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ORIGINAL PAPER

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Reproductive response of the copepod *Rhincalanus gigas* to an iron-induced phytoplankton bloom in the Southern Ocean

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Abstract The reproductive response of *Rhincalanus gigas* to the build up of a phytoplankton bloom in the Southern Ocean was studied during the European iron fertilization experiment (EIFEX). Egg production experiments were conducted over a period of approximately 5 weeks during development of a diatom dominated bloom. *R. gigas* showed a clear response to increasing chlorophyll *a* concentrations and the total egg production of the *R. gigas* population was highest just after the peak of the bloom at day 29 after fertilization. The average peak production was 50 eggs female^{-1} day^{-1}. The percentage of egg producing females increased from about 0 to 90% during the course of the experiment. Accordingly, the maturation of the gonads reflected the positive response towards enhanced chlorophyll *a* concentrations. The fast reproductive response indicate that *R. gigas* was food limited during the period of this study in the Antarctic Polar Front region (APF).

Introduction

The Southern Ocean is known as a high nutrient—low chlorophyll (HNLC) ecosystem where production is generally low, even though macronutrients like nitrate and phosphate are available all year round (Martin et al. 1990). In recent years, it has been shown that primary production in the Southern Ocean is limited by iron availability (Martin et al. 1990). Large scale artificial iron fertilization experiments of HNLC waters have induced phytoplankton blooms (e.g. Boyd et al. 2000; Gervais et al. 2002; Coale et al. 2004). Copepods dominate the zooplankton communities of the Southern Ocean in terms of numbers and biomass (Voronina 1998; Pakhomov et al. 2000), but our understanding of copepod population dynamics in this area is still poor. As a consequence of the short growth season and low primary production, zooplankton growth and reproduction in the Southern Ocean may be limited by food availability. A productive area within the Southern Ocean is associated with the Antarctic Polar Front (APF) where blooms are frequently reported (Laubscher et al. 1993). In the Atlantic sector of the APF *Rhincalanus gigas* is one of the most abundant large copepod species (e.g. Ommanney 1936; Atkinson 1991; Pakhomov et al. 2000), and is known to have a protracted period of recruitment through the summer into the autumn (Ommaney 1936). In recent years it has become more and more apparent, that growth and development of populations of large calanoid copepods within the Southern Ocean depend on the availability of food (Ward and Shreeve 1999; Shreeve et al. 2002). However, most of the reported studies give only snapshot results where temporal development is not taken into consideration.

The European iron fertilization experiment (EIFEX) provided an unique opportunity to follow the reproductive response of *R. gigas* during the entire development of a diatom dominated phytoplankton bloom. This Lagrangian type experiment made it possible to study the same zooplankton assemblage for 38 consecutive days, which has so far not been achieved for the Southern Ocean.

Material and methods

Study location

The iron fertilization experiment EIFEX was carried out during austral autumn (21 Jan 2004–25 Mar 2004) in the Atlantic sector of the Southern Ocean on *RV* Polarstern. We choose a cyclonic eddy (extending over

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60 × 100 km) for our experiment, embedded in a meander of the APF and centred at approximately 49.5°S and 02°E (Strass et al. 2005). On the 10th of February the centre of this eddy was marked with a drifting buoy and an area of 150 km^2 around the buoy was fertilized with seven tons of iron sulphate solution ($FeSO_4$). The first indication of algal response to iron enrichment was a small but detectable increase in the photosynthetic efficiency (F_v/F_m) after about 24 h (Röttgers et al. 2005). On board measurements of in situ phytoplankton F_v/F_m and later measurements of the partial pressure of CO_2 (pCO_2) served as markers of the fertilized patch. Sampling was carried out within the eddy, inside and outside the fertilized patch throughout the duration of the experiment (38 days).

Egg production experiments

Net samples from the upper 50 m of the water column were taken with Bongo nets (100 µm mesh size) around dusk. Net catches were diluted in 20 l of surface water. Sub-samples were transferred to a Petri dish and sorted under a binocular. For the egg production experiments, healthy *R. gigas* females were handpicked individually and transferred to 100 ml beakers filled with filtered seawater. The females were incubated individually at in situ temperature in the dark. Depending on how many females were present in the net haul, 12–36 parallel incubations were set up (Table 1). After 24 h, the females were removed from the beakers and eggs in the beakers were counted immediately under the binocular. For each station the average egg production rate was derived using data from all incubations, including those where females did not spawn.

