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Effect of food composition on egg production and hatching success rate of two copepod species (*Calanoides carinatus* and *Rhincalanus nasutus*) in the Benguela upwelling system

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We have analysed the daily egg production (EPR) and hatching success rates of the calanoid copepods Calanoides carinatus and Rhincalanus nasutus as a function of nano- and microplankton concentration and composition in the northern Benguela upwelling system off Namibia. Food concentration explained 55% (R. nasutus) to 62% (C. carinatus) of the EPR variability. We found no relation between the residuals of the food concentration–EPR regression and the percentage of the different taxonomic components of the nano- and microplankton. Nor was there a relation with the proportion of the diatom Skeletonema costatum that dominated the major blooms or with the number of nano- and microplankton species. We conclude that food quality differences could not be attributed to the relative composition of microplanktonic particles of the different groups (i.e. taxonomic composition).

INTRODUCTION

The importance of food quality for copepod production is a subject that has attracted researchers' attention in recent years (e.g. Kleppel *et al.*, 1998; Koski *et al.*, 1998; Laabir *et al.*, 1999; Jones *et al.*, 2002; Koski and Klein Breteler, 2003). This research has resulted in a new understanding of the key physiological role of biomolecules, such as vitamins, amino acids and lipids (Poulet *et al.*, 1989; Kattner and Hagen, 1995; Kleppel *et al.*, 1998), and in their potentially limiting role (Anderson and Pond, 2000).

However, it is important to distinguish between quality, as described by taxa composition with respect to the presence or absence of a specific biochemical component in that taxa (hereafter 'taxonomic food quality'), from the quality of any species as affected by its nutritional status (hereafter 'stoichiometric food quality', Mitra and Flynn, 2005). Most of the studies on taxonomic food quality analyse the quality of a single taxonomic food item, and therefore the ecological implications of taxonomic quality in a system where species diversity has merited a paradox (Huisman and Weissing, 1999) are difficult to evaluate. Furthermore it is often difficult to distinguish between the effects of food quality and food quantity (Koski and Klein Breteler, 2003). In addition to the difficulty of distinguishing between food quantity and quality limitation, we have to add the potential toxicity of many phytoplankton species (cyanobacteria, dinoflagellates, diatoms) and the difficulties to differentiate between toxicity and low quality in some cases (Jónasdóttir *et al.*, 1998). Also, toxicity is often a function of poor nutrient status, and hence 'quality' becomes all the more confusing because toxicity is enhanced in species of poor (nutritional) quality.

Recent research suggests that it is at a very low or very high phytoplankton concentrations where the low diversity found might have an effect in terms of taxonomic food quality (Irigoien *et al.*, 2004). At very low

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concentrations, quantity is much more likely to be the limiting factor than quality. Therefore, it is in dense phytoplankton blooms that taxonomic food quality limitation is most likely to be clearly identified. Furthermore, upwelling systems with high food concentrations have shorter trophic chains and a lower number of links per species, making them more sensitive to extinctions or changes in the basal species composition (Dunne *et al.*, 2004).

The objectives of this study are (i) to analyse the relation between nano- and microplankton taxonomic composition and copepod egg production (EPR) in an upwelling area where few species dominate the phytoplankton and (ii) to analyse the effect of the diatom *Skeletonema costatum* on copepod EPR and hatching success rates; this diatom has recently been suggested to be toxic (Ianora *et al.*, 2004).

METHOD

Data were collected during voyage M48/5 onboard the German *RV Meteor* along cross-shelf transects in the northern Benguela upwelling region off Namibia in October 2000 (*Fig. 1*). Animals for EPR measurements were collected by slow (<0.5 m s^{-1}) and short duration (5 min) oblique tows in the upper 100 m using a 500-μm WP-2 net. *Calanoides carinat*us and *Rhincalanus nasutus* were selected for the EPR estimation because these two species shared the highest abundances (*C. carinatus*) and widest distribution (*C. carinatus* and *R. nasutus*) in the study area. Daily EPR was measured by placing, usually 1–2 females of each species in 1 L glass bottles filled with 80-μm screened seawater (to avoid contamination with eggs spawned in the sea) from the depth of maximum chlorophyll *a* concentration. Preliminary experiments showed that *C. carinatus* ceased spawning when placed in filtered seawater, therefore the incubations had to be carried out with suspended food, and measured EPR should be considered as the result from previous feeding and feeding during the incubation. In any case, the individuals were incubated in water from the station at which they were captured, and therefore the EPR value is likely to be representative for the area. About 30 min elapsed from collection of the copepods to the start of the incubation. Two to five replicates were incubated on a rotating wheel to avoid particle settling (0.2 rpm rotating speed) at ambient temperatures (14–16°C) in a temperature-controlled laboratory. Resuspension of eggs in rotating bottles may increase the egg cannibalism rates (Runge and Roff, 2000). However, the maximum EPR rates measured were comparable with maximum values obtained in the laboratory (see *Results* and *Discussion*), suggesting that cannibalism did not significantly affect the measurement. After 24 h, the condition of the females was checked, and the eggs and hatched nauplii enumerated. Experiments with dead or moribund females were discarded from further analyses.

