed in clutch size and egg production rate between treatments with and without
552 egg separation in Halsband-Lenk (2005) and Hopcroft et al. (2005). Plourde and Joly
553 (2008) also suggest that freshly laid eggs could be damaged by sinking through the egg
554 separator mesh, causing abnormal development and death. Thus, the strongly
555 fluctuating egg production and hatchability data of *M. pacifica* in the present study are
556 of questionable value. As an alternative explanation, since *M. pacifica* has several
557 cohorts in one sampling period, there could be great differences in feeding history,
558 gonad maturation and reproductive condition. These individual differences in
559 reproductive condition may mask a clear synchronization of reproduction like that seen
560 in *E. bungii* mentioned above.

561 *Metridia okhotensis*

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

571 followed by reproduction is the pattern confirmed by the OECOS data set.

572 **Gut pigment**

573 Diel changes in gut pigment (greater at night) were only detected in the present study
574 for *M. pacifica* and *M. okhotensis* (Table 2). Individuals of these relatively small
575 copepods contain more phytoplankton pigment at night in March, than individuals of
576 the much larger *Neocalanus* spp. or *E. bungii* (Fig. 7b). The diel cycling indicates that
577 *M. pacifica* undertook a nocturnal ascent to feed, while the large *N. cristatus* and *E.*
578 *bungii* did not show clear DVM (Yamaguchi et al., this issue) or strong diel feeding
579 cycles (but see below for *N. cristatus*). Gut pigment in *Neocalanus* spp. is known to
580 be determined by the availability of phytoplankton (cf. Dagg et al., 2006). For
581 *Neocalanus* spp. and *E. bungii*, the lower gut pigment values in March than that in April
582 in this study (Fig. 7a, d, e) are considered to have been mainly governed by the temporal
583 changes in environmental chlorophyll *a* (greater in April, Fig. 1c). In contrast,
584 nighttime gut pigment of *M. pacifica* did not vary between March and April (Fig. 7b).
585 Since March was pre-bloom and April was during the massive bloom, similar gut
586 pigment contents of *M. pacifica* between March and April are surprising. Their active
587 DVM and swimming behavior are keys to the mechanism by which they apparently fed
588 at the same rates in March and April, despite large differences in available
589 phytoplankton. Bearing this in mind, in April when the food conditions were sufficient,
590 *M. pacifica* may have met its food quota with less activity than was required in March.
591 This could have enabled the cessation of DVM behavior of both *M. pacifica* and *M.*
592 *okhotensis* (except C6F) in late April (Yamaguchi et al., this issue). Terminating DVM
593 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.

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.

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 evaluated. For *E. bungii*, domination by C3-C6F/M in March is considered to be the last phase of their

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

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662

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830 copepods during the spring phytoplankton bloom in the Oyashio region.

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832

833 Figure captions

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

838 Fig. 2. Temporal changes in zooplankton wet biomass in the 0-150 m (a) and 0-500 m
839 (b) water column in the Oyashio region both day and night during 9-14 March
840 and 6-30 April 2007. Night:day (N:D) ratios are shown, and horizontal dashed
841 lines indicate the position of N:D=1. Temporal changes in depth composition
842 of zooplankton biomass in the 0-150 m and 150-500 m during day (c) and night
843 (d) during the same period.

844 Fig. 3. *Eucalanus bungii*. Temporal changes in abundance (circles) and composition
845 of copepodid stages (a), of naupliar stages (b), sex ratio (C4-C6) (c) and gonad
846 maturation phases of C6F (d) in the Oyashio region during 9-14 March and 6-30
847 April 2007. Horizontal lines in (c) indicate means of each copepodid stage.
848 For gonad maturation of C6F (d), eight categories were applied.

849 Fig. 4. *Metridia pacifica* (a) and *M. okhotensis* (b). Temporal changes in abundance
850 (circles) and composition of copepodid stages in the Oyashio region during 9-14
851 March and 6-30 April 2007.

852 Fig. 5. *Neocalanus cristatus* (a), *N. flemingeri* (b) and *N. plumchrus* (c). Temporal
853 changes in abundance (circles) and composition of copepodid stages in the
854 Oyashio region during 9-14 March and 6-30 April 2007.

855 Fig. 6. *Eucalanus bungii* (a), *Metridia pacifica* (b) and *M. okhotensis* (c). Time
856 series of egg production rate estimates (upper panels), proportion of normal eggs

857 and hatchability (second panels), individual recruitment (ind. female^{-1} day^{-1})
858 (third panels) and population recruitment (ind. m^{-3} day^{-1}) (bottom panels) in the
859 Oyashio region during 9-14 March and 6-30 April 2007.
860 Fig. 7. *Eucalanus bungii* C6F (a), *Metridia pacifica* C6F (b), *M. okhotensis* C6F (c),
861 *Neocalanus cristatus* C5 (d) and *N. flemingeri* C5 (e). Temporal changes in gut
862 pigment content of individuals collected in the upper 150 m during day (open
863 circles) and night (solid circles) in the Oyashio region during 9-14 March and
864 6-30 April 2007. Vertical bars indicate standard deviations.
865 Fig. 8. *Metridia pacifica* C6F (a, b) and *Neocalanus cristatus* C5 (c, d). Temporal
866 changes in individual body mass (WM, DM and Ash) of specimens collected
867 during daytime in the upper 150 m (a, c) and their water content (%WM) and
868 AFDM content (%DM) (b, d) in the Oyashio region during 9-14 March and 6-30
869 April 2007. Vertical bars indicate standard deviations. For body composition,
870 a significant relationship with time was recognized only for N. *cristatus* (d).
871 Fig. 9. *Neocalanus cristatus* (a) and *N. flemingeri* (b). Temporal changes in
872 cumulative stage composition of *N. cristatus* (a) and proportions of each stage in
873 *N. flemingeri* (b) in the Oyashio region during 9-14 March and 6-30 April 2007.
874 Horizontal dashed line in (a) indicates position of 50% in each of younger and
875 older stages, and arrows in (b) indicate peaks of each copepodid stage.
876 Fig. 10. Schematic diagram showing reproductive and developmental timing of large
877 copepods (*Eucalanus bungii, Metridia pacifica, M. okhotensis, Neocalanus*
878 *cristatus, N. flemingeri* and *N. plumchrus*) in the Oyashio region during the
879 spring phytoplankton bloom in 2007.