Carbon mass (CM) analyses

Rhincalanus gigas females for carbon analysis were handpicked from additional Bongo net catches (100 µm mesh size; 50–0 m). Females were kept in filtered seawater for 24 h before the carapace length was measured under a binocular. Subsequently, animals were transferred individually onto pre-combusted GFF-filters and stored at −80°C. Back on land, samples were dried overnight at 60°C and analysed for carbon and nitrogen on a C/N-Analyser (Carlo Erba NA-1500).

Maturation of the gonads

Zooplankton was sampled using a Mulitnet (100 µm mesh size) at five depth intervals: 400–160, 160–100, 100–50, 50–25 and 25–0 m, respectively. The samples were preserved in buffered formaldehyde (4% final concentration) and stored at 4°C until further analysis. Between 40 and 60 *R. gigas* females from selected stations were examined to determine the stage of the ovarian development. Stations were selected to give good temporal coverage, irrespective of the time of the day at capture. Gonad maturation stages can be established from whole preserved specimens without staining (Niehoff and Runge 2003) in copepods with transparent carapaces. This is the case for female *R. gigas*. Therefore, gonads of whole, unstained animals were studied under a dissecting microscope and five developmental stages (GS) were distinguished (Table 2; modified after Niehoff and Hirche 1996; Niehoff and Runge 2003).

Results

Before fertilization near-surface (8 m depth) chlorophyll *a* distribution in the eddy was patchy with concentrations ranging from 0.5 up to 1.2 µg chlorophyll *a* l^{-1}. During the course of the experiment samples outside the fertilized patch showed high variability with chlorophyll *a* values ranging from 0.3 µg l^{-1} up to about 1 µg l^{-1}. Inside the fertilized patch, chlorophyll *a* concentrations increased and maximum integrated values (0–100 m depth) were found around day 30. Because of the patchy chlorophyll *a* distribution within the eddy, clear differences between in-patch and out-patch values were not observed until about day 10 after fertilization when in-patch chlorophyll *a* concentrations exceeded those found outside the fertilized patch. Throughout the experiment the phytoplankton assemblage was dominated by chain-forming (*Chaetoceros* spp., *Fragilariopsis kerguelensis*, *Pseudo-nitzschia* spp.) and large single

Table 1 Mean (± SE) egg production rates (EPR) of *Rhincalanus gigas* inside and outside the fertilized patch (out). Station numbers are given with corresponding average chlorophyll *a* concentrations over the upper 60 m of the water column (mg m^{-3}). *n* number of incubated females

Station	*t* (day after fertilization)	Chl *a*	*n*	Mean EPR ± SE
424	0	0.76	20	0
508	10	1.7	36	23.7 ± 3.6
513	15	1.97	12	28 ± 8.1
514 out	17	0.61	12	0
544	23	2.45	20	22 ± 5.4
560	29	2.7	18	49.8 ± 10
561 out	29	1.02	20	0.3 ± 0.3
579	32	1.65	20	15.4 ± 4.3
593	36	2.05	15	8 ± 4.8

Table 2 Classification of the gonad developmental stage (GS) based on macroscopic criteria, modified for *Rhincalanus gigas* after Niehoff and Hirche (1996) and Niehoff and Runge (2003)

GS 1	Oocytes present in the ovary; oviduct empty or only with single, transparent, small oocytes
GS 2	Transparent oocytes in the oviduct in one or maximal two layers
GS 3	Transparent oocytes in the oviduct in several layers; all oocytes similar in size; no nucleus visible
GS 3.5	Oocytes in the oviduct in several layers, all similar in size; ventral row with visible nucleus, but still transparent
GS 4	Several rows of oocytes in the oviduct; oocytes increase in size in ventral direction, ventral row is larger, darker and with visible nucleus

celled diatoms (*Thalassiothrix antarctica*, *Corethron inerme*, *Proboscia* spp., *Rhizosolenia* spp.; P. Assmy, personal communication).