Where eggs were spawned in sufficient numbers, they were incubated for hatching success-rate measurements. Eggs were selected randomly, and batches of 30–100 eggs were gently transferred to 60-mL tubes filled with filtered seawater (in order to evaluate only maternal effects). Eggs were incubated at ambient sea surface temperature conditions for 24–48 h. Following the incubation period, the samples were examined microscopically to determine the number of nauplii and unhatched eggs.

Water samples for species identification and carbon estimation of nano- and microplankton (>2 μm) were collected at the depth of maximum chlorophyll *a* concentration, screened through a 80-μm mesh and preserved in a 1% final concentration of Lugol's iodine solution. Subsamples (100 mL) were settled (Utermöhl technique) and counted with an inverted microscope. Phytoplankton carbon biomass was estimated from cell volume (Eppley *et al.*, 1970) and using a factor of 0.21 pg C μm^{-3} for ciliates (Ohman and Runge, 1994). Heterotrophic dinoflagellates were separated from autotrophic forms based on taxonomical considerations.

The effect of nano- and microplankton taxonomic composition on EPR was analysed using the Jónasdóttir *et al.* (Jónasdóttir *et al.*, 1998) method with the adaptation to field data proposed by Irigoien *et al.* (Irigoien *et al.*, 2000a). Briefly, it is similar to a multiple regression approach which would include as explanatory factors the amount of microplanktonic carbon and the proportion of the phytoplankton group whose effect is to be investigated. The analysis is divided in two steps, regression fitting and residuals analysis, so non-linear regressions can be used to fit the EPR–food concentration relationship. In comparison with performing a multiple regression directly on the data, this two-step approach has the advantage of not presupposing the 'shape' of a potential negative effect (which could be linear, non-linear or in steps) or that all negative effects would present the same type of relation to the percentage of the different food items. To represent this approach graphically, the residuals of the EPR versus microplankton regression are plotted against the proportion of each microplankton group considered relative to the total microplanktonic carbon. Therefore, the approach is basically the same as that of Jónasdóttir *et al.* (Jónasdóttir *et al.*, 1998), though we consider the effect of a component in the diet relative to the rest of the microplankton assemblage as reference food. The results should be interpreted in the same way: if the component considered has a negative effect, the residuals would tend to be

