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Title	Mesozooplankton response to iron enrichment during the diatom bloom and bloom decline in SERIES (NE Pacific)
Author(s)	Tsuda, Atsushi; Saito, Hiroaki; Nishioka, Jun et al.
Citation	Deep Sea Research Part II Topical Studies in Oceanography, 53(20-22), 2281-2296 https://doi.org/10.1016/j.dsr2.2006.05.041
Issue Date	2006
Doc URL	https://hdl.handle.net/2115/17204
Type	journal article
File Information	E-SRpIITS053-20-22.pdf

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Mesozooplankton response to iron enrichment during the diatom bloom and bloom decline in SERIES (NE Pacific)

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Suggested running title: Mesozooplankton response in SERIES

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Abstract

A mesoscale iron-fertilization experiment was carried out in the eastern subarctic Pacific during summer 2002. The iron-patch was traced for 25 days after the enrichment, and the abundance and behavior of mesozooplankton was compared with those outside of the patch during the diatom bloom and its period of decline (day 14-25). The chlorophyll-*a* concentration in the patch was high between day 14 and day 16 (6 mg m^{-3}) and decreased to 1.4 mg m^{-3} at the end of the observation. Dominant zooplankton species in the upper 200 m depth were copepods: *Eucalanus bungii*, *Pseudocalanus* spp. *Neocalanus plumchrus, N. cristatus*, and *Metridia pacifica*. Species composition did not change significantly in the patch over the observation period. However, shallower distribution depths were observed in *E. bungii, N. cristatus* and *M. pacifica* in the patch triggered by the diatom bloom. Especially, *E. bungii* was mainly distributed in the subsurface layer outside of the patch, but it was mainly distributed in the surface mixed layer inside the patch, which was accompanied by enhanced growth rate and increased biomass. We also propose the accumulation processes of zooplankton in the patch due to the upward immigration. Moreover, the abundance of the first copepodite stage of *E. bungii* and calyptopis larvae of euphausiids increased several fold in the patch compared to the densities outside the patch. The increases of

both species are considered to be due to lowered mortality during the egg and nauplius stages, which was caused by lowered relative importance of eggs and nauplii in the diets of the suspension-feeding omnivores in the patch due to increased diatom abundance during the diatom bloom. Gut-pigment contents of dominant copepods in the patch increased 6 to 8 times, and the maximum values were observed during the bloom peak. The grazing impact on phytoplankton was low during the bloom period, but increased in the declining period of the diatom bloom.

Key words

Iron-enrichment, copepod, vertical distribution, grazing, growth, North Pacific

Introduction

Several ecosystem-scale iron fertilization experiments have been carried out in the major HNLC (high nitrate low chlorophyll) oceans to test the iron deficiency hypothesis (Martin and Fitzwater, 1988) and to investigate the ecosystem responses to iron addition (Martin et al., 1994, Coale et al. 1996; Boyd et al. 2000; Tsuda et al., 2003). In all experiments, phytoplankton standing stocks largely increased with iron addition accompanied with significant drawdown of pCO_2 and macronutrients. The time scales of the iron-induced blooms ranged from a few days to over a month; presumably depending on the temperature, light availability and retention mechanisms of the bio-available iron concentration (Abraham et al., 2000, Tsumune et al., in press). In the HNLC oceans, pico- and nano-phytoplankton are dominant throughout the year (e.g. Booth et al., 1993), and the production of these organisms is considered to be balanced with the grazing by microzooplantkon (Strom and Welschmeyer1991, Tsuda and Kawaguchi 1997). However, diatoms, micro-sized phytoplankton, became the most dominant taxa in the phytoplankton communities in the iron-enriched water masses, although pico- and nano-phytoplankton such as Prymnesiophytes also show enhanced growth rates with iron-addition (e.g. Gall et al. 2001; Suzuki et al., in press; Tsuda et al., in press a). The enhanced growth rate and increases of size and

abundance of phytoplankton are considered to improve the food availability to meso-zooplankton.

In IronEX II (the second, mesoscale iron enrichment experiment, Coale et al., 1996), abundance of small copepods increased due to the improved food availability and behavioral change (diel vertical migrators remaining in the surface layer during the daytime in the iron-patch) during a week and then decreased in the later half of the experiment, presumably due to predation (Rollwagen Bollens and Landry, 2000). Arrested migration of mesozooplankton was considered as an important cause of the relatively small increase of phytoplankton abundance and small decrease of macronutrients and pCO_2 in the enriched water of IronEx II (van Scoy and Coale, 1994; Banse, 1995; Cullen, 1995). In contrast, Southern Ocean mesozooplankton, which was dominated by large-sized copepods, showed neither a detectable biomass change during the two weeks observation period or a significant contribution to the export flux from the euphotic layer (Zeldis, 2001). Moreover, in SEEDS which was conducted in the western subarctic Pacific, detectable changes in biomass, vertical distribution, diel vertical migration were not observed during the two weeks period (Tsuda et al. in press b). However, in SEEDS abundances of the first copepodite stage of surface resident copepods (*Neocalanus plumchrus* and *Eucalanus bungii*) significantly increased after

the formation of the diatom bloom. The increases of both species were considered to be due to lowered predation mortality during the egg and nauplius stages, which was caused by the increase of alternative foods (diatoms).

Responses by mesozooplankton to diatom blooms induced by iron addition have been different in each experiment, which presumably is caused by differences in species composition of mesozooplankton and phytoplankton, and the seasonal timing of the experiments. More importantly, these functional and numerical responses are only possible to be observed by ecosystem-scale experiments.

Similar species of mesozooplankton are distributed in both Alaskan gyre (AG) and western subarctic gyre (WSG) (Mackas and Tsuda, 1999). However, there are considerable differences between east and west of the subarctic Pacific in timing of the seasonal stratification (Parsons and LeBrasseur, 1968), chlorophyll concentration (Sugimoto and Tadokoro, 1997, Banse and English 1999), phytoplankton taxonomic composition (Obayashi et al., 2001), iron deficiency indicated by photochemical quantum efficiencies of the algal photosystem II (Suzuki et al. 2002) and dust flux (Duce and Tindale, 1991). Moreover, some species of dominant copepods shows different life cycles between AG and WSG (Miller et al. 1984; Miller and Clemons, 1988, Tsuda et al., 1999; Tsuda et al., in press c).

The iron enrichment experiment in the eastern subarctic Pacific (SERIES) was carried out in the summer of 2002 with a relatively long observation period which covered the period of decline of the bloom (Boyd et al. 2004). Iron fertilization firstly caused an increase of prymnesiophytes, and then an increase of diatoms after the crash of prymnesiophytes bloom (Marchetti et al. in this volume). Mesozooplankton responses in the first half of the experiment are presented by Sastri and Dower (in this volume). We investigated the functional and numerical responses of mesozooplankton during the artificially created diatom bloom and its declining phase to add to our knowledge of the effects of iron enrichment on marine ecosystems.

Materials and Methods

A meso-scale *in situ* iron-enrichment experiment, SERIES (Subarctic Ecosystem Response to Iron Enrichment Study), was conducted in the Alaskan gyre of the North Pacific (50º20'N, 145º45'W) from 9 July to 4 August 2001 by R.Vs. J.P. Tully, El Puma and *Kaiyo-Maru*. The experiment consisted of multiple additions of iron as $FeSO_4$ with an inert tracer gas, sulphur hexafluoride (SF_6), over a 77 km^2 patch on 9 July 2001. Day 0 (D0) is defined as the 24-h period starting at 00:00 10 July 2001. The iron-patch was followed from 10 July to 4 August 2002, by tracking the SF_6 signals and

pCO_2 signals (Boyd et al. 2004). We observed the mesozooplankton responses from 24 July to 4 August (D14 to D25) by R.V. *Kaiyo-Maru*, Fisheries Agency of Japan. In-patch net samplings during the daytime were carried out every second day, basically, from D14, and out-patch samples were carried out on D14, D20 and D25. Nighttime samplings were carried out during the mapping of the patch at every second day from D15. Locations of the inside and outside stations were determined by pCO_2 monitoring, because SF_6 signals were too low to determine the shape and position of the patch and pCO_2 drawdown were distinctive during the observation period of *Kaiyo-Maru* (Wong et al this volume). Locations of out-patch sampling were distributed to the south-east from the patch center (Fig. 1), because we observed different water mass characterized by low temperature and high pCO_2, located to the north and western area of the patch (Wong et al. this volume).

A VMPS net (opening-closing multi-layer net: 50x50 cm mouth opening, 0.1mm mesh opening, Terazaki and Tomatsu, 1997) was towed from 200 m depth for estimations of standing stock, vertical distribution and diel vertical migration. The sampling layer was divided into 0-30, 30-80, 80-200 m. The samples were immediately preserved with 10 % buffered formalin seawater. In the laboratory, all individuals except small copepods (mainly *Oithona* spp. and non-adult stages of

Pseudocalanus spp.) were identified, counted and sorted for measurements of wet weight. Especially, the dominant copepods *Calanus pacificus, Neocalanus cristatus, N. plumchrus, Eucalanus bungii* and *Metridia pacifica* were separated into each developmental stage and counted. When the sample was heavily contaminated with diatom cells, which occurred in the surface layer, the sample was subsampled and one fourth of the original samples were enumerated. The carbon biomasses were estimated using the wet weight and a conversion factor of 0.08 (Peters and Downing, 1984).

Vertical tows for gut pigment measurements were conducted with a NORPAC net (45 cm mouth opening and 0.33 mm mesh opening with 1 L codend). The net was towed from 50 m depth, and the samples were poured onto a gauze with the same mesh, then frozen in liquid nitrogen immediately and stored in a dark refrigerator (-80 ^{o}C). In the laboratory, the samples were thawed with chilled, filtered seawater. The sixth copepodite stages of *Calanus pacificus* (C6 female), *Neocalanus cristatus* (C5), *N. plumchrus* (C5), *Eucalanus bungii* (C5 and C4) and *Metridia pacifica* (C6 females) were sorted under a dissecting microscope with dim light, rinsed twice in filtered seawater, then dipped in 6 ml N,N-dimethylformamide for pigment extraction. Three and five individuals were used for each extraction for *N. cristatus* and the other copepods, respectively. Chlorophyll-*a* and its degradation products were measured with

a Turner Designs fluorometer. Gut pigment was calculated as the sum of chlorophyll-*a* and phaeopigments, and expressed as the chlorophyll-*a* equivalent weight. Estimated gut content pigments were converted to grazing rates and filtering rates assuming gut evacuation rates of 0.045, 0.042, 0.042, 0.048 h^{-1} for *N. cristatus*, *N. plumchrus*, *E. bungii* and *M. pacifica*, respectively (Saito, 1996) and average concentration of nano- and micro-sized chlorophyll-*a* in the surface mixed layer on the sampling day. We also assumed a pigment denaturing factor (0.8) during the freezing/thawing process (Tsuda and Sugisaki, 1994).

The gut pigment contents were measured in relatively large and dominant species and stages. The grazing rates of younger stages were estimated using the allometric functions (Peters and Downing, 1984):

$$\text{Log}\ (V_1/V_2)=0.534\ \text{log}\ (W_1/W_2) \qquad (1)$$

$$W=9.86L^{2.1} \qquad (2)$$

V_1 and V_2 are the filtering rates of copepods with weights of W_1 and W_2, respectively, and L is the copepod body length. The body lengths of *Neocalanus* spp. and *Eucalanus bungii* were taken from Tsuda et al. (1999) and Tsuda et al. (in press c). For *Metridia pacifica* and *Calanus pacificus*, prosome length was measured under a

dissecting microscope attached with a CCD camera and image analyzer for at least 30 individuals for each developmental stage. Then, the community grazing rates were estimated by abundances and individual grazing rates for each developmental stage.

