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Meso- and microzooplankton responses to an *in situ* iron fertilization experiment (SEEDS II) in the northwest subarctic Pacific

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ABSTRACT

A mesoscale iron fertilization experiment was carried out in the western subarctic Pacific during summer 2004. The iron-patch was traced for 26 days after the enrichment, and the abundance and behavior of meso- and microzooplankton was compared with those outside of the patch. The surface chlorophyll-*a* concentration in the patch was high between days 10 and 13 (2.5 mg m^{-3}) and decreased to the initial level after day 20. Microzooplankton grazing rates, estimated by a dilution method, was mostly balanced with phytoplankton growth rates throughout the observed period. Dominant mesozooplankton species in the upper 200 m were copepods: dominated by *Eucalanus bungii*, *Neocalanus plumchrus* and *Metridia pacifica*. Species composition did not change in the patch over the observation period. The copepod biomass was 3–5 times higher than in Subarctic Pacific Iron Experiment for Ecosystem Dynamics Study (SEEDS), the previous iron-enrichment experiment in the same area, before the bloom, and exponentially increased both inside and outside the patch, which was mainly brought by the development of *N. plumchrus*. The development rates of *N. plumchrus* were not significantly different between inside and outside the patch. Estimated grazing rate suggest that the copepod grazing was main cause of the low accumulation of phytoplankton biomass, and dominance of grazing-resistant organisms such as large ciliates, large diatoms and diatoms with extremely long setae. "Arrested migration" for *M. pacifica* and upward shift of vertical distribution by *E. bungii* were observed during the bloom period, even if the accumulation of phytoplankton biomass was very low compared to other iron-enrichment experiments. These results indicate that the copepod grazing shaped the food-web structure of the lower trophic levels (biomass and species composition) in SEEDS II.

1. Introduction

In high nitrate low chlorophyll (HNLC) oceans, microzooplankton grazing is recognized as an important mechanism to keep phytoplankton biomass low (e.g., Strom and Welschmeyer, 1991; Tsuda and Kawaguchi, 1997). In addition, phytoplankton growth is highly stressed by the low iron availability which is clearly demonstrated by mesoscale iron addition experiments in the equatorial Pacific (Coale et al., 1996), the Southern Ocean (Boyd et al., 2000; Coale et al., 2004) and the subarctic North Pacific (Tsuda et al., 2003; Boyd et al., 2004). Recently, "the ecumenical iron hypothesis" has been accepted in general and suggests that iron controls the growth of large phytoplankton and the microzooplankton grazing controls the smaller phytoplankton having a lower requirement for iron and insensitivity to mesozooplankton grazing (Cullen, 1995; Landry et al., 1997; Dagg et al., 2009). More than 10 mesoscale iron-enrichment experiments have been performed in major HNLC oceans, and increases of phytoplankton abundance dominated by diatom species have been observed in most experiments (Boyd et al., 2007). These findings support the ecumenical iron hypothesis. Moreover, a major part of the fixed carbon were respired to inorganic carbon in the surface mixed layer by microzooplankton and bacterial activities (Boyd et al., 2004; Coale et al., 2004; Saito et al., 2006).

In contrast, responses of mesozooplankton to the phytoplankton increase caused by iron enrichment were variable. Mesozooplankton grazing is an important loss factor for phytoplankton, although the zooplankton grows slowly and cannot markedly increase in abundance during a fertilization experiment. In IronEx II in the equatorial Pacific, increase of mesozooplankton in the surface mixed layer was observed caused by arrested migration (diel vertical migrators stayed in the surface layer during daytime; Rollwagen Bollens and Landry, 2000). A similar concentration mechanism was suggested for subsurface resident copepods in the eastern subarctic Pacific (Tsuda et al., 2006). These functional (behavioral) responses are important to determine the fate of carbon cycle in the iron-enriched water mass because

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behavioral time scales (hours to day) are much shorter than life-cycle time scales (weeks to years). In Subarctic Pacific Iron Experiment for Ecosystem Dynamics Study (SEEDS) in the western subarctic Pacific and SOIREE in the Southern Ocean, almost no response was observed in the biomass and behavior of mesozooplankton (Zeldis, 2001; Tsuda et al., 2005), although abundance of first copepodite of surface living copepods increased during the diatom blooming period. The higher recruitment was suggested to be caused by the lowered mortality rate during the egg and naupliar stages because of the increase of alternative food (diatom) for omnivorous predators (Tsuda et al., 2005, 2006). The same mechanism was suggested for the increase of small copepods in the iron-patch of EisenEx (Henjes et al., 2007). Moreover, a clear enhancement of egg production rate of a dominant copepod in the patch was observed in the same experiment (Jansen et al., 2006).

Subarctic Pacific Iron Experiment for Ecosystem Dynamics Study II was the second iron-enrichment experiment in the western subarctic Pacific. The experiment was carried out at almost same location and same season to SEEDS. However, the response of phytoplankton was quite different to those of SEEDS. The chlorophyll-*a* concentration in the iron-enriched area only increased 2.5 times of ambient concentration and returned to low algal concentrations dominated by pico-phytoplankton (Tsuda et al., 2007). More importantly, the main cause of low accumulation of phytoplankton biomass was suggested as the copepod grazing on the phytoplankton. In this paper, we will describe the detailed information on meso- and microzooplankton response to the iron enrichment in SEEDS II and discuss about causes of response and its consequences.