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. 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.

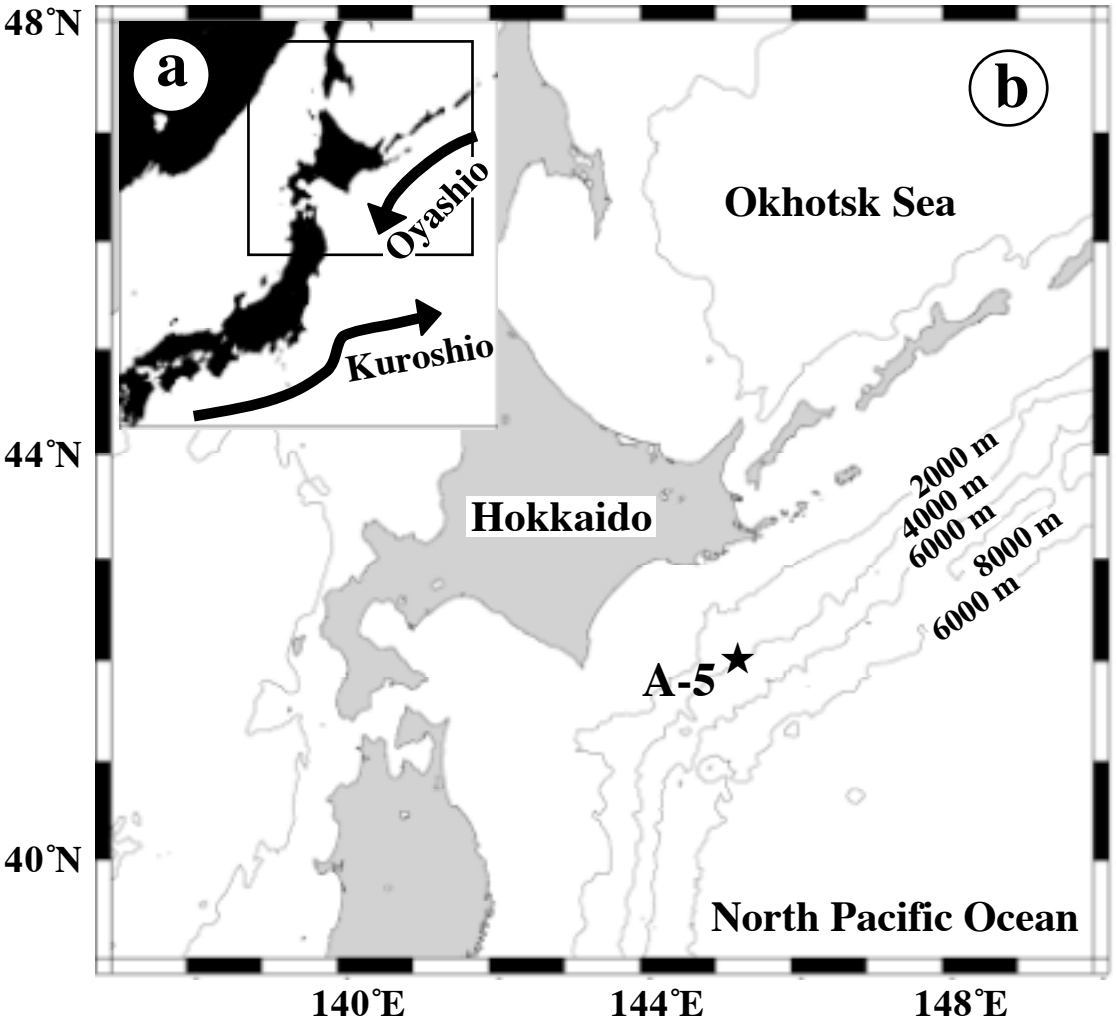
	Stage						
Species	Nauplii	C1	C2	C3	C4	C5	C6
Eucalanus bungii	<u>79.4</u>	1.2	0.7	1.2	1.2	0.8	1.4
Metridia pacifica		<u>43.9</u>	<u>11.3</u>	<u>5.2</u>	1.6	1.6	1.7
Neocalanus cristatus		0.9	3.6	1.9	1.5	1.0	
Neocalanus flemingeri		1.2	1.0	1.0	0.6	0.9	0.4
Neocalanus plumchrus		1.0	0.8	0.6	1.5	0.7	

Table 2. Day and night comparison on gut pigment of late copepodid stages of dominat copepods in the Oyashio region during 9 March-30 April 2007. Values are mean±1sd. **: p <0.01, ns: not significant. Details of data see Fig. 7.

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

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. *: p <0.05, **: p <0.01, ***: p <0.001, ns: not significant.