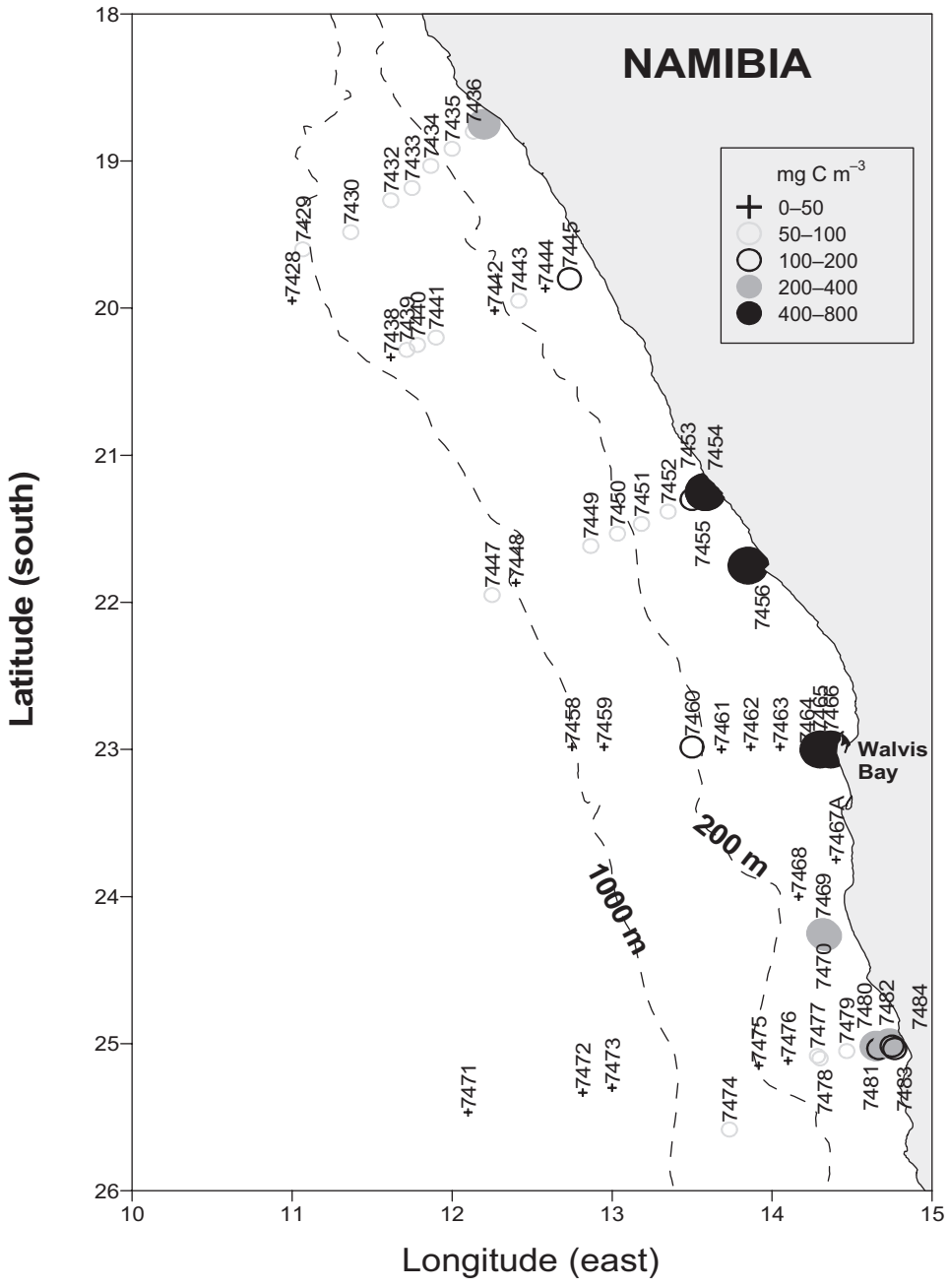

Fig. 1. Map of the study area and station positions showing the concentration of nano- and microplankton (mg C m^{-3}).

negative when this component increases proportionately in the diet.

RESULTS

There was a clear onshore–offshore gradient in phytoplankton concentration, reaching up to 800 mg C m^{-3} at coastal stations (Fig. 1). The concentrations in excess of 300 mg C m^{-3} were generally because of *S. costatum* blooms (Fig. 2), where this species always represented >80% of the total nano- and microplankton C-biomass (including ciliates and heterotrophic dinoflagellates).

Daily EPR was positively related to nano- and microplankton concentration, this significant relationship explaining 62% of the variance with *C. carinatus* and 55% with *R. nasutus* (Fig. 3). The daily EPR per individual was higher for *C. carinatus* than *R. nasutus* (Fig. 3); this difference remains the same, if EPR is expressed in

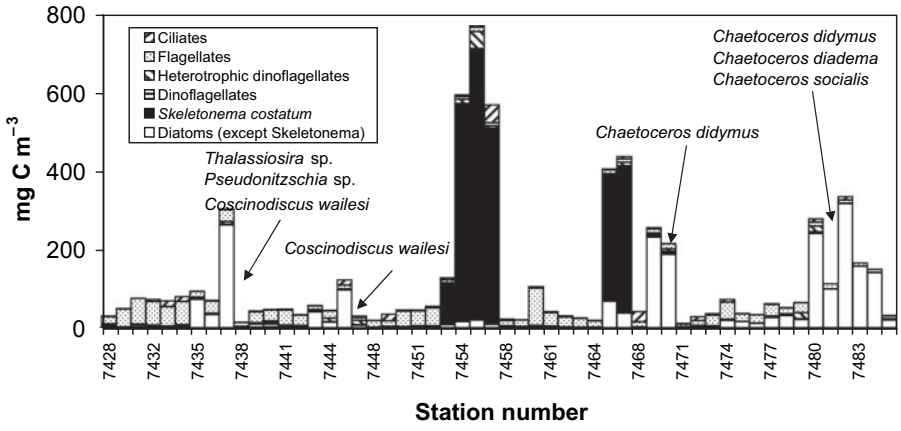

Fig. 2. Taxonomic composition of the nano- and microplankton at each of the stations sampled.