Water samples for primary productivity were collected with teflon coated, trace metal clean Niskin X type samplers attached on a Kevlar line at 6 light depths at 100, 35, 25, 10, 6 and 1% of the surface irradiance. The sampling depth was decided from the irradiance profile obtained just before the sampling. Surface irradiance was measured with LiCor LI-190SA cosine collector. Underwater irradiance was measured with PRR-600 (Biospherical Instruments Inc.). The samples were dispensed into acid-cleaned Nalgene Polycarbonate bottles (250 ml) in a tent on board. An aliquot (1% of total inorganic carbon) of ^{13}C-labelled Na$H^{13}CO_3$ (99 at-%; Shoko Co., Ltd.) was spiked to each bottle within an hour after sampling. Incubation was conducted in triplicate for each depth in a running sub-surface seawater tank (100%) or in light attenuated, thermo-controlled tanks (the other light depths) for 24 hours. Light attenuation was achieved with a blue film and black mesh screens. After the incubation, each sample was filtered with a pre-combusted GF/F filter, and the filter was stored in a -80°C deep freezer. The measurement principles, equipment and calculations are described in Hama et al. (1983), and the method followed is detailed in

Imai et al. (2002).

Results

Composition and biomass

For the 0-200m water column, copepods were most dominant taxa in the carbon biomass both inside and outside of the patch throughout the observation period (Fig. 2). They accounted for 71 and 65 % of the mesozooplankton biomass, inside and outside of the patch, respectively, and the difference between inside and outside was significant (*t*-test, $p<0.05$). Moreover, significant increase of copepods biomass was observed for both inside and outside of the iron-patch. The slope of the biomass increase was higher inside the patch than that for the outside. Euphausiids accounted for 8 and 10% of the mesozooplankton biomass, inside and outside of the patch, respectively. Other mesozooplankton, dominated by chaetognaths, accounted for 21 and 25 % of the mesozooplankton biomass, inside and outside of the patch, respectively. Significant differences between inside and outside were not observed in euphausiid and other zooplankton biomasses. Almost the same results were obtained for the 0-30 m layer biomass. Copepods biomass in the patch were significantly higher than that of outside ($p<0.05$), but biomasses of euphausiids and other zooplankton did not show significant

differences. Moreover, the relative contribution of the mixed layer biomass to the 0-200 m biomass of copepods was significantly higher in the patch (43%) than that outside (33%) (*t*-test, $p<0.05$), which suggests that an upward shift in the vertical distribution of copepods in the patch.

Numerical composition of the mesozooplankton did not change between inside and outside of the patch throughout the observation period, although there were some variations among samples (Fig. 3). The community was dominated by *Pseudocalanus* spp. We observed two types of *Pseudocalanus*, one is relatively large (prosome length; 1mm) with anteriorly angular cephalosome and another is relatively small (PL: 0.8 mm) with anteriorly rounded cephalosome. Detailed identification with some individuals suggested that the large individuals were dominated by *Pseudocalanus mimus* and small individuals were *P. newmanii*. *Eucalanus bungii* and *Calanus pacificus* were also abundant throughout the observation period. Copepodite stage 4 (C4)-5 and C2-5 were abundant for *E. bungii* and *C. pacificus*, respectively. We identified three species of *Neocalanus*. *N. plumchrus* was most abundant and major part of the population consisted of C4 and C5. Second most dominant species was *N. cristatus*, and C5 individuals were most abundant. We scarcely observed *N. flemingeri*.

Abundance

Significant differences ($p<0.05$) of abundance between inside and outside of the patch were observed in *N. cristatus* C5, *E. bungii* C5, C1, *P. mimus* C6 female and euphausiid calyptopis larvae in the 0-200 m water column (Fig. 4). *P. mimus* showed the apparent increases of abundance during the observation period both inside and outside of the patch, statistical test (*t*-test) was performed after the offset of the trends by linear regression. *M. pacifica* C3, C4 and C6 female from the surface mixed layer in the patch significantly higher abundance than outside (Fig. 4, only C3 was shown), although they did not show the differences in the samples collected from 0-200m water column, suggesting an upward immigration into the surface mixed layer of *M. pacifica* in the patch occurred during the observation period. Increases of abundance of C5 individuals were observed in *N. cristatus* and *E. bungii*, and slopes of the increases were significantly higher inside the patch than those outside. We could not observe any differences between the inside and outside of the patch in abundances of these copepods at the beginning of the observation (D14 and D15), but the differences became larger with time. Abundance of C2 and 3 of *E. bungii* showed decreases with time both inside and outside of the patch (not shown), suggesting that the observation period agreed with the growth period of young to preadult copepodites. Then, we calculated

the average stage of *E. bungii*, *N. cristatus* and *N. plumchrus* in the samples collected from the 0-200 m water column. Positive slopes were observed in all species of ontogenetic migrating copepods both inside and outside of the patch (Fig. 5). A higher growth rate of *E. bungii* was estimated inside the patch than that outside, although the slopes were not different statistically (ANCOVA, $p>0.1$). However, the average stage in the patch during the later observation period (D23 to D25) was significantly larger than that outside (*t*-test $p<0.05$). Stage duration times (inverse of the slope) were 11 and 26 days, inside and outside the patch, respectively. Significant differences also were not detected for *N. cristatus* and *N. plumchrus*. However, shorter stage duration was also observed inside the patch for *N. cristatus* (20 vs. 26 days).

Vertical distribution and migration

Averaged distribution depths inside the patch were significantly shallower than those outside for *E. bungii* (C3-C5) and *N. cristatus* (C2-C4) (*t*-test, $p<0.05$) (Fig. 6). These differences of distribution depths were observed throughout the observation period, and we could not detect a significant deepening or rising of the distribution depth with time. Upward movements of the copepods were more clearly shown in the averaged vertical profiles of their distribution (Fig. 7). *E. bungii* was distributed mainly below the surface mixed layer outside the patch, while they were distributed to

the surface mixed layer inside the patch. *N. cristatus* was almost homogeneously distributed below the surface mixed layer, while they were mainly distributed in the 30-80 m layer in the patch. Moreover, some individuals of *N. cristatus* appeared even in the surface mixed layer in the patch, especially during night time (Fig. 7). Although a significant difference of the averaged distribution depths of *Metridia pacifica* was not observed between the inside and outside of the patch, they were more abundantly distributed in the surface mixed layer in the patch, especially during night time (Fig. 7).

Gaetanus sp. and *Pleuromamma* spp. only appeared during night time (not shown). However, vertical migration behaviors of these copepods were not different between the inside and outside of the patch. In addition, nocturnal rises of the vertical distribution were observed in *N. cristatus* C5 and *M. pacifica* C4-C6 female. Distance of diel migration (difference of averaged distribution depth between night and day) increased from 8 m to 43 m with developmental stages.

Grazing

Gut pigment contents of the copepods examined were 6 to 8 times higher in the patch than those of outside (Fig. 8). Gut pigment contents outside of the patch were low and constant throughout the observation period. In contrast, the gut pigment contents in the patch were variable. *N. plumchrus* showed a significant decreasing

trend (Spearman's rank correlation, $P<0.01$) and also *C. pacificus* showed a weakly significant trend ($p=0.1$). All copepods showed low gut pigment contents on D26 when the chlorophyll concentration was low (Fig. 9).

Filtering rates of the copepods were estimated from the gut pigment content, evacuation rates and nano- and micro-sized chlorophyll concentration in the surface mixed layer (see Methods). Filtering rates during the diatom blooming period (D14 to D21), indicated by high chlorophyll concentration, of *C. pacificus* and *E. bungii* in the patch were 45 to 59 % of rates outside, and the differences were significant (*t*-test, $p<0.05$) in C4 and C5 of *E. bungii* (Fig. 9). Then, filtering rates of the copepods increased 1.3 to 3.5 times during the decline phase of the bloom (D23 and D25) compared with the average filtering rates during the bloom period.

Estimated community grazing rates in the surface mixed layer ranged between 189 and 643 mgC $m^{-2}d^{-1}$ (average: 328 mgC $m^{-2}d^{-1}$). The community grazing rate peaked on D19, which was basically correlated with copepod abundance (Fig. 10). *E. bungii* was the most dominant grazer and accounted for 44 to 63 % of the community grazing rates. *Pseudocalanus* spp. was the second most dominant grazer and accounted for 15 to 35 %. Primary production was high (1940 mgC $m^{-2}d^{-1}$) at the beginning of the observation (D14) and rapidly decreased to 207 mgC $m^{-2}d^{-1}$ at the end

of the observation (Fig. 10). The community grazing rates by copepods was a minor component of phytoplankton mortality during the diatom bloom, but its relative importance increased with the decrease of the primary production. During the period of bloom decline (D23 and D25), the community grazing rates were almost balanced with the primary production (Fig. 10).

Discussion

Community composition and seasonal timing

Copepods that show vertical migration as part of their ontogenetic development, especially *Eucalanus bungii*, accounted for major part of mesozooplankton biomass in this study. These copepods have annual or biennial life cycles in the subarctic Pacific (Miller et al. 1984, Miller and Clemons 1988; Tsuda et al. 1999; Tsuda et al. in press c). Miller et al. (1984) suggested that main spawning period of *E. bungii* was the beginning of July and the peak abundance of young copepodites (C1 and C2) was end of July. We observed a decrease of C2 abundance during the observation period (24 July to 4 August), which suggests that the main spawning period was before our observation as suggested by Miller et al. (1984). Tsuda et al. (in press c) suggested that reproduction and growing season of *E. bungii* was different between AG and the Oyashio region and

this agreed with the productive season of each region. *Neocalanus cristatus* and *N. plumchrus* were dominated by C4 and C5 individuals, which also agrees well with the life histories described in previous works (Miller et al. 1984; Miller and Clemons 1988). The growing season of *N. plumchrus* is later than that of *N. flemingeri* and that of *N. cristatus* is longest among the ontogenetic migrating copepods from January to August (Miller et al., 1984; Tsuda et al., 1999; Kobari and Ikeda, 1999). These agreements suggests that the iron-enrichment experiment was carried out during the local production maximum and later half of the growing period of the ontogenetic migrators in this area. A remarkable difference in the mesozooplankton composition between previous works and this study was the abundance of *N. plumchrus*. In this study, averaged abundance of C5 and C4 were 74 and 44 copepods m^{-2}, respectively. These abundances were an order of magnitude lower than that of Miller and Clemons (1988). However, Mackas et al. (1998) suggested that timing of certain developmental stages of *N. plumchrus* occurred earlier from 1970s to 1990s. Mackas et al. showed that zooplankton biomass maximum, which was estimated from C5 individuals of *N. plumchrus* accounting for 50% of the total number of the species, was observed at the end of June to mid July in the early 1970s and it was observed in mid May in the late 1990s in the eastern subarctic Pacific. This suggests that most of the population of *N.*

plumchrus had left the epipelagic layer for overwintering by our sampling period.

The averaged biomass of the copepod community in the 0-200 m water column were 0.95 and 0.63 gC m^{-2} inside and outside of the patch, respectively. The equivalent values in SEEDS (iron-enrichment experiment in WSG) were 0.86 and 1.12 gC m^{-2} (Tsuda et al in press), which showed that the copepod biomass in the patch was similar to that of SEEDS and outside biomass of copepod was a little lower than SEEDS. Moreover, non-copepod biomass, mainly composed of chaetognaths and euphausiids, in this study were much lower than those of SEEDS. In contrast, copepod biomass in the surface mixed layer (0-30 m) were 0.41 and 0.21 gC m^{-2}, inside and outside of the patch, respectively, and they were 1.4 and 2.7 times higher than those of SEEDS. Higher biomass in this study was partly caused by upward immigration of dominant copepods in the iron-enriched patch.