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



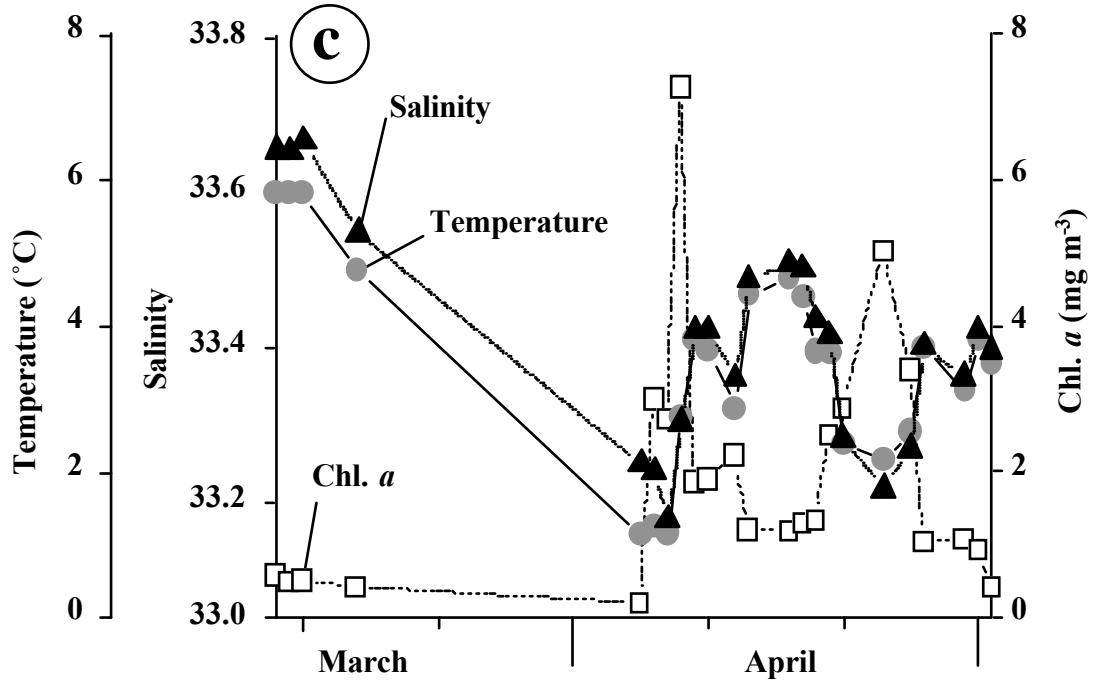
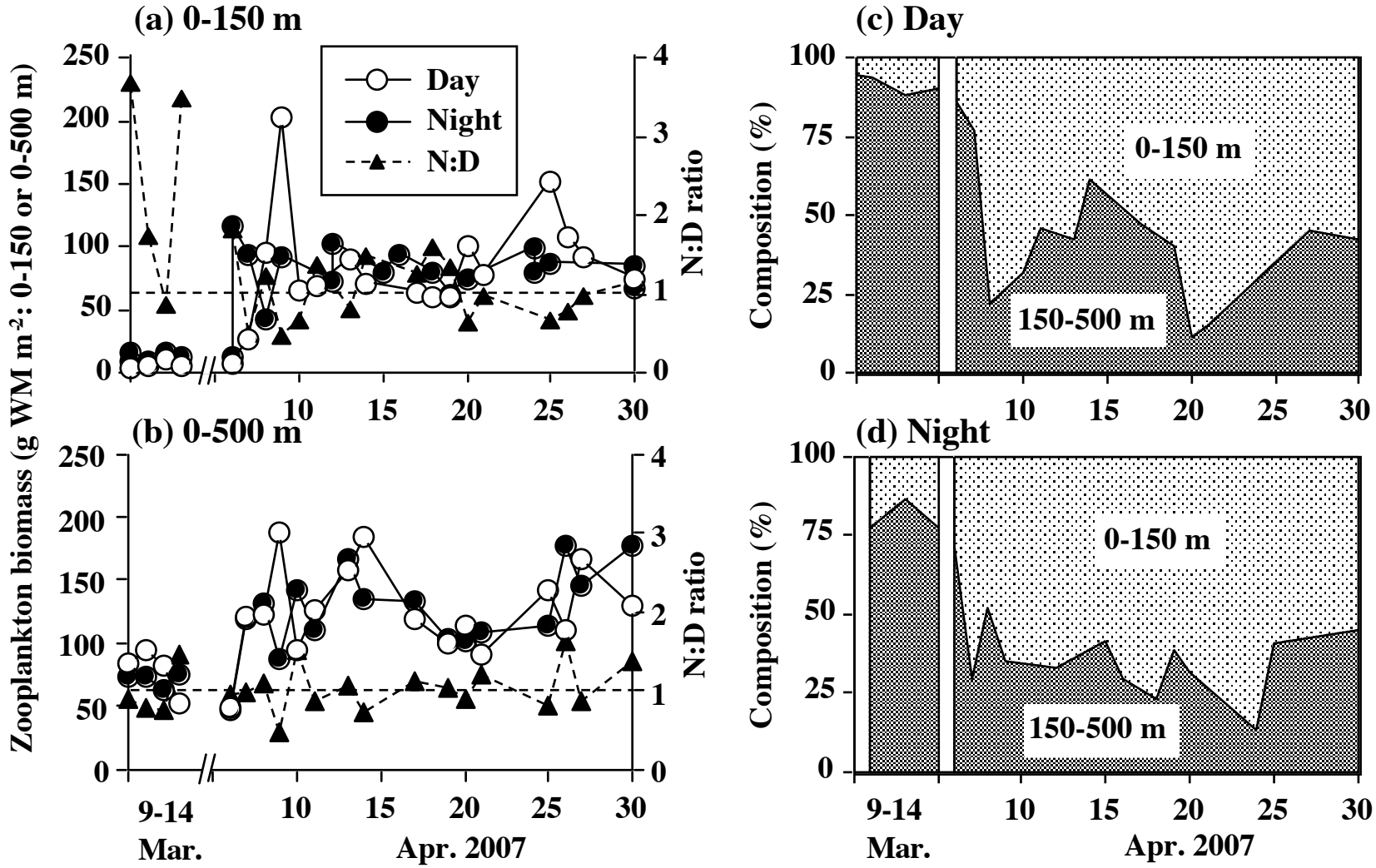


Fig. 1 (Yamaguchi et al.)