Temperature profiles taken inside and outside the fertilized patch over the duration of the experiment indicated that the mixed layer often extended down to 100 m, temperatures between 3.5 and 4.5°C were stable within the nearly closed eddy circulation (V. Strass, personal communication).

Egg production and CM analyses

Figure 1 shows the egg production rates of *R. gigas* over the course of the experiment. First measurements were conducted at the time of iron release and represent conditions before fertilization. *R. gigas* did not produce eggs at this time. Egg production and the proportion of egg producing females increased significantly inside the fertilized patch with increasing chlorophyll *a* concentrations (Table 1). At day 29 after fertilization an average of 50 eggs female^{-1} day^{-1} were produced, with about 90% of the incubated females producing eggs. The highest individual egg production rate (153 eggs female^{-1} day^{-1}) was also observed at day 29 after fertilization. Outside the fertilized patch, the number of egg producing females as well as egg production rates remained close to zero (Fig. 1, Table 1).

Carbon content of *R. gigas* varied considerably from 220 to 1,780 µg C female^{-1} (Table 3). The mean carbon content of the females did not change significantly over the course of the fertilization experiment (ANOVA: $F = 6.22$, $P = 0.067$), although a slight increase in carbon content was observed.

Maturation of the gonads

The proportion of females in the different gonad developmental stages (GS 1–GS 4) is shown in Fig. 2. The corresponding integrated chlorophyll *a* values for the upper 100 m of the water column are indicated. Until day 12 after fertilization, females in all GS were present in changing proportions with no significant difference between in- and out-patch stations. From day 16 on, about 90% of the *R. gigas* females were in GS 4 at all in-patch stations. In the out-patch stations the relative contribution of GS 4 females remained low (about 10%).

Discussion

Several studies have examined the relationship between reproduction of *R. gigas* and chlorophyll *a* as an indicator of food availability. Published results show high variability in the correlation between egg production rates and surface chlorophyll levels (Ward and Shreeve 1995; Ward et al. 1996; Shreeve et al. 2002). However, although chlorophyll *a* concentration may represent actual food availability it does not give information on past feeding conditions or food quality. Hence, interpretation of the correlation found in previous studies can be problematic. The EIFEX cruise presented an unique opportunity to study the complete build up of a phytoplankton bloom with the corresponding reproductive response of the copepods investigated.

Egg production in relation to food

Although maturing females require food supply for oocyte maturation and egg production, some studies have shown that temperature rather than food controls egg production rates for different species (e.g. Kiørboe et al. 1988; White and Roman 1992). Our survey of the reproductive response of copepods within an iron fertilized patch and non-fertilized water in a nearly closed eddy allows us to separate the temperature effect from that of food supply. Temperature changes were only minor during the experiment (± 1°C) and the temperature field was the same within the eddy. During the present study, females started to produce eggs within the fertilized patch and egg production rate increased concurrent with increasing chlorophyll *a* concentrations (Fig. 1a). In contrast, at the two out-patch stations where female *R. gigas* could be obtained for egg production experiments, chlorophyll *a* values and egg production remained low (Table 1, Fig. 1a). The average egg production of *R. gigas* during the course of the experiment showed a significant positive relationship with chlorophyll *a* concentrations ($r^2 = 0.714$; $P < 0.005$; Fig. 1b). Although the present study was a Lagrangian experiment, some of the *R. gigas* sampled may have just recently turned into adult females or immigrated into the patch (S. Krägefsky, unpublished data). Both factors could affect the correlation between egg production rates and chlorophyll *a*, and may lead to the relatively high variability observed between and within each station (Fig. 1b).