Functional responses

The most remarkable functional response to the iron-induced bloom was the shallower depth of the vertical distributions in the dominant copepods. Upward shifts of the vertical distribution were clearly observed in *Eucalanus bungii* (C3-C5) and *Neocalanus cristatus* (C2-C4). We also observed a relatively abundant distribution of *Metridia pacifica* in the surface mixed layer in the patch, although it was not detectable

in the analysis of averaged distribution depth (Fig. 7). Differences in distribution depth were detected from the beginning of the observation (D14), and lasted throughout the observation period. The SERIES experiment was divided into two phases: Phase I (D0 to D10) was characterized by low chlorophyll standing stock (up to 2mg m^{-3}) dominated by pico and nano-phytoplankton, especially prymnesiophytes, and phase II (D11 to D25) was characterized by high chlorophyll standing stock (up to 6.5 mg m^{-3}) dominated by micro-sized diatom (Marchetti et al. this volume; Saito et al., this volume). During the phase I period, the density of *E. bungii* in the surface mixed layer of the patch was relatively stable, as well as *M. pacifica* and *Neocalanus* spp., and high density of *E. bungii* was observed on day 17 (Sastri and Dower, this volume). These data suggested that upward shift of vertical distribution was triggered by the diatom bloom which started around D10. *Eucalanus bungii* and *Neocalanus cristatus* are usually distributed below the thermocline (Mackas et al. 1993) and mainly graze on sinking particles (Dagg, 1993). The sinking particle flux was low and stable until day 20 (Boyd et al. 2004), therefore, the sinking particles could not have been a cue for the upward movement. Light attenuation is a possible cue for this behavior. One percent light-depth was located between 56 and 32 m during the phase I and became shallower to around 20 m at the peak of the diatom bloom (Marchetti et al., in this volume).

However, light intensity changes depend on the weather conditions and the time of day, and considering these factors it was not clear if the copepods used light intensity as a cue for the change in vertical distribution. In the iron-enrichment studies, the iron-enriched water mass, usually the surface mixed water, is traced using SF_6 as an inert tracer (Law et al., 1998). In SERIES, the surface mixed layer depth defined by the sigma-*t* increase over 0.02 m^{-1} and SF_6 depth defined as the 50% of the concentration of average mixed layer SF_6 concentration basically agreed well (Law et al. this volume). These SF_6 profiles suggested that the water mass of the surface mixed layer moved differently from the water mass below the thermocline. Nevertheless, we observed the change of the vertical distribution and increased abundance of *N. cristatus* in the 30-80-m layer of the patch (Fig. 7). This result strongly suggests that upward immigration took place at a time scale of less than a day. Rollwagen Bollens and Landry (2000) observed an increase of copepods biomass and suggested an arrested migration in the IronEx II. The observed changes of vertical dsitribution of *E. bungii*, *M. pacifica* and *N. cristatus* was different from the arrested migration, because we could not observe any significant changes of vertical distributions between day and night. However, the effects on the material flow and ecosystem dynamics are the same because time scales of these behaviors are much shorter than numerical responses by these

copepods.

The vertical distribution of these copepods being shallower, especially *E. bungii*, led to two consequences: one was the improvement of food availability and the second was the accumulation of the individuals in the iron-enriched patch. Gut pigment content increased 6 to 8 times compared to individuals outside, but these changes were mainly caused by the increase of chlorophyll concentration in the patch. Filtering rates of the copepods in the patch were about half the rate outside the patch (Fig. 9) and POC in the patch increased about 4 times the level outside the patch (Boyd et al., 2004). These results suggest that ingestion rates during the diatom-blooming period doubled in the patch. Improved food availability in the patch led to higher developmental rates in *E. bungii* and presumably in *N. cristatus* (Fig. 5). Although there has been discussion on whether the ocean is a dilute environment in food for mesozooplankton or not (e.g. Mullin and Brooks, 1976), the enhanced growth rate in the patch suggested that the growth rate of *E. bungii* was food-limited before the iron-enrichment.

If the water mass of the iron-enriched surface water moved differently from the water mass below the thermocline and copepods migrated from below the thermocline into the surface mixed water, accumulation of the copepods would have occur.

However, the total density of *E. bungii* (C1 to C6) in the 0-200 m water column of the patch did not increase with time (Fig. 11), and the average density was not significantly different from the outside one (*t*-test, $p>0.05$). In contrast, *N. cristatus* showed a significant increase in the total density with time (Spearman's rank correlation, $p<0.05$), and the averaged density between D22 and D26 was 1.8 times higher than the outside one (*t*-test, $p<0.05$). In our sampling period, the SF_6 depth was deeper than the mixed layer depth estimated by sigma-*t* profiles between D18 and D23 (Fig. 12), suggesting that the water mass of the surface mixed layer moved with the water mass below the thermocline. In addition, the horizontal movement of the patch was slow during our sampling period compared with the earlier half of the experiment (Law et al., in this volume) and the area of the patch much increased by dilution (Law et al., this volume). Therefore, we could not detect a significant increase of the total density of *E. bungii*. On the other hand, as the vertical distribution of *N. cristatu*s is deeper than *E. bungii*, then, individuals of *N. cristatus* distributed in the deeper layer may have migrated into our sampling range (0-200 m) triggered by the diatom bloom.

We observed a significant increase of copepod biomass in the patch. Total density of *N. cristatu*s increased with time (Fig. 11), but *N. cristatus* was a minor component in the mesozooplankton biomass in the patch (Sastri and Dower, this

volume). Therefore, the increase of copepod biomass mainly came from the enhanced growth rate of *E. bungii* in the patch. The averaged copepodite stage in the patch during the declining phase of the diatom bloom was about one stage older than that of the outside (Fig. 5). The body volume of *E. bungii* increases by about 3 times for each developmental stage. The increase of body volume approximates the observed increase of the copepod biomass.

The community grazing rates by mesozooplankton was a minor component of the phytoplankton mortality during the high primary production period (Fig. 10) as in the past iron-enriched experiments in cold waters (Zeldis, 2001; Tsuda et al in press). However, the community grazing rates were almost the same level or a little higher than the primary production during the declining phase of the diatom bloom. This relationship was mainly caused by the decrease of the primary production, but the increase of copepod biomass and the increase of filtering rates during the declining period in the patch also affected the increase of the community grazing rates.

Numerical responses

In the subarctic Pacific, dominant zooplankton have an annual or biennial life cycles (Conover, 1988; Mackas and Tsuda 1999). Moreover, reproduction of *Neocalanus* copepods, most dominant copepods in biomass, take place in mesopelagic

layer using the organic matter accumulated in the previous year (Fulton, 1972, Saito and Tsuda, 2000). Therefore, it should be relatively hard to detect the numerical responses of these organisms. However, Tsuda et al. (in press b) observed significant increases of C1s of *N. plumchrus* and *E. bungii* in the patch during a relatively short observation period (13 days) of SEEDS. They suggested that the increase of C1 individuals occurred due to the lowered mortality during their egg and nauplius stages caused by an increase of alternative food (diatoms) for suspension-feeding omnivores. In this study, we observed significantly higher abundances of euphausiid calyptopis larvae and C1 of *E. bungii*. These organisms are at early stages of development and mainly distributed in the surface mixed layer. Therefore, the same mechanism as in SEEDS are concluded to have led to a higher abundance of these organisms. Actually, the filtering rates of large suspension feeders such as *Eucalanus bungii* and *Calanus pacificus* decreased compared to rates outside. Accumulation process caused by change of their vertical distribution which was mentioned earlier, seems unlikely for calyptopis larvae and C1 of *E. bungii* because they were mainly distributed in the surface mixed layer throughout the observation period and we did not observed their changes in vertical distribution. Moreover, SERIES had a relatively long observation period (25 days) compared with SEEDS (13 days), and, increases of young larvae might have partly been caused by

enhanced reproduction in the high food-availability environment.

Conclusions

We observed enhanced growth rates of subsurface living copepods associated with change of their vertical distribution, and increases of young stages of euphausiids and *Eucalanus bungii*. These findings clearly suggest that effects of iron-enrichment cascade to the mesozooplankton level. More importantly, these responses can only be elucidated by ecosystem-scale iron-enrichment experiments.

Acknowledgements

The authors express their thanks to the captains and crew members of the FRV *Kaiyo-Maru* of the Fisheries Agency of Japan and the all participants of the cruise for their cooperation at sea. This research was supported by Global Environment Research Fund from the Ministry of Environment.

References

Abraham, E.R., Law, C.S., Boyd, P.W., Lavender, S.J., Maldonaldo, M.T. and Bowie, A.R., 2000. Importance of stirring in the development of an iron fertilized phytoplankton bloom. Nature 407, 727-730.

Banse, K., 1995. Community response to IRONEX. Nature 375, 112.

Banse, K., English, D.C., 1999. Comparing phytoplankton seasonality in the eastern and western subarctic Pacific and the western Bering Sea. Progress in Oceanography 43, 235-288.

Booth, B.C., Lewin, J., Postel, J.R., 1993. Temporal variation in the structure of autotrophic and heterotrophic communities in the subarctic Pacific. Progress in Oceanography 31, 57-99.

Boyd, P.W., Watson, A.J., Law, C.S., Abraham, E.R., Trull, T., Murdoch, R., Bakker, D.C.E., Bowie, A.R., Buesseler, K.O., Chang, H., Charette, M., Croot, P., Downing, K., Frew, R., Gall, M., Hadfield, M., Hall, J., Harvey, M., Jameson, G., La Roche, J., Liddicoat, M., Ling, R., Maldonado, M.T., McKay, R.M., Nodder, S., Pickmere, S., Pridmore, R., Rintoul, S., Safi, K., Sutton, P., Trezepek, R., Tanneberger, K., Turner, S., Waite, A., Zeldis, J., 2000. A mesoscale phytoplankton bloom in the polar Southern Ocean stimulated by

iron fertilization. Nature 407, 695-702.

Boyd, P.W., Law, C. S., Wong, C.S., Nojiri, Y., Tsuda, A., Lavasseur, M., Takeda, S., Rivkin, R., Harrison, P.J., Strzepek, R., Gower, J., McKay, R.M., Abraham, E., Arychuk, M., Barwell-Clarke, J., Crawford, W., Crawford, D., Hale, M., Harada, K., Johnson, K., Kiyosawa, H., Kudo, I., Marchetti, A., Miller, W., Needoba, J., Nishioka, J., Ogawa, H., Page, J., Robert, M., Saito, H., Sastri, A., Sherry, N., Soutar, T., Sutherland, N., Taira, Y., Whitney, F., Wong, S.-K. E., Yoshimura, T., 2004. The decline and fate of an iron-induced subarctic phytoplankton bloom. Nature 428, 549–553.

Coale, K.H., Johnson, K.S., Fitzwater, S.E., Gordon, R.M., Tanner, S., Chavez, F.P., Ferioli, L., Sakamoto, C., Rogers, P., Millero, F., Steinberg, P., Nightingale, P., Cooper, D., Cochlan, W.P., Landry, M.R., Constantinou, J., Rollwagen, G., Trasvina, A., Kudela, R., 1996. A massive phytoplankton bloom induced by an ecosystem-scale iron fertilization experiment in the equatorial Pacific Ocean. Nature 383, 495-501.

Conover, R.J. 1988. Comparative life histories in the genus *Calanus* and *Neocalanus* in high latitudes of northern hemisphere. Hydrobiologia 167/168, 127-142.