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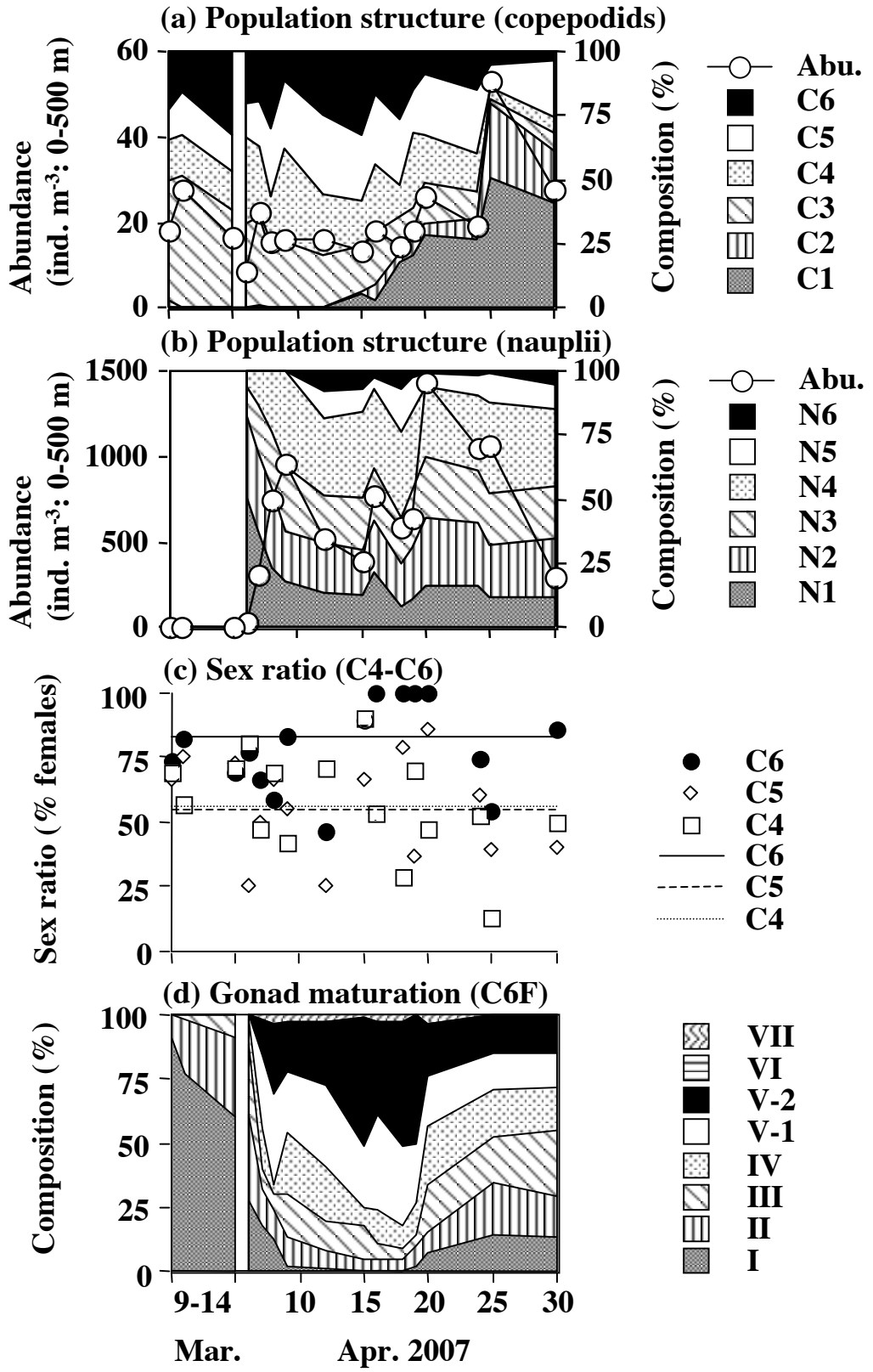

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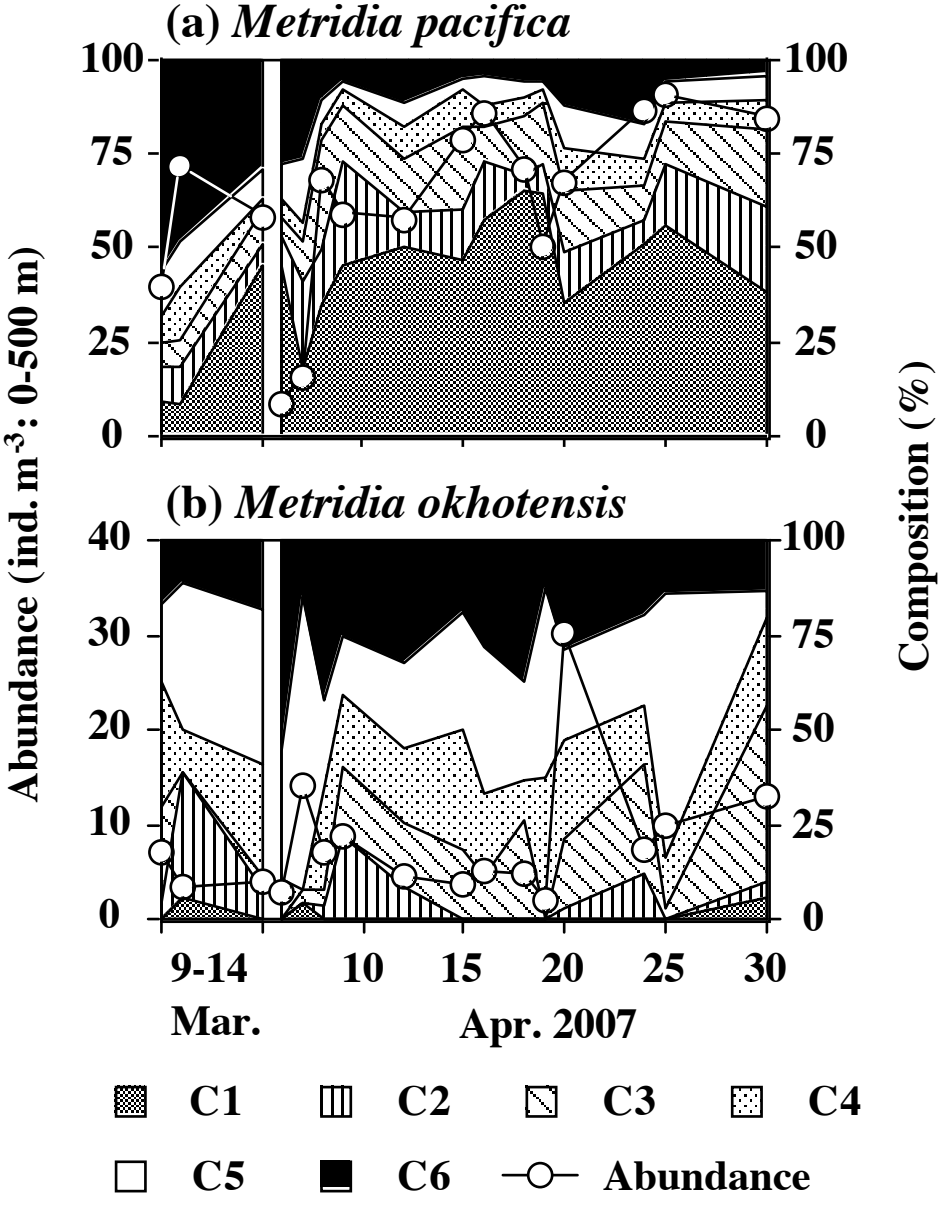