2. Materials and methods

A mesoscale *in situ* iron-enrichment experiment, SEEDS II, was conducted in the western subarctic gyre of the North Pacific (48°N, 166°E) from 20 July to 20 August 2004 by R.V. *Hakuho-Maru* (HM) and R.V. *Kilo Moana* (KM). The experiment consisted of an addition of 322 kg iron as $FeSO_4$ with an inert tracer gas, sulphur hexafluoride (SF_6), over an $8\times8\,km^2$ patch with a mixed layer depth of ~35 m on 20 July 2004. Day 1 is defined as the 24-h period starting at 00:00 21 July 2004. The second iron addition (159 kg) without SF_6 was performed on day 6. The iron-patch was followed from 21 July to 14 August 2004, by tracking the SF_6 and pCO_2 signals (Tsumune et al., 2009). Net samplings were carried out at an interval of 1–3 days in the patch and at intervals of 1–5 days outside the patch until day 24. Additionally, a net sampling was carried out outside the patch on day 32. Location of the inside stations were determined by the SF_6 and pCO_2 monitoring.

A VMPS net (opening–closing multi-layer net: $50\times50\text{-}cm^2$ mouth opening, 0.1-mm mesh opening, Terazaki and Tomatsu, 1997) was towed from 200-m depth for estimations of standing stock, vertical distribution and diel vertical migration by HM from days 1–12 to 23–24. The sampling layer was divided into 0–20, 20–50, 50–100 and 100–200 m, which were identical layers to SEEDS (Tsuda et al., 2005). A Norpac net (mouth diameter: 45 cm, 0.1-mm mesh opening) was towed from 20- and 200-m depths in KM from days 15 to 22. The samples were immediately preserved with 10% buffered formalin seawater. In a laboratory, all individuals except small copepods (mainly *Oithona* spp. and non-adult stages of *Pseudocalanus* spp.) were identified, counted and sorted out for measurements of wet weight. Especially, the dominant copepods *Neocalanus cristatus*, *Neocalanus plumchrus*, *Calanus pacificus*, *Metridia pacifica* and *Eucalanus bungii* were counted into each developmental stage. Maturity stages of adult female *E. bungii* were determined according to Miller et al. (1984). Maturity stage 1, 2 and 3 of Miller et al. (1984) were grouped as the early maturity stage in this study. The carbon biomasses were estimated using the wet weight and a conversion factor of 0.08 (Peters and Downing, 1984).

In order to examine the microzooplankton grazing rate and phytoplankton gross growth rate, dilution experiments (Landry and Hassett, 1982; Landry et al., 1995) were carried out once before the iron fertilization on 19 July 2004 (we defined as D0), 9 times inside the iron-patch. For the experiments, all the sampling tubes, containers, incubation bottles were cleaned after the method of Saito et al. (2005). Duplicate dilution treatments of 0.25, 0.5, 0.75 and 1.0 × of natural seawater were prepared in 1.2-l polycarbonate bottles with filtered seawater (FSW). The FSW was obtained by gravity filtration with acid-cleaned cartridge filter (Gelman Versapor, 0.2 μm pore size) attached to the spigot of the Niskin bottle via a silicon tube.

Nutrients (nitrate and iron) were not added to the bottles because the injection effect was negligible in the dilution experiments during SEEDS (Saito et al., 2005). Incubations for the dilution experiment were performed for 24 h in a water bath with running sea surface water. Each experimental bottle was covered by a neutral density filter to reduce irradiance to 55% of the surface irradiance. Microzooplankton grazing rate and gross growth rate of phytoplankton were calculated for total phytoplankton by fluorometric analysis of chlorophyll-*a* at the beginning (only for undiluted samples) and the end of the incubations. For determination of chlorophyll-*a* concentration, triplicate (initial) or duplicate (final) water samples (116 ml) were filtered through 25-mm GF/F filters with low vacuum pressure (<100 mmHg). The filters were immediately soaked in 6 ml of *N*,*N*-dimethylformamide and pigments were extracted in the dark at −20 °C for a day (Suzuki and Ishimaru, 1990). Chlorophyll-*a* concentration was determined with a Turner Designs fluorometer by the non-acidification method of Welschmeyer (1994). The phytoplankton growth rate and microzooplankton grazing rate were estimated as the *Y*-axis intercept and the slope of the linear regression line of the apparent growth rate of the phytoplankton as a function of the dilution rate (Landry and Hassett, 1982).

Water samples for *in situ* abundance of microzooplankton were obtained at intervals of 1–2 and 3–8 days, inside and outside the patch, respectively, with Niskin bottles attached to a CTD system from the 5, 10 and 30 m depths. For enumeration of micro-sized zooplankton (ca. >10 μm), 1 l of water was transferred into a black plastic bottle and preserved with 5% acid-Lugol's solution (final concentration). Certain fractions of water samples were allowed to settle and concentrated. Ciliates, heterotrophic dinoflagellates (*Gyrodinium* spp.) and crustacean nauplii were counted with an inverted microscope at a magnification of 200 ×. For ciliates, aloricate ciliates and tintinnids were counted separately. For each sample, length and width of animals in each category were measured for up to 30 individuals using an image analyzer with a CCD camera attached to the microscope. Cell volumes of ciliates and dinoflagellates were estimated with an ellipsoid approximation of cell shape. A carbon weight for aloricate ciliates was calculated with the carbon weight–volume relationship of 0.19 pg C μm^{-3} (Putt and Stoecker, 1989). Carbon weights of tintinnids were calculated with the equation of Verity and Langdon (1984). For dinoflagellates, the relationship of 0.14 pg C μm^{-3} was used (Lessard, 1991). Carbon weights of nauplii were calculated from the body length (Uye, 1988). These methods were same as those of SEEDS (Saito et al., 2005).

Saito et al. (2009) estimated the mesozooplankton grazing rates from the exponential increase of the mesozooplankton biomass in the surface layer. In this study, we try to estimate the major copepod grazing rate by an independent method from Saito