Cullen, J.J., 1995. Status of the iron hypothesis after the open-ocean enrichment

experiment. Limnology and Oceanography 40, 1336-1343.

Dagg, M.J., 1993. Sinking particles as a possible source of nutrition for the calanoid copepod *Neocalanus cristatus* in the subarctic Pacific Ocean. Deep-Sea Research 40, 1431-1445.

Duce, R.A., Tindale, N.W., 1991. Atmospheric transport of iron and its deposition in the ocean. Limnology and Oceanography 36, 1715-1726.

Fulton, J., 1972. Some aspects of the life history of *Calanus plumchrus* in the Strait of Georgia. Journal of the Fisheries Research Board of Canada 30, 811-815.

Gall, M.P., Boyd, P.W., Hall, J., Safi, K.A., Chang, H., 2001. Phytoplankton processes. Part 1: Community structure during the Southern Ocean Iron Release Experiment (SOIREE). Deep-Sea Research Part II 48, 2551-2570.

Hama, T., Miyazaki, T., Ogawa, Y., Iwakuma, T., Takahashi, M., Otsuki, A., Ichimura, S., 1983. Measurement of photosynthetic production of a marine phytoplankton population using a stable ^{13}C isotope. Marine Biology 73, 31-36.

Imai, K., Nojiri, Y., Tsurushima, N., Saino, T., 2002. Time series of seasonal variation of primary productivity at station KNOT (44ºN, 155ºE) in the sub-arctic western North Pacific. Deep-Sea Research Part II 49, 5395-5408.

Kobari, K., Ikeda, T., 1999. Vertical distribution, population structure and life cycle of *Neocalanus cristatus* (Crustacea: Copepoda) in the Oyashio region, with notes on its regional variations. Marine Biology 134, 683-696.

Law, C. S., Watson, A. J., Liddicoat, M. I., Stanton, T., 1998. Sulphur hexafluoride as a tracer of biogeochemical and physical processes in an open-ocean iron fertilization experiment. Deep Sea Research Part II 45, 977-994.

Law, C.S. (this volume)

Marchetti, A., Sherry, N.D., Kiyosawa, H., Tsuda, A., Harrison, P.J., (this volume) Phytoplankton processes I: changes in biomass and community composition due to a mesoscale iron enrichment in the NE subarctic Pacific. Deep-Sea Research Part II.

Mackas, D.L., Goldblatt, R., Lewis, A.G., 1998. Interdecadal variation in developmental timing of *Neocalanus plumchrus* population at Ocean Station P in the subarctic North Pacific. Canadian Journal of Fisheries and Aquatic Science 55, 1878-1893.

Mackas, D.L., Sefton, H., Miller, C.B., Raich, A., 1993. Vertical habitat partitioning by large calanoid copepods in the oceanic subarctic Pacific during spring. Progress in Oceanography 32, 259-294.

Mackas, D. L., Tsuda, A., 1999. Mesozooplankton in the eastern and western subarctic Pacific: community structure, seasonal life histories, and interannual variability. Progress in Oceanography 43, 335-363.

Martin, J.H., Coale, K.H., Johnson, K.S., Fitzwater, S.E., Gordon, R.M., Tanner, S.J., Hunter, C.N., Elrod, V.A., Nowicki, J.L., Coley, T.L., Barber, R.T., Lindley, S., Watson, A.J., Van Scoy, K., Law, C.S., Liddicoat, M.I., Ling, R., Stanton, T., Stockel, J., Collins, C., Anderson, A., Bidigare, R., Ondrusek, M., Latasa, M., Millero, F.J., Lee, J., Yao, W., Zhang, J.Z., Friederich, G., Sakamoto, C., Chavez, F., Buck, K., Kolber, Z., Greene, R., Falkowski, P., Chisholm, S.W., Hoge, F., Swift, R., Yungel, J., Turner, S., Nightingale, P., Hatton, A., Liss, P., Tindale, N.W., 1994. Testing the iron hypothesis in ecosystems of equatorial Pacific Ocean. Nature 371, 123-129.

Martin, J.H., Fitzwater, S.E., 1988. Iron deficiency limits phytoplankton growth in the north-east Pacific subarctic. Nature 331, 341-343.

Miller, C.B., Clemons, M.J., 1988. Revised life history analysis for large grazing copepods in the subarctic Pacific Ocean. Progress in Oceanography 20, 293-313.

Miller, C.B., Frost, B.W., Batchelder, H.P., Clemons, M.J., Conway, R.E., 1984. Life

histories of large, grazing copepods in a subarctic ocean gyre: *Neocalanus plumchrus, Neocalanus cristatus* and *Eucalanus bungii* in the northeast Pacific. Progress in Oceanography 13, 201-243.

Obayashi, Y., Tanoue, E., Suzuki, K., Handa, N., Nojiri, Y., Wong, C.S., 2001. Spatial and temporal variabilites of phytoplankton community structure in the northern North Pacific as determined by phytoplankton pigments. Deep-Sea Research Part I 48, 439-469.

Parsons, T.R., LeBraseur, R.J. 1968. A discussion of some critical indices of primary and secondary production for large-scale ocean surveys. California Marie Research Comm CalCOFI Report 12, 54-63.

Peters, R.H., Downing, J.A., 1984. Empirical analysis of zooplankton filtering and feeding rates. Limnology and Oceanography 29, 763-784

Rollwagen Bollens, G.C., Landry, M.R., 2000. Biological response to iron fertilization in the eastern equatorial Pacific (IronEx II). II. Mesozooplankton abundance, biomass, depth distribution and grazing. Marine Ecology Progress Series 2001, 43-56.

Saito, H., 1996. Study on the seasonal variability in the productivity of lower-trophic levels and diel feeding rhythm of copepods in the Oyashio region. Bulletin of

Hokkaido National Fisheries Research Institute 60, 1-144.

Saito, H., Tsuda, A., 2000. Egg production and early development of large subarctic copepods *Neocalanus cristatus*, *N. plumchrus* and *N. flemingeri*. Deep-Sea Research Part I 47, 2141-2158.

Saito, H. et al. (this volume)

Sastri, A.R., J.F. Dower, (this volume). Mesozooplankton community response to a phytoplankton bloom in the NE subarctic Pacific. Deep-Sea Research Part II,

Strom, S.L., Welschmeyer, N.A., 1991. Pigment-specific rates of phytoplankton growth and microzooplankton grazing in the open subarctic Pacific Ocean. Limnology and Oceanography 36, 50-63.

Sugimoto, T., Tadokoro, K., 1997. Interannual-interdecadal variations in zooplankton biomass, chlorophyll concentration and physical environment in the subarctic Pacific and Bering Sea. Fisheries Oceanography 6, 74-93.

Suzuki, K., Liu, H., Saino, T., Obata, H., Takano, M., Okamura, K., Sohrin, Y., Fujishima, Y., 2002. East-west gradients in the photosynthetic potential of phytoplankton and iron concentration in the subarctic Pacific Ocean during early summer. Limnology and Oceanography 47, 1581-1594.

Suzuki, K., Hinuma, A., Saito, H., Kiyosawa, H., Liu, H., Saino, T., Tsuda, A., (in press)

Responses of phytoplankton and heterotrophic bacteria in the northwest subarctic Pacific to in situ iron fertilization as estimated by HPLC pigment analysis and flow cytometry. Progress in Oceanography

Terazaki, M., Tomatsu, C., 1997. A vertical multiple opening and closing plankton sampler. Journal of Advanced Marine Science Technological Society 3, 127-132.

Tsuda, A., Kawaguchi, S., 1997. Microzooplankton grazing in the surface water of Antarctic Ocean during an austral summer. Polar Biology 18, 240-245.

Tsuda, A., Kiyosawa, H., Kuwata, A., Mochizuki, M., Shiga, N., Saito, H., Chiba, S., Imai, K., Nishioka, J., Ono, T., (in press a). Responses of diatoms to iron-enrichment (SEEDS) in the western subarctic Pacific, temporal and spatial comparisons. Progress in Oceanography,

Tsuda, A., Saito, H., Kasai, H., 1999. Life histories of *Neocalanus flemingeri* and *Neocalanus plumchrus* in the western subarctic Pacific. Marine Biology 135, 533-544.

Tsuda, A., Saito, H., Kasai, H., (in press c). Life histories of *Eucalanus bungii* and *Neocalanus cristatus* (Calanoida: Copepoda) in the western subarctic Pacific. Fisheries Oceanography,

Tsuda, A., Saito, H., Nishioka, J., Ono, T., (in press b). Mesozooplankton responses to iron-fertilization in the western subarctic Pacific (SEEDS2001). Progress in Oceanography,

Tsuda, A., Sugisaki, H., 1994. *In-situ* grazing rate of the copepod population in the western subarctic North Pacific during spring. Marine Biology 120, 203-210.

Tsuda, A., Takeda, S., Saito, H., Nishioka, J., Nojiri, Y., Kudo, I., Kiyosawa, H., Shiomoto, A., Imai, I., Ono, T., Shimamoto, A., Tsumune, D., Yoshimura, T., Aono, T., Hinuma, A., Kinugasa, M., Suzuki, K., Sohrin, Y., Noiri, Y., Tani, H., Deguchi, Y., Tsurushima, N., Ogawa, H., Fukami, K., Kuma, K., Saino, T., 2003. A mesoscale iron enrichment in the western subarctic Pacific induces large centric diatom bloom. Science 300, 958-961.

Tsumune, D., Nishioka, J., Shimamoto, A., Takeda, S., Tsuda, A., (in press) Physical behavior of the iron fertilized patch by SF_6 tracer release experiment. Progress in Oceanography,

van Scoy, K., Coale, K., 1994. Pumping iron in the Pacific. New Scientist 3, 32-35.

Wong, C.S., Timothy, D., Nojiri, Y., Xie, L., Wong, S.-K. E., Page, J.S., (this volume). Carbon flux, distribution and budget with special reference to the fugacity of carbon dioxide (fCO_2) in the SERIES iron fertilization experiment. Deep Sea

Research Part II

Zeldis, J., 2001. Mesozooplankton community composition, feeding, and export production during SOIREE. Deep-Sea Research Part II *48*, 2615-2634.

Legends of figures

Fig. 1. Relative horizontal locations of the out-patch sampling of mesozooplankton to the patch center during SERIES. The numbers in the figure indicate the elapsed number of days from the iron fertilization and the lower case characters indicate daytime (d) and nighttime (n) samplings.

Fig. 2. Community composition of mesozooplankton collected with a VMPS net from 200 m depth to the surface inside and outside of the iron-enriched patch during the later half of SERIES. Filled circles above the column indicate the nighttime sampling.

Fig. 3. Temporal changes of carbon biomass of copepods, euphausiids and other mesozooplankton in a 0-200 m (left panels) and 0-30 m (right panels) water column inside (solid lines) and outside (broken lines) of the iron-enriched patch during the later half of SERIES. Filled circles indicate nighttime sampling.

Fig. 4. Representative temporal changes of abundance of mesozooplankton inside (solid lines) and outside (broken lines) of the iron-enriched area which showed higher

abundance in the patch than those of outside. Filled circles indicate nighttime sampling.

Fig. 5. Relations between elapsed time (d) from the iron fertilization and averaged copepodites stage of *Eucalanus bungii*, *Neocalanus plumchru*s and *N. cristatus* during the later half of SERIES. Closed circles are inside and open circles outside. Solid and broken lines denote regression lines for inside and outside of the patch, respectively.

Fig. 6. Temporal changes of average depth of vertical distributions for dominant copepodites stages of *Eucalanus bungii* (left panels) and *Neocalanus cristatus* (right panels) inside and outside the iron-enriched area. Solid lines are the in-patch variation and broken lines are out-patch variations. Filled circles indicate nighttime sampling.