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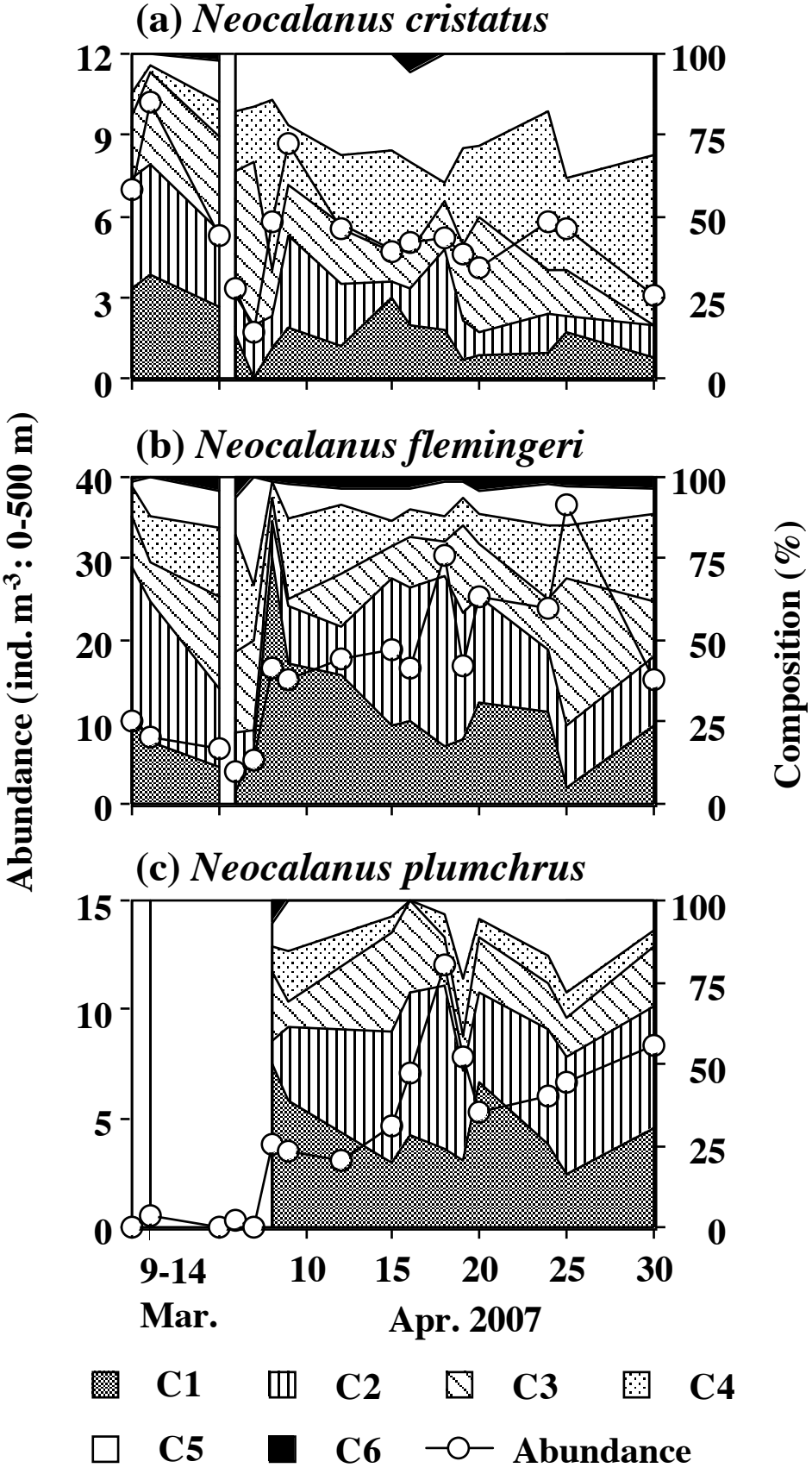