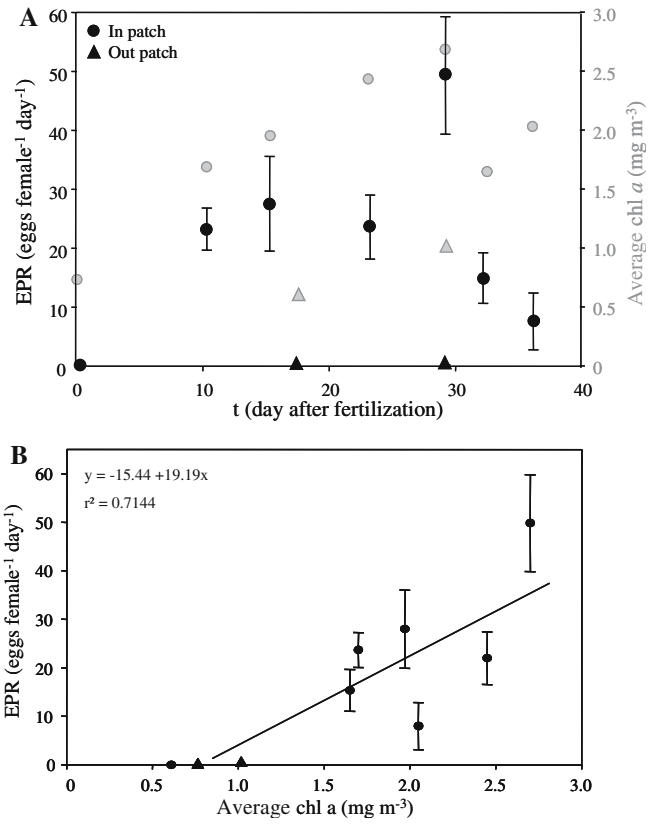

Fig. 1 Mean (± SE) egg production rates (EPR) of *Rhincalanus gigas* after iron fertilization, **a** inside the fertilized patch (*black circles*) and outside the fertilized patch (*black triangles*), with corresponding average chlorophyll *a* concentrations over the upper 60 m of the water column (*grey symbols*). **b** Mean (± SE) egg production rates (EPR) of *Rhincalanus gigas* in relation to the average chlorophyll *a* concentration (0–60 m). Regression line fitted ($P < 0.005$)

In this study we found no saturation level for egg production rate as a function of chlorophyll *a*. It is therefore possible that with even higher chlorophyll *a* the egg production rate may exceed the maximum of ~50 eggs female^{-1} day^{-1}. Indeed, the highest daily egg production rate by a single female was 153 eggs female^{-1} day^{-1}, exceeding the number of ripe eggs counted in preserved females with full oviducts. Consequently, under conditions of abundant food supply, the spawning cycle may take even less than 24 h. This has also been observed for another Antarctic copepod species, *Calanoides acutus*, during a study in Gerlache Strait (Lopez et al. 1993). Assuming a carbon content of 420 ng C per egg (Ward and Shreeve 1999) and an egg production rate of 153 eggs female^{-1} day^{-1}, this corresponds to a daily rate of 5–11% of measured body carbon for day 29 after fertilization. The high variability in the carbon content of adult females found in this study is within the range given by Shreeve et al. (2002; 288–1,791 µg C female^{-1}). Given the high variability of results and the small amount of samples analysed, no significant changes in body carbon could be found during this study.

Gonad maturation with increasing chlorophyll *a* concentration

Different gonad stages for *R. gigas* were already described and illustrated by Ommanney (1936). Similar to the increase in egg production rates, gonad maturation took place concurrent with increasing chlorophyll *a* concentrations (Fig. 2). Females in GS 4 were also found at the beginning of the fertilization experiment and at the out-patch stations which may indicate favourable past feeding conditions for these animals. Consequently, no significant differences between the stages of gonad development between in- and out-patch were found until day 12 (Fig. 2). In contrast, the out-patch station at day 17 after fertilization and all following days show significant differences in *R. gigas* gonad development compared to the in-patch stations where almost all females were found in GS 4. The fact that all other GS stages were more or less absent in the in-patch stations after day 17, show that it took at most 1 week for *R. gigas* to use the increasing food availability during the present study for the completion of the gonad maturation.

The reproductive flexibility of *R. gigas* depending on food conditions, as shown in this study, can be seen as a behavioural response to the episodic and patchy food supply in the Southern Ocean environment.