Fig. 7. Day and night vertical distributions of *Eucalanus bungii* C5, *Neocalanus cristatus* C4 and *Metridia pacifica* C4 during the later half of SERIES. Horizontal bars indicate 1 S.D.

Fig. 8. Temporal changes of gut pigment content *Calanus pacificus* C6fem, *Eucalanus*

bungii C5, C4 and *Neocalanus plumchrus* C5 inside and outside of the patch. Solid lines are the inside-patch variation and broken lines are outside-patch variations. Filled circles indicate nighttime sampling and vertical bars indicate 1 S.D.

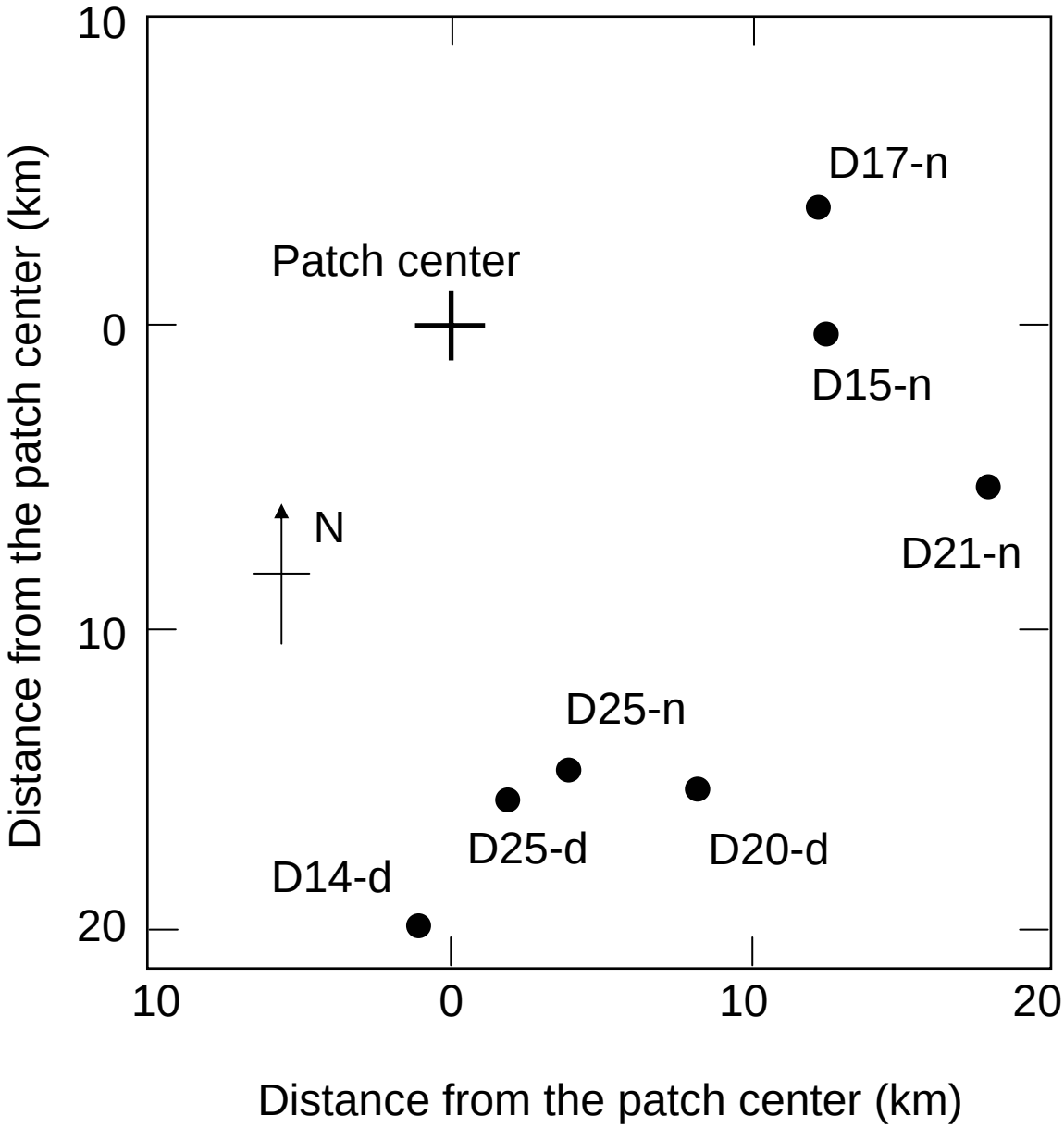
Fig. 9. Temporal changes of filtering rates of *Calanus pacificus* C6fem, *Eucalanus bungii* C5, C4 and *Neocalanus plumchrus* C5 inside and outside of the patch. Solid lines are the inside-patch variation and broken lines are outside-patch variations. Filled circles indicate nighttime sampling and vertical bars indicate 1 S.D.

Fig. 10. Temporal changes of water column integrated primary production and the copepod community grazing rate in the iron-enriched patch during diatom-bloom and decline phase of SERIES. The carbon consumption by copepod community was estimated from gut pigment contents and numerical abundance of the copepods with C/chl ratio of 51.

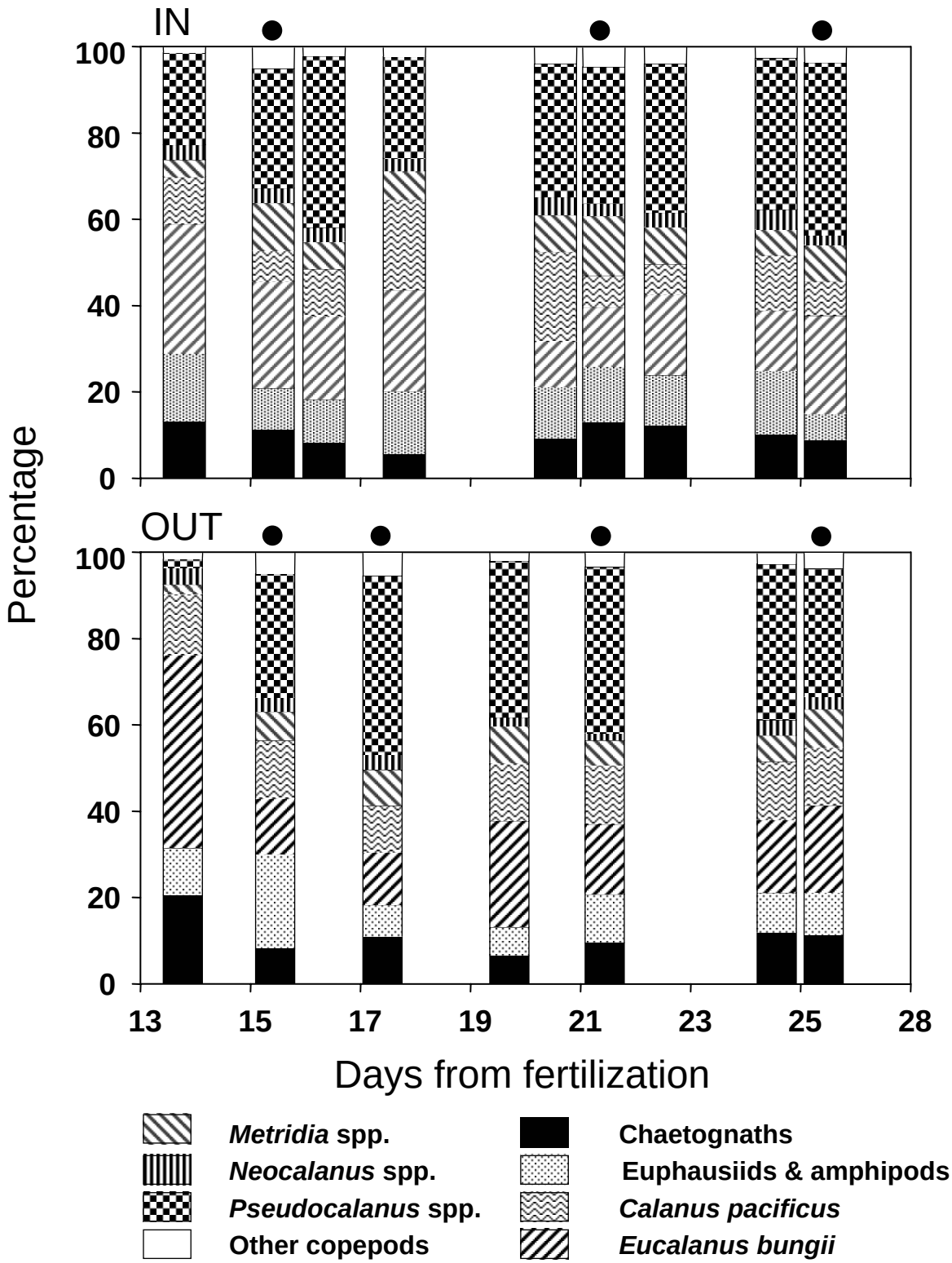
Fig. 11. Temporal changes of total abundances of *Eucalanus bungii* (C1-C6) and *Neocalanus cristatus* (C1-C5) in the 0-200 m water column in the iron-enriched patch. Solid lines with filled circles indicate inside and broken lines with open circles indicate

outside of the iron-enriched patch.

Fig. 12. Temporal changes of the bottom of the surface mixed layer estimated from the sigma-*t* profiles (broken line) and the SF_6 depth (solid line) during the diatom bloom and period of bloom decline of SERIES.