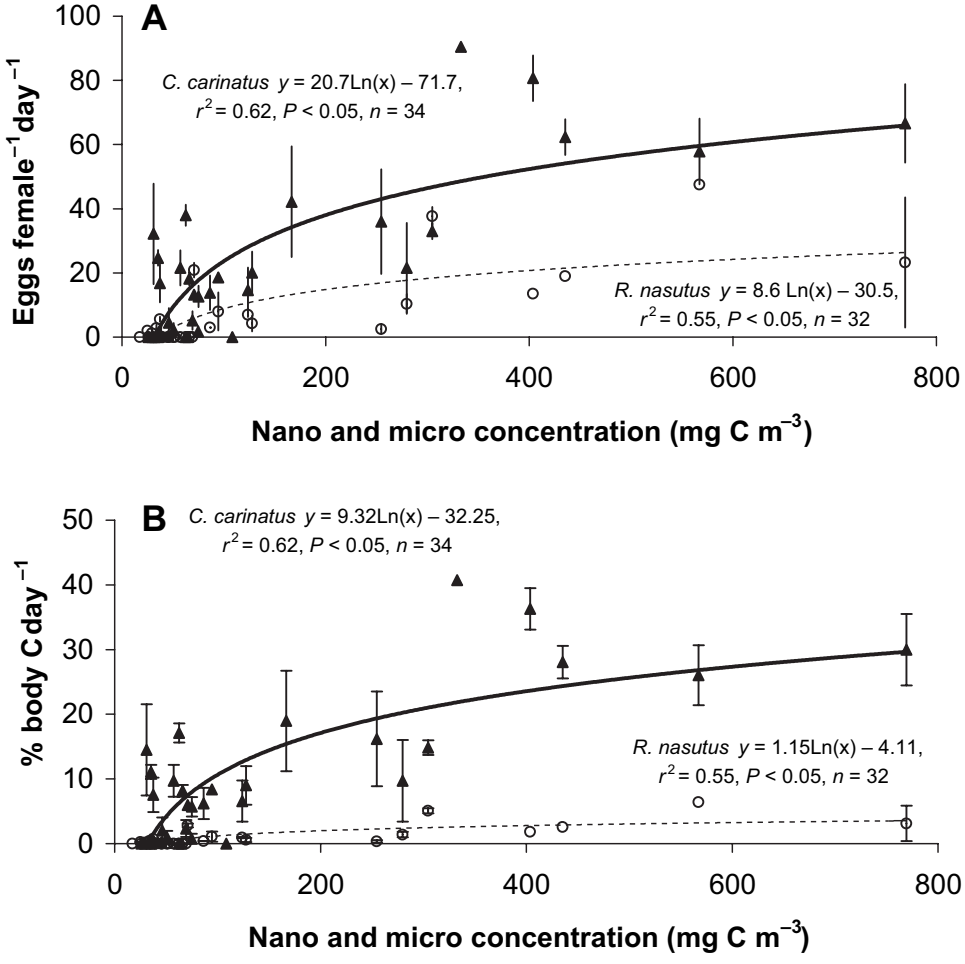

Fig. 3. (**A**) Relation between nano- and microplankton concentration (mg C m^{-3}) and daily egg production (EPR) (eggs $female^{-1}$ day^{-1}) and (**B**) relation between nano- and microplankton concentration (mg C m^{-3}) and daily EPR expressed as % of the female body carbon produced per day. Female body and egg carbon from Richardson *et al.* (Richardson *et al.*, 2001). Filled triangles and solid line for *Calanoides carinatus*, and open circles and dashed line for *Rhincalanus nasutus*. The vertical bars indicate the standard error of the average.