Table 3 Carbon and nitrogen analyses of *Rhincalanus gigas* females during the European iron fertilization experiment (EIFEX)

Station	*t*	Carapax length (µm)	CM (µg)	C:N	*n*
424	0	7,140 (6,720–7,600)	245 (220–267)	3.97 (3.71–4.16)	4
508	10	7,965 (6,743–10,600)	1,034 (636–1,656)	4.89 (2.67–6.79)	23
513	16	7,400 (6,560–8,080)	790 (352–1,780)	6.43 (4.2–9.46)	45
514 out	17	7,300 (6,975–7,518)	760 (519–1,083)	4.38 (3.17–5.95)	5
544	22	6,975 (6,433–7,363)	847 (657–906)	4.82 (4.25–5.73)	6
560	29	7,380 (7,200–7,500)	877 (576–1,218)	4.77 (3.62–5.62)	5
579	33	7,392 (6,975–7,750)	1,102 (899–1,399)	5.13 (4.56–5.73)	8

Mean values of the carapace length, carbon mass (CM) and carbon to nitrogen mass ratio (C:N) are shown, with their range in parentheses
n number of analysed individuals, *t* number of days after the first iron addition

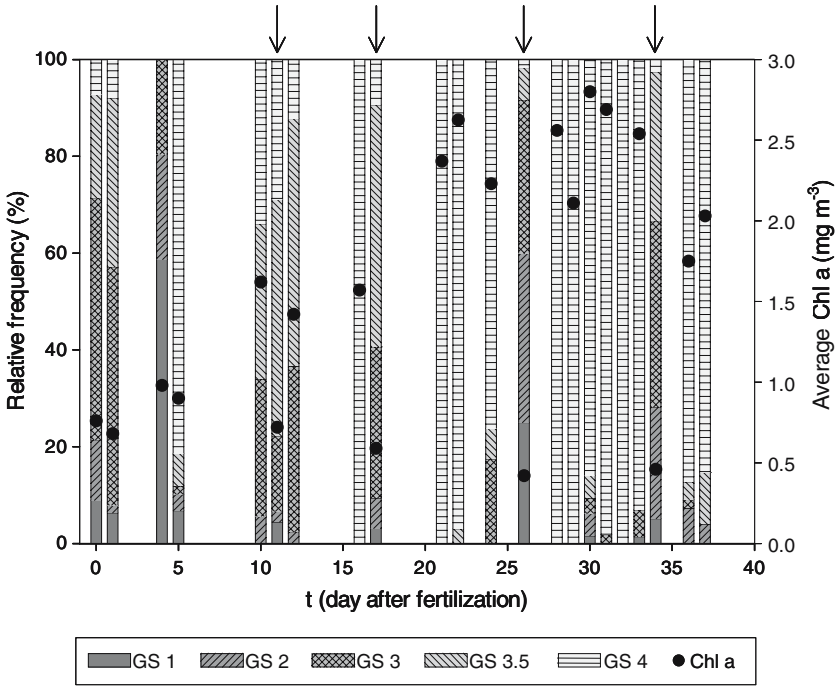

Fig. 2 Percent composition of *Rhincalanus gigas* female population gonad development stages (GS) from multinet samples with corresponding average chlorophyll *a* concentrations over the upper 100 m of the water column (*black dots*). *Arrows* mark the out-patch stations

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References

Atkinson A (1991) Life cycles of *Calanoides acutus*, *Calanus simillimus* and *Rhincalanus gigas* (Copepoda: Calanoida) within the Scotia Sea. Mar Biol 109:79–91

Boyd PW, Watson AJ, Law CS, Abraham ER, Trull T, Murdoch R, Bakker DCE, Bowle AR, Buesseler KO, Chang H, Charette M, Croot P, Downing K, Frew R, Gall M, Hadfield M, Hall J, Harvey M, Jameson G, LaRoche J, Liddlcoat M, Ling R, Maldonado MT, McKay RM, Nodder S, Pickmere S, Pridmore R, Rintoul S, Safl K, Sutton P, Strzepek R, Tanneberger K, Turner S, Walte A, Zeldis J (2000) A mesoscale phytoplankton bloom in the polar Southern Ocean stimulated by iron fertilization. Nature 407:695–702