Tsuda et al. Fig. 1



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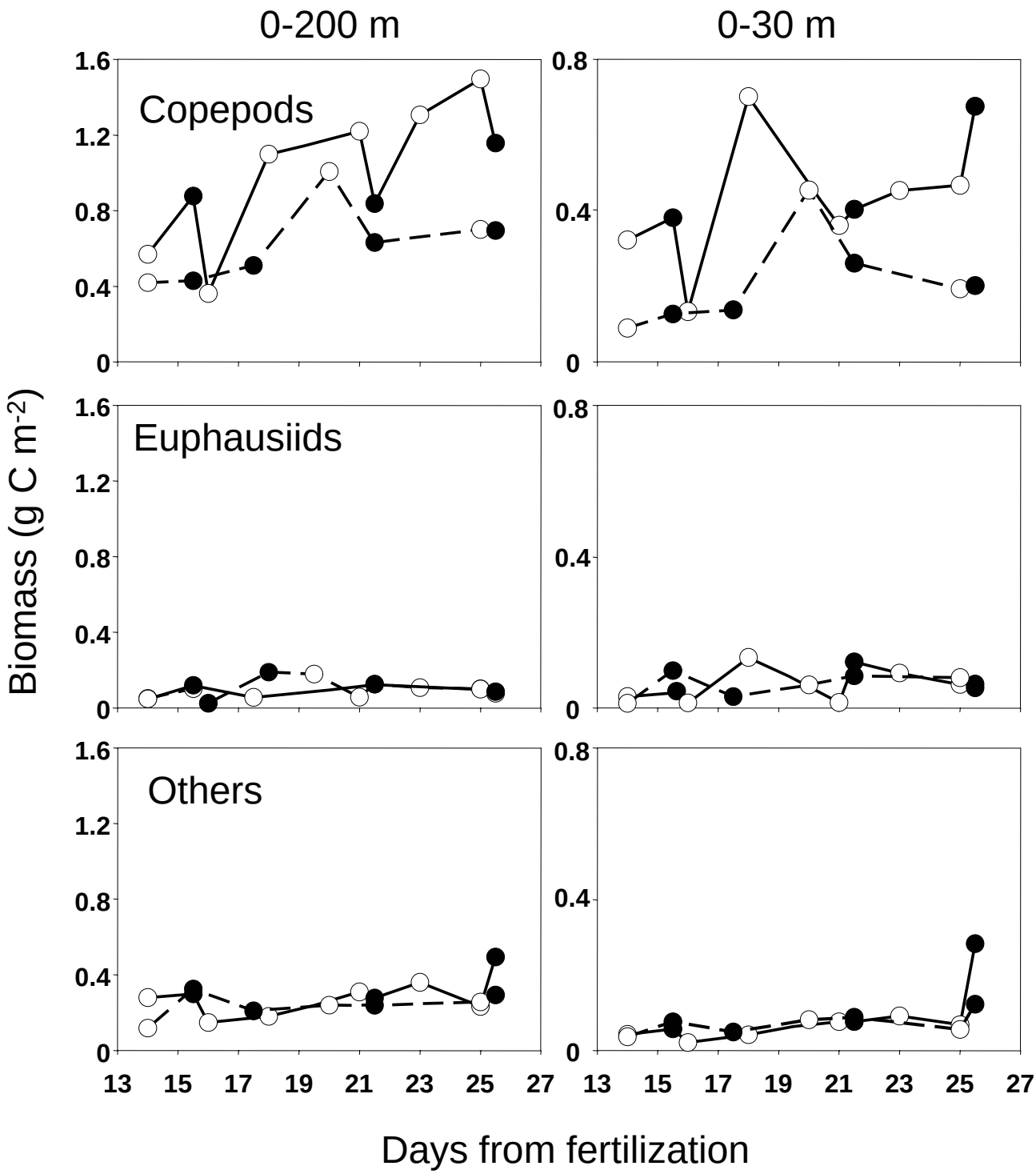
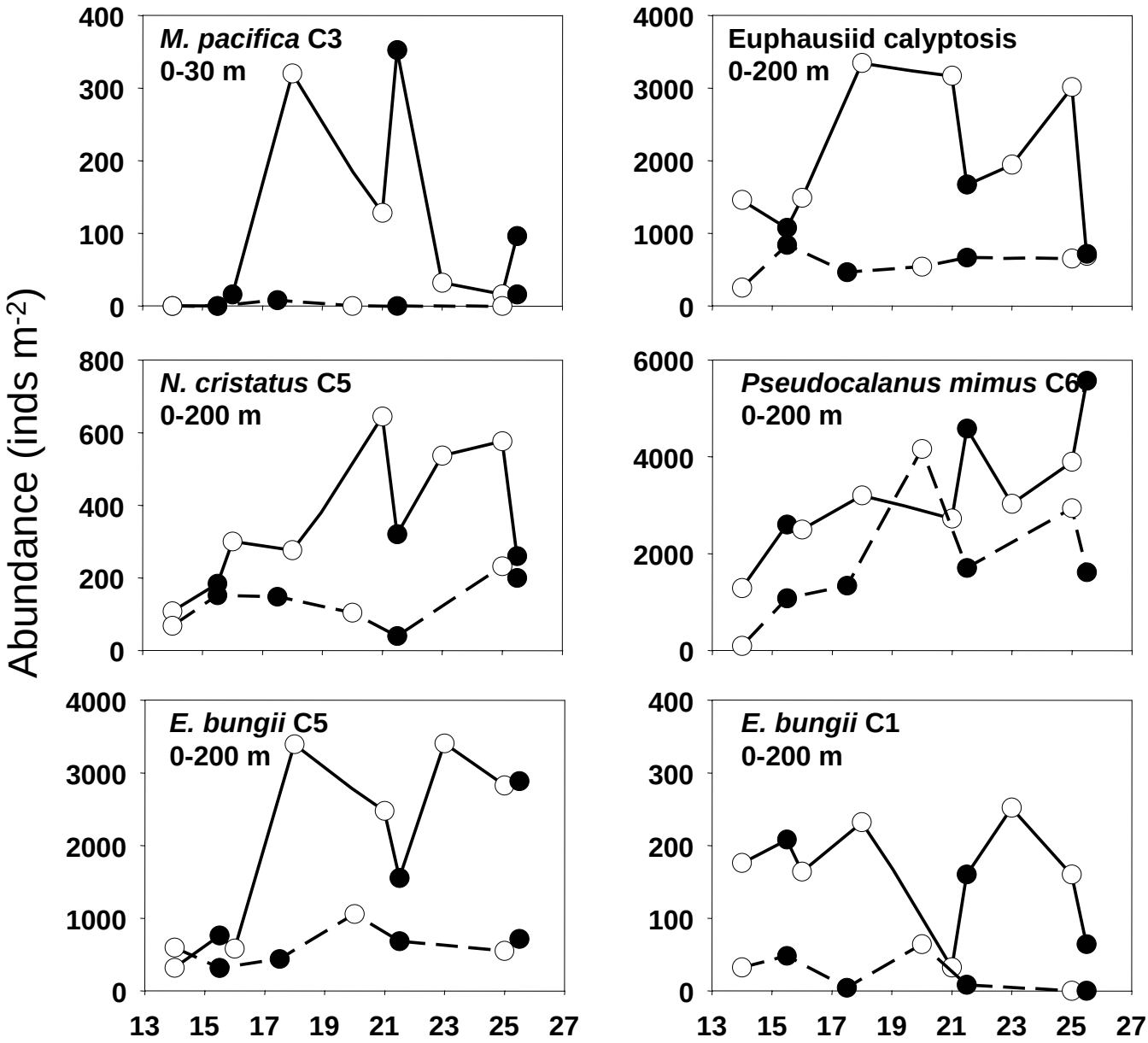


Tsuda et al. Fig. 3



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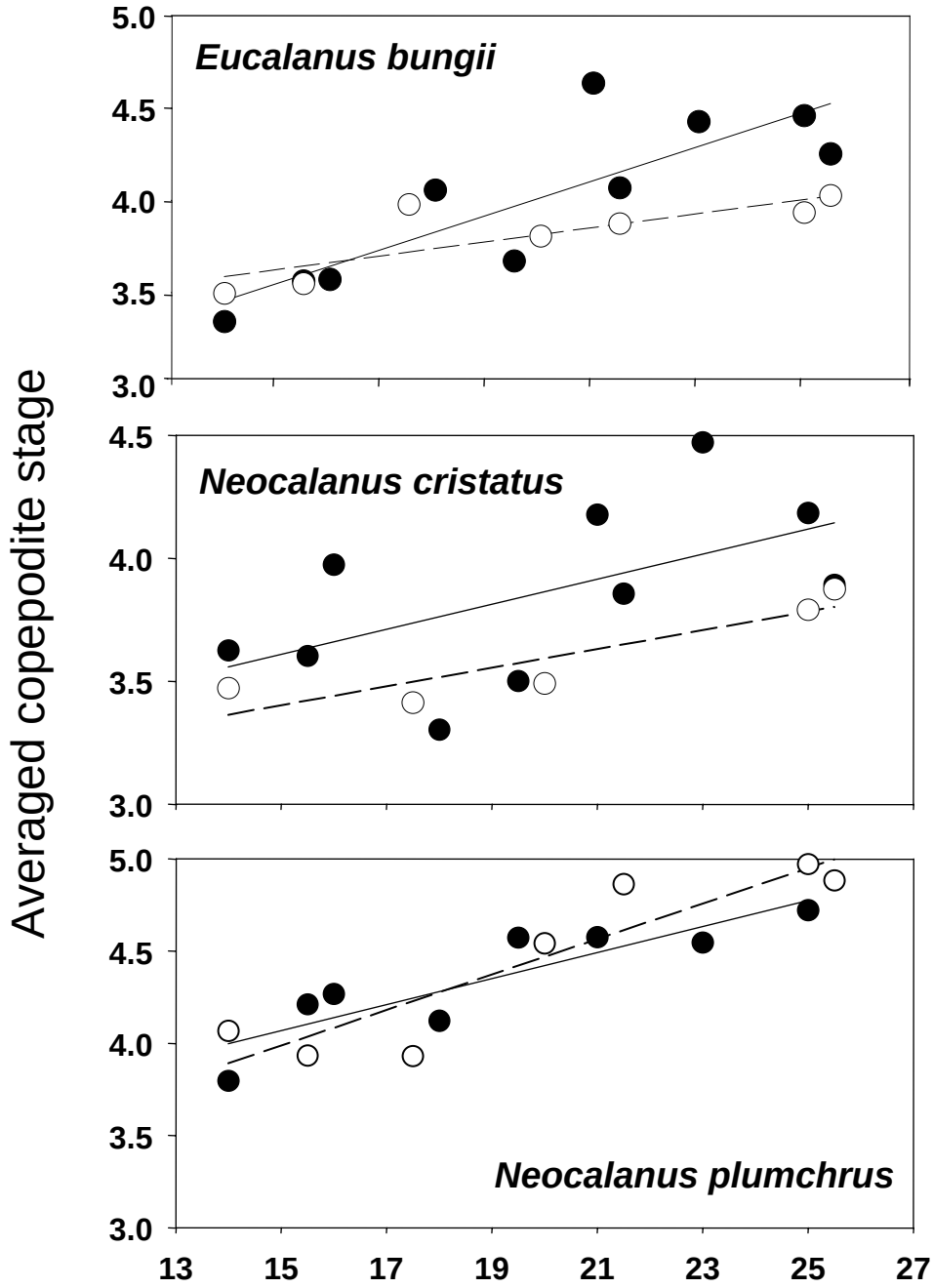

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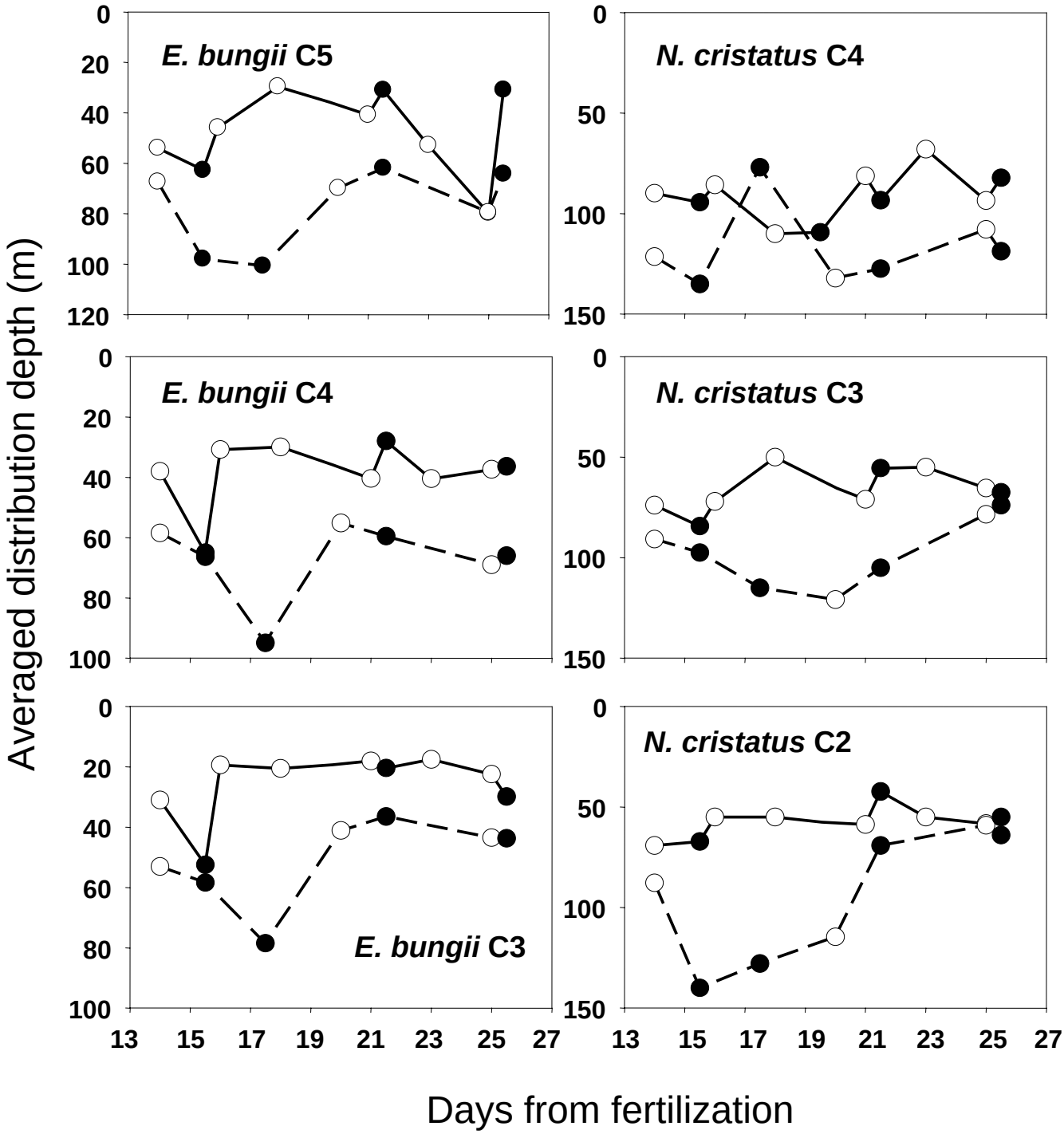