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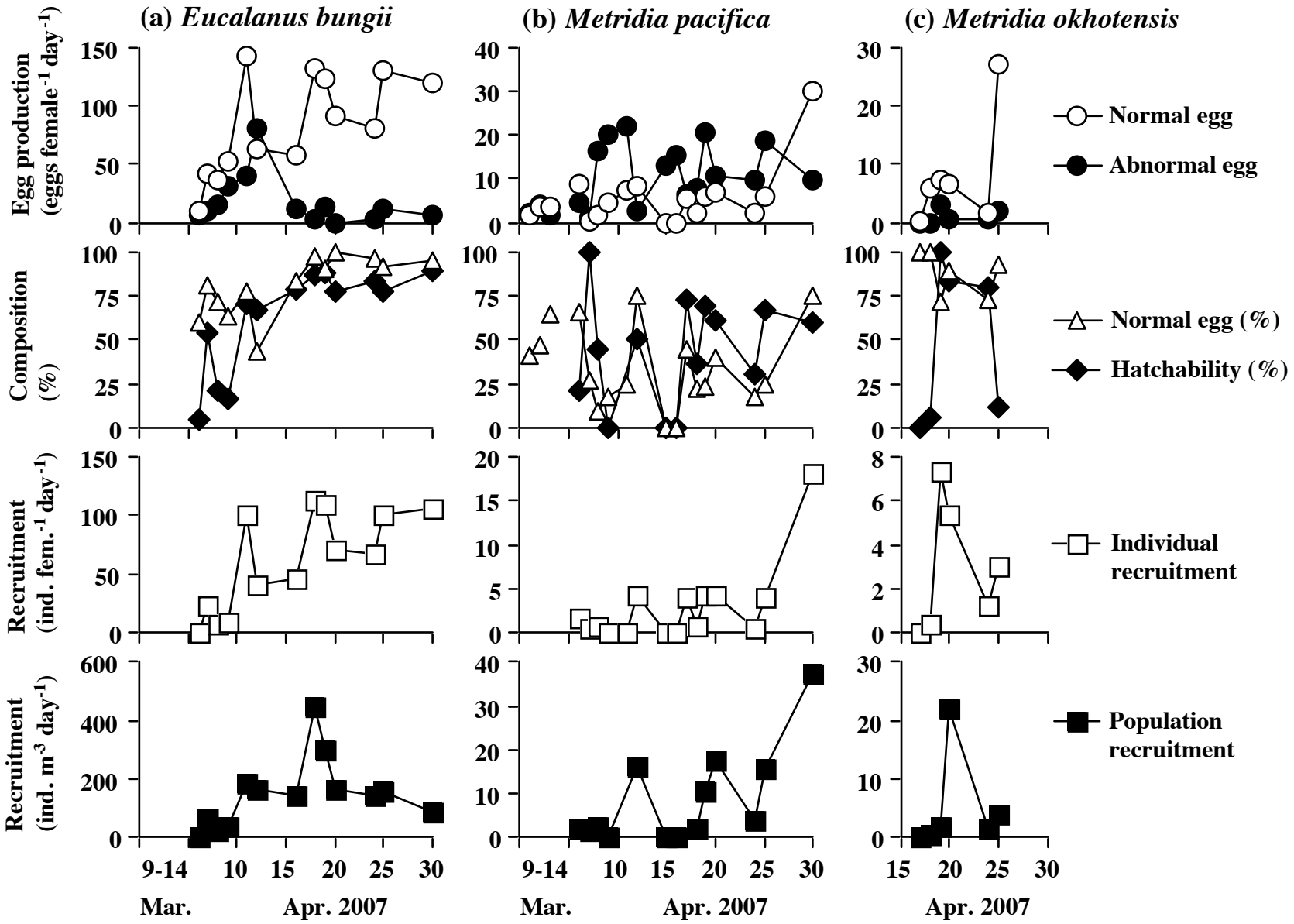


Fig. 6 (Yamaguchi et al.)