Coale KH, Johnson KS, Chavez FP, Buesseler KO, Barber RT, Brzezinski MA, Cochlan WP, Millero FJ, Falkowski PG, Bauer JE, Wanninkhof RH, Kudela RM, Altabet MA, Hales BE, Takahashi T, Landry MR, Bidigare RR, Wang X, Chase Z, Strutton PG, Friederich GE, Gorbunov MY, Lance VP, Hilting AK, Hiscock MR, Demarest M, Hiscock WT, Sullivan KF, Tanner SJ, Gordon RM, Hunter CN, Elrod VA, Fitzwater SE, Jones JL, Tozzi S, Koblizek M, Roberts AE, Herndon J, Brewster J, Ladizinsky N, Smith G, Cooper D, Timothy D, Brown SL, Selph KE, Sheridan CC, Twining BS, Johnson ZI (2004) Southern Ocean iron enrichment experiment: carbon cycling in high- and low-Si waters. Science 304:408–414

Gervais F, Riebesell U, Gorbunov MY (2002) Changes in primary productivity and chlorophyll a in response to iron fertilization in the Southern Polar Frontal Zone. Limnol Oceanogr 47:1324–1335

Kiørboe T, Møhlenberg F, Tiselius P (1988) Propagation of planktonic copepods: production and mortality of eggs. Hydrobiologia 167/168:219–225

Laubscher RK, Perissinotto R, McQuaid CD (1993) Phytoplankton production and biomass at frontal zones in the Atlantic Sector of the Southern Ocean. Polar Biol 13:471–481

Lopez MDG, Huntley ME, Lovette JT (1993) *Calanoides acutus* in Gerlache Strait, Antarctica. I. Distribution of late copepodite stages and reproduction during spring. Mar Ecol Prog Ser 100:153–165

Martin JH, Gordon RM, Fitzwater SE (1990) Iron in Antarctic waters. Nature 345:156–158

Niehoff B, Hirche H-J (1996) Oogenesis and gonad maturation in the copepod *Calanus finmarchicus* and the prediction of egg production from preserved samples. Polar Biol 16:601–612

Niehoff B, Runge JA (2003) A revised methodology for prediction of egg production *Calanus finmarchicus* from preserved samples. J Plankton Res 25:1581–1587

Ommanney FD (1936) *Rhincalanus gigas* (Brady) a copepod of the southern macroplankton. Discov Rep 13:277–384

Pakhomov EA, Perissinotto R, McQuaid CD, Froneman PW (2000) Zooplankton structure and grazing in the Atlantic sector of the Southern Ocean in late austral summer 1993: Part 1. Ecological zonation. Deep Sea Res I 47:1663–1686

Röttgers R, Colijn F, Dibbern M (2005) Algal physiology and biooptics. In: Smetacek V, Bathmann U, Helmke E (eds) The expedition ANTARKTIS XXI/3-4-5 of the research vessel Polarstern in 2004. Reports on Polar and Marine Research, 500, pp 82–88

Shreeve R, Ward P, Whitehouse MJ (2002) Copepod growth and development around South Georgia: relationships with temperature, food and krill. Mar Ecol Prog Ser 233:169–183

Strass V, Cisewski B, Gonzalez S, Leach H, Loquay K-D, Prandke H, Rohr H, Thomas M (2005) The physical setting of the European Iron fertilization Experiment EIFEX in the Southern Ocean. In: Smetacek V, Bathmann U, Helmke E (eds) The expedition ANTARKTIS XXI/3-4-5 of the research vessel Polarstern in 2004. Reports on Polar and Marine Research, 500, pp 15–46

Voronina NM (1998) Comparative abundance and distribution of major filter-feeders in the Antarctic pelagic zone. J Mar Syst 17:375–390

Ward P, Shreeve RS (1995) Egg production in three species of Antarctic Calanoid Copepods during austral summer. Deep Sea Res I 42:721–735

Ward P, Shreeve RS (1999) The spring mesozooplankton community at South Georgia: a comparison of shelf and oceanic sites. Polar Biol 22:289–301

Ward P, Shreeve RS, Cripps GC, Trathan PN (1996) Mesoscale distribution and population dynamics of *Rhincalanus gigas* and *Calanus simillimu*s in the Antarctic Polar Open Ocean and Polar Frontal Zone during summer. Mar Ecol Prog Ser 140:21–32

White JR, Roman MR (1992) Egg production by the calanoid copepod *Acartia tonsa* in the mesohaline Chesapeake Bay: the importance of food resources and temperature. Mar Ecol Prog Ser 86:239–249