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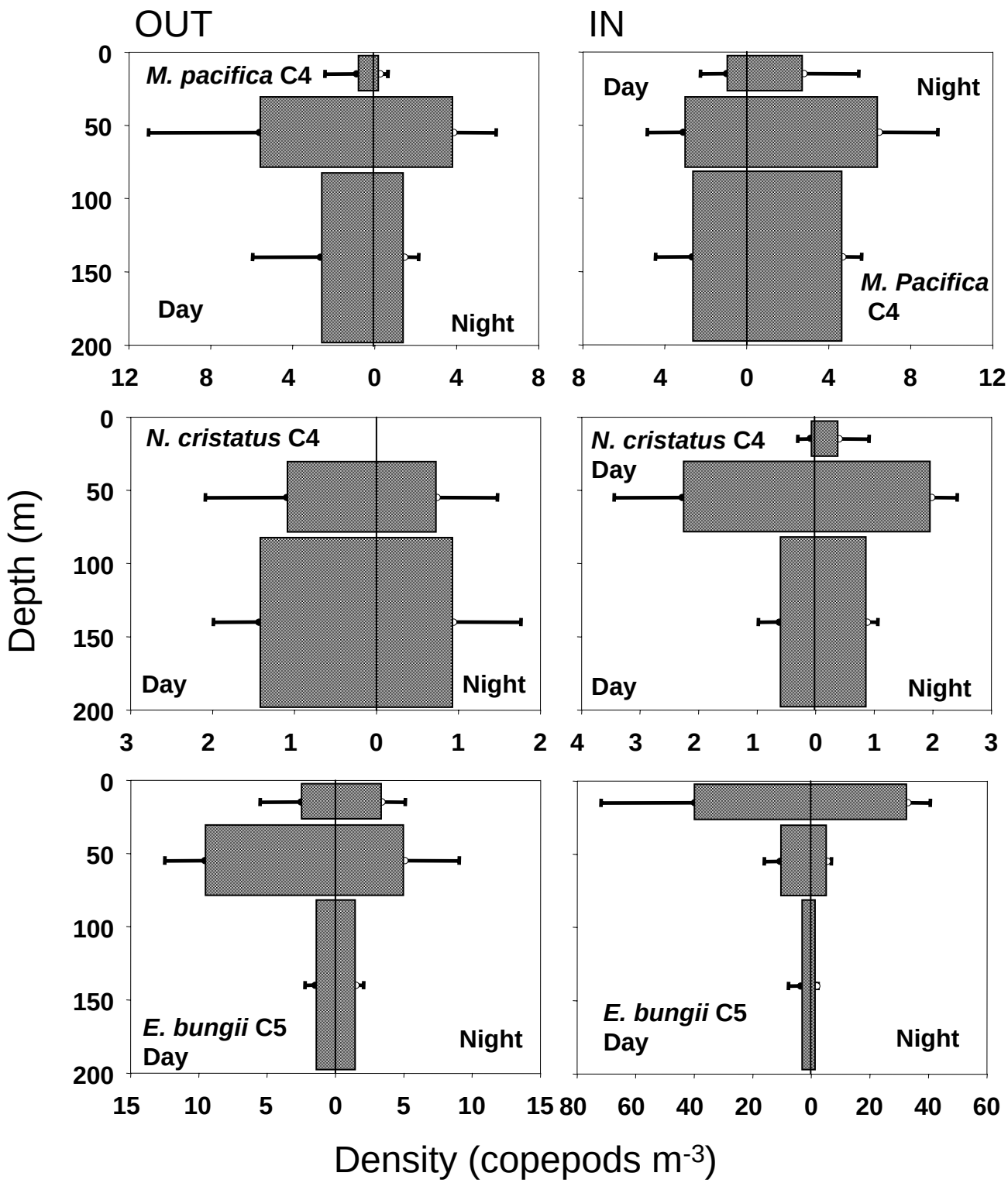

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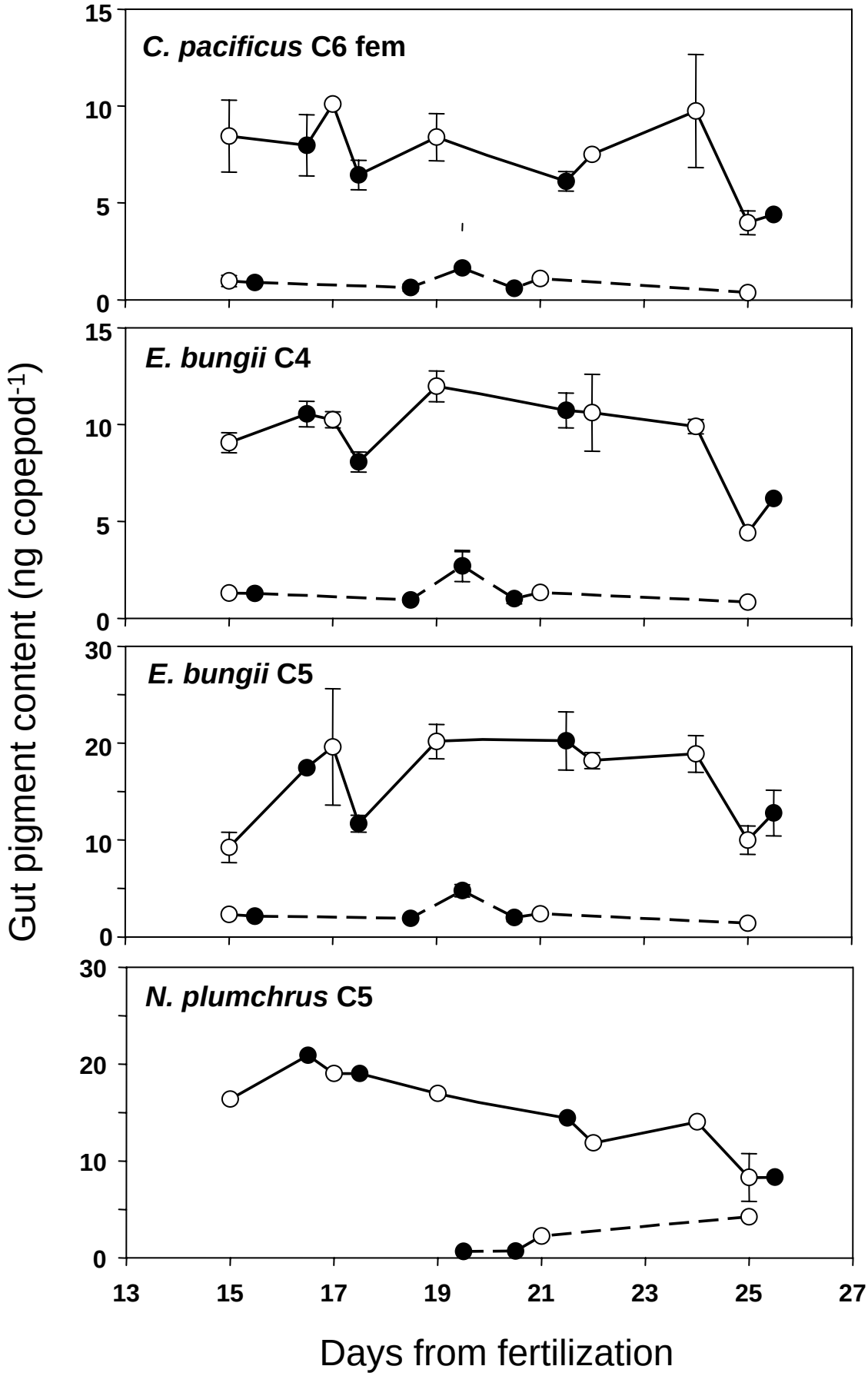

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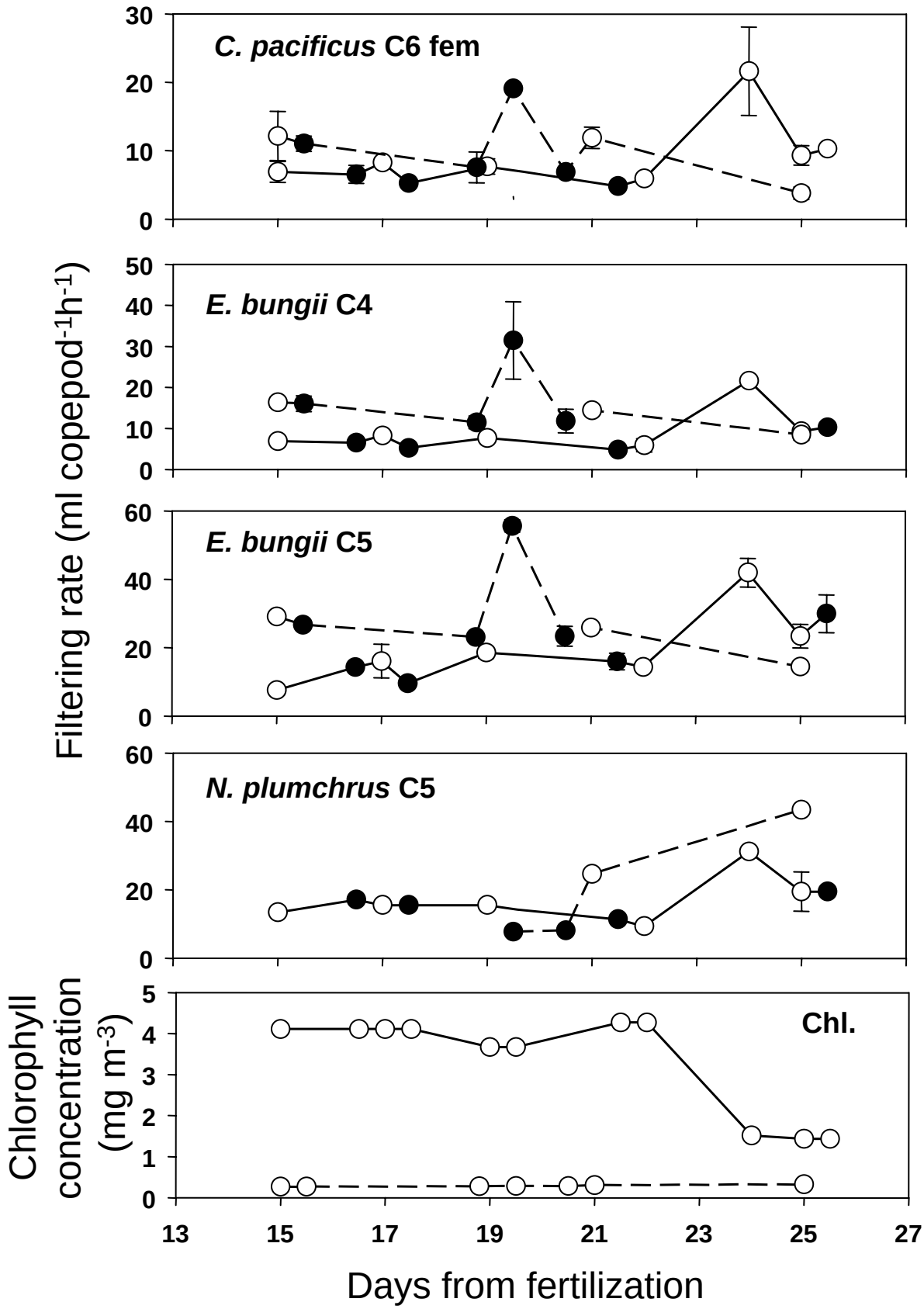