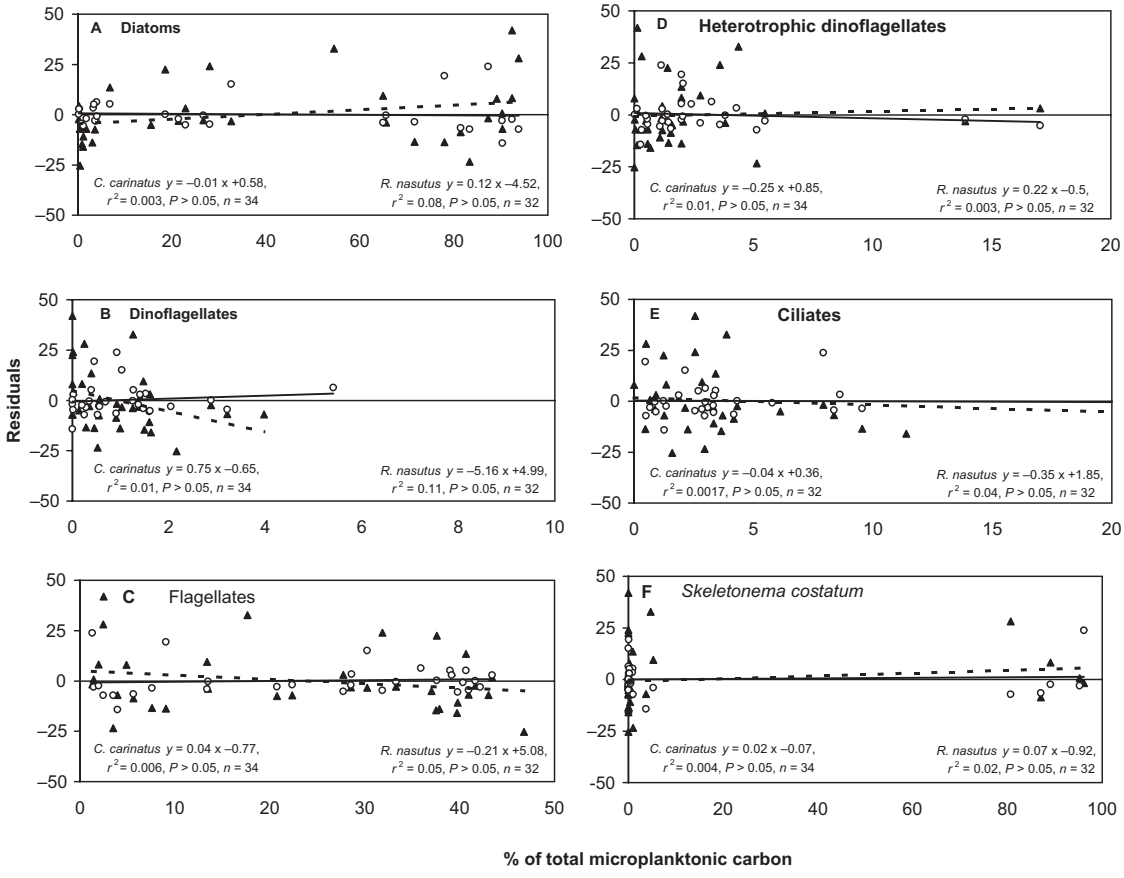

Fig. 4. Relation between the residuals of the regressions presented in Fig. 3 and the proportion (%) of different groups in the nano- and microplankon: (**A**) diatoms, (**B**) dinoflagellates, (**C**) flagellates, (**D**) heterotrophic dinoflagellates, (**E**) ciliates and (**F**) *Skeletonema costatum*. Symbols as in Fig. 3.

terms of productivity [egg carbon mass produced as a fraction of female body carbon mass using female body and egg carbon values from Richardson *et al* (Richardson *et al.*, 2001); Fig. 3B].

The residuals of the relationship of daily EPR versus nano- and microplankton concentration were neither related to the dominance of any of the major taxonomic groups (Fig. 4A–E) nor to that of *S. costatum* (Fig. 4F) or to the species richness of the nano- and microplankton assemblage (Fig. 5). Likewise, egg hatching success rates of both species were not related to either the concentration of *S. costatum* (Fig. 6A) or its proportion in the nano- and microplankton community (Fig. 6B).

DISCUSSION

Sampling along cross-shelf transects in an upwelling area afforded the opportunity to measure EPR over a wide range of natural food concentrations, which is usually restricted to laboratory experiments using simplified diets. Our results show a classical curve of EPR versus food concentration for both *C. carinatus* and *R. nasutus*. The average daily EPR rates of the two species at high food concentrations were comparable with previous laboratory and field measurements in the region (Borchers and Hutchings, 1986; Peterson *et al.*, 1990; Armstrong *et al.*, 1991; Richardson and Verheye, 1998; Richardson *et al.*, 2001), indicating that the copepods

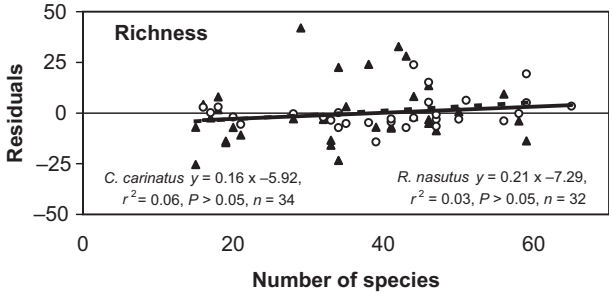

Fig. 5. Relation between the residuals of the regressions presented in Fig. 3 and the number of species in the nano- and microplankon. Symbols as in Fig. 3.