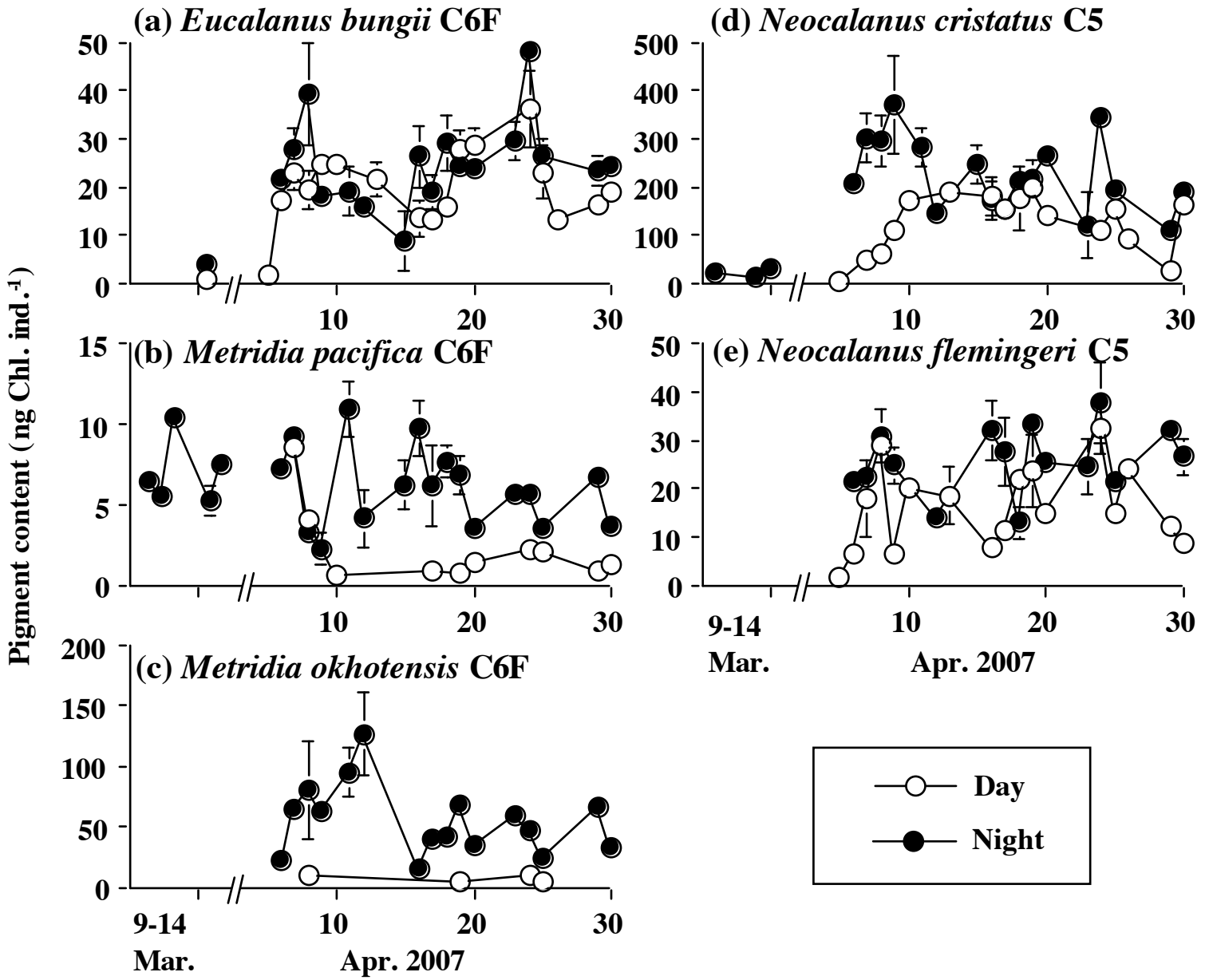
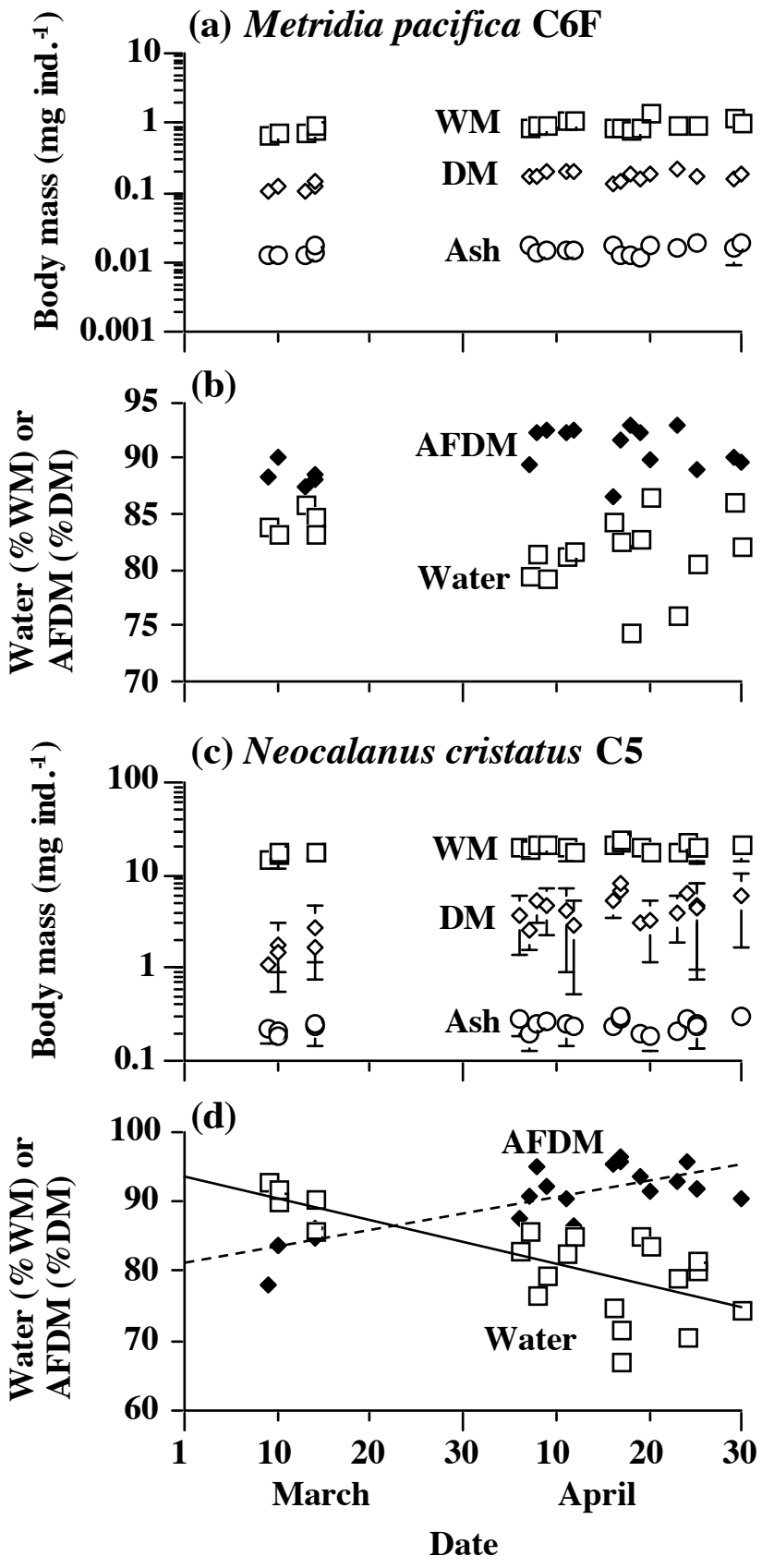


Fig. 7 (Yamaguchi et al.)