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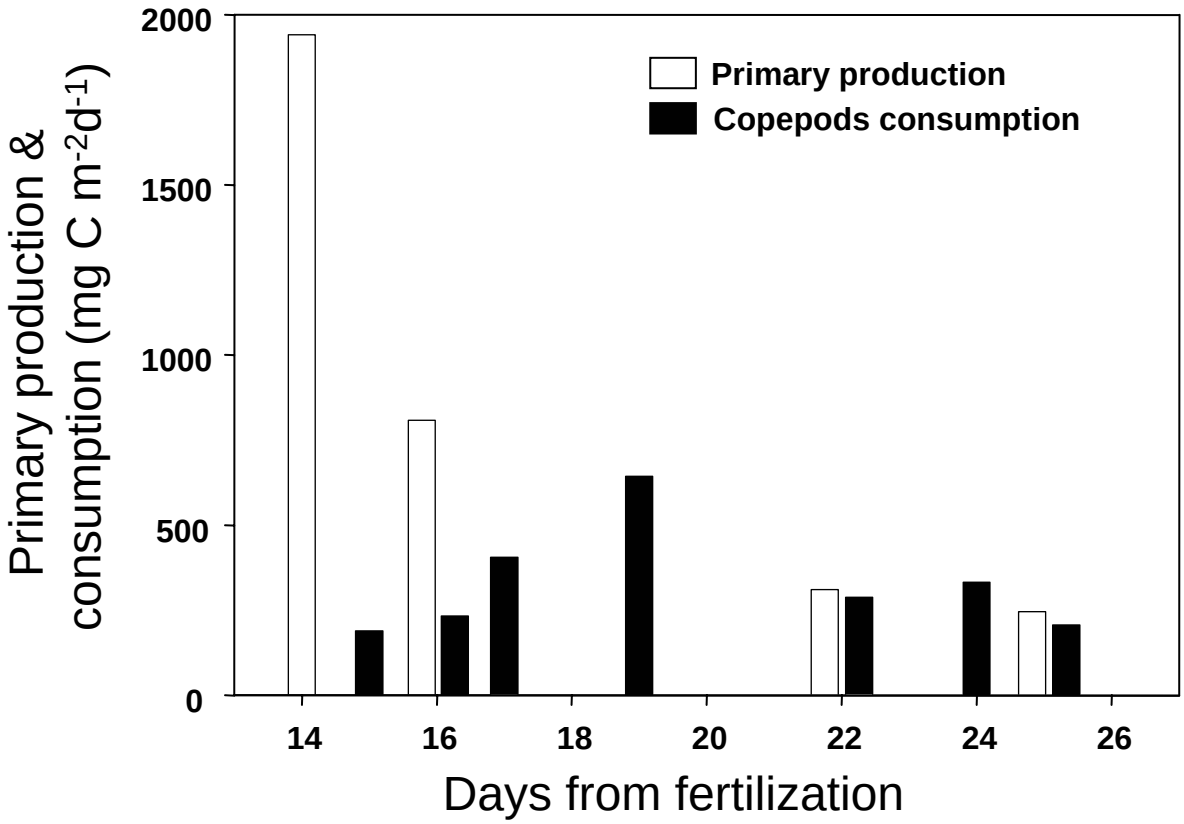

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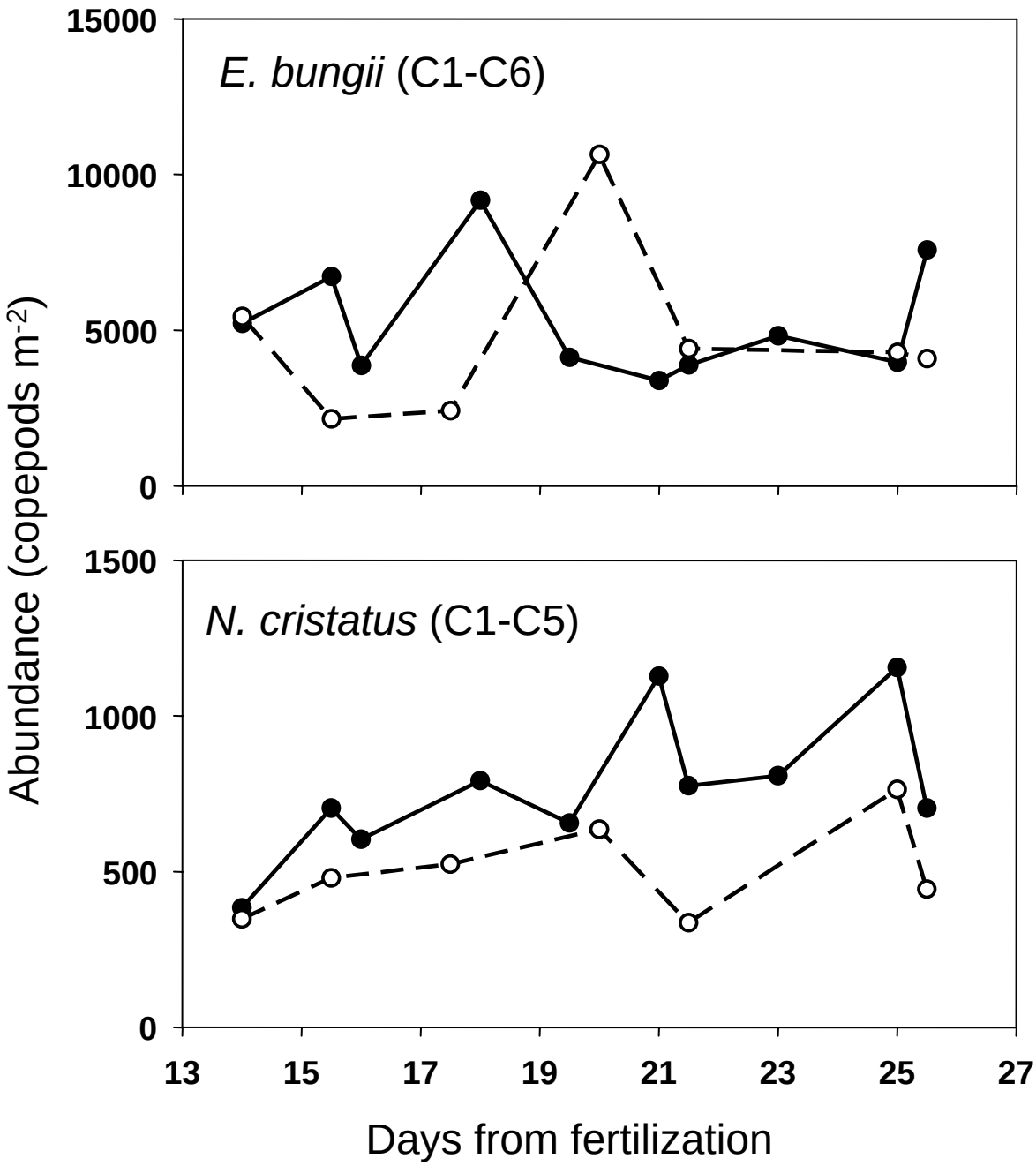

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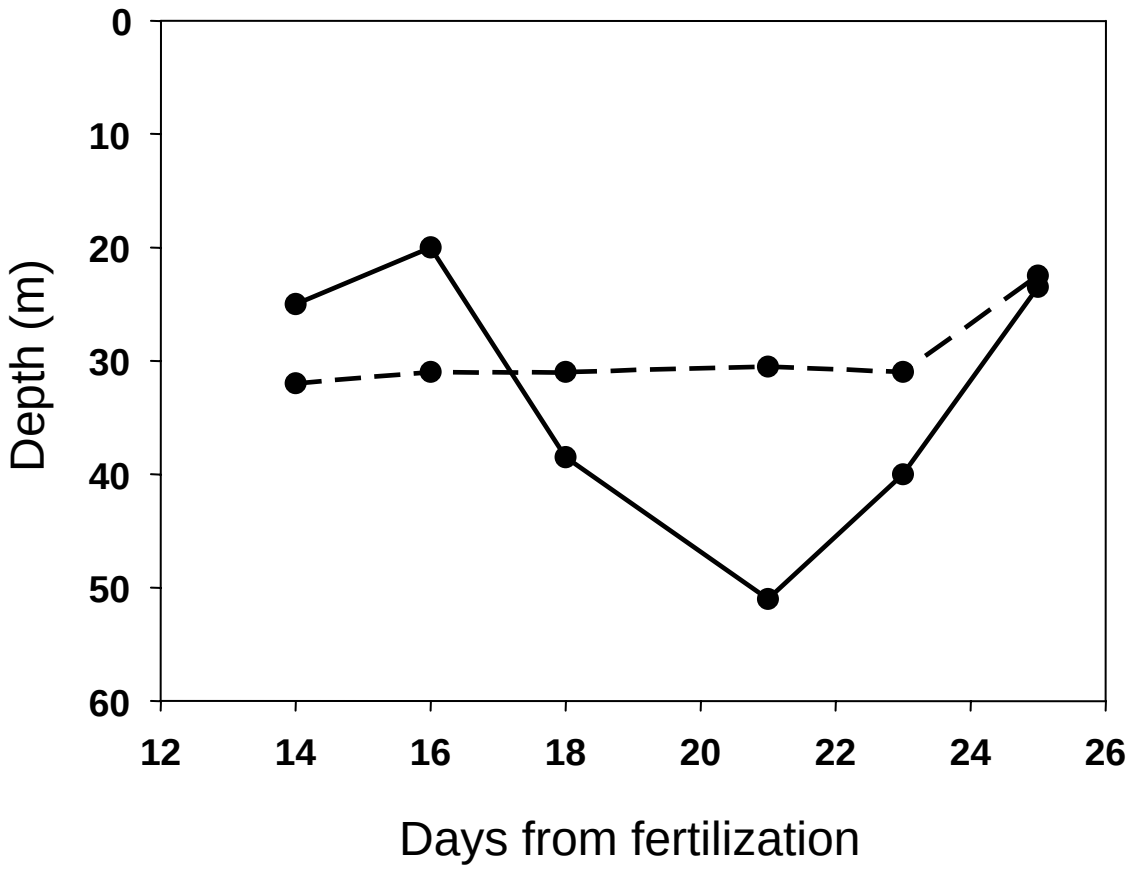

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