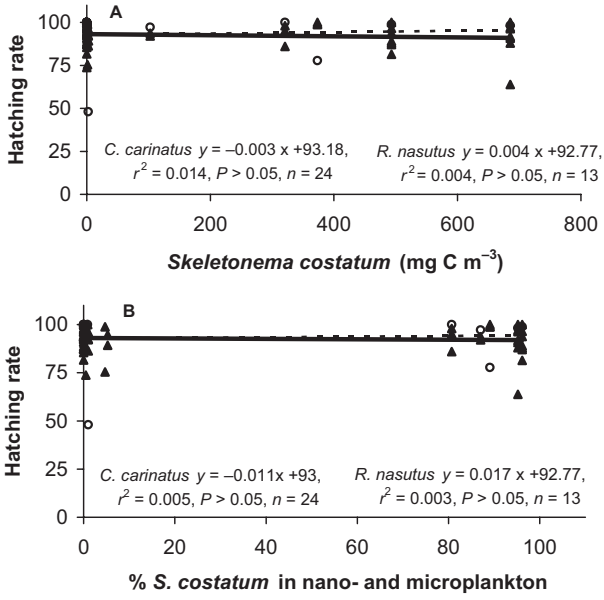

Fig. 6. Egg-hatching success rates of *Calanoides carinatus* (filled triangles) and *Rhincalanus nasutus* (open circles) in relation to (**A**) the concentration of *Skeletonema costatum* (mg C m^{-3}) and (**B**) the percentage contribution of *S. costatum* to the total nano- and microplankton carbon.

were utilizing the available resources. The difference between both species also agrees with previous results, where the EPR of *R. nasutus* was consistently lower than those of *Calanus pacificus* (Mullin, 1993). This difference was attributed to the high wax ester accumulation by *R. nasutus* making its EPR less directly coupled to the instantaneously available food supply (Ohman *et al.*, 1998).

However, we were not able to detect any effect of the food composition, even when a single phytoplankton species accounted for >80% of the total nano- and microplankton biomass (including ciliates). This result suggests that unless the dominant species has a clear deleterious effect (e.g. Irigoien *et al.*, 2000a), production limitations because of taxonomic food quality differences among the analysed groups are not important or that within group variability was larger than between-group differences. It is likely that, at high food concentrations where the bulk of the required energy is provided by the main prey species, the ability for selective feeding on other prey by copepods can compensate for a hypothetical poor quality of the main prey. Some results suggest that when one prey dominates in the environment, copepods do increase their selectivity towards other food items (Irigoien *et al.*, 2000b). This may explain the difference with freshwater planktonic herbivores, such as *Daphnia* where quality limitation has been shown (Muller-Navarra, 1995). There is also the possibility of a trophic upgrading of the food quality by microzooplankton (Klein Breteler *et al.*, 1999; Park *et al.*, 2003), although this also seems to be species dependent (Klein Breteler *et al.*, 2004).

Using a multiple-regression approach, Irigoien *et al.* (Irigoien *et al.*, 2000a) were able to detect a negative effect of the dinoflagellate *Karenia mikimotoi* (formerly *Gyrodinium aureolum*) in the field, a species previously shown to have a deleterious effect in the laboratory (Gill and Harris, 1987). However, in the present study, this approach was unable to establish that *S. costatum*, a diatom shown to have a negative effect in the laboratory (Ianora *et al.*, 2004), impacted negatively on either EPR or egg-hatching success rate. This may be because of the negative effect being not only species but also strain or growing conditions specific (Pohnert *et al.*, 2002; Orsini *et al.*, 2004; Wichard *et al.*, 2005), or simply because the negative effect in the laboratory was owing to low food quality and not toxicity, and therefore not detectable in the field (Irigoien *et al.*, 2002; Jones and Flynn, 2005). In any event, our results indicate that if laboratory results are to be extrapolated to the field and interpreted in ecological terms, the tests have to be carried out with mixed diets following the approach suggested by Jónasdóttir *et al.* (Jónasdóttir *et al.*, 1998).