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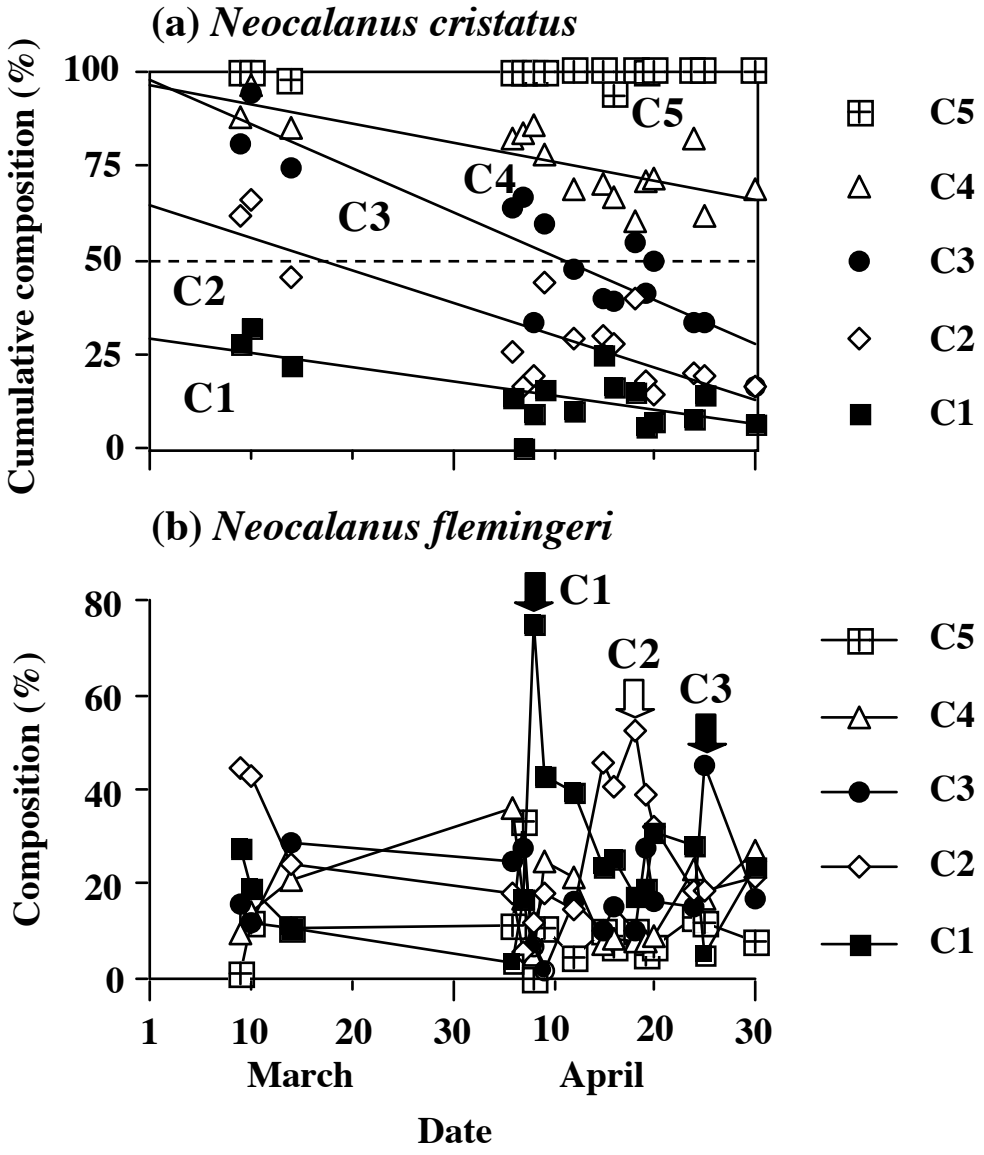

Fig. 9 (Yamaguchi et al.)

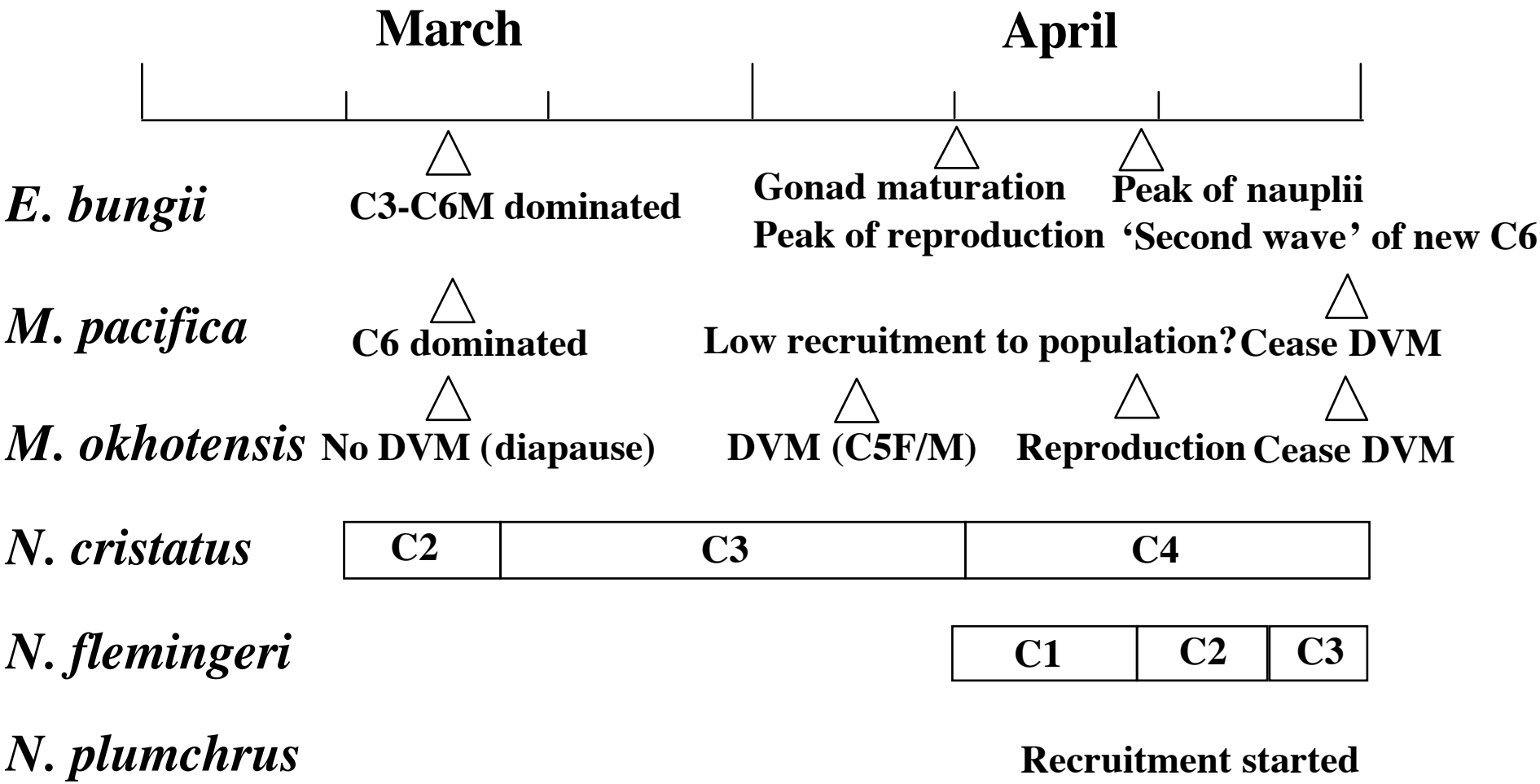

Fig. 10 (Yamaguchi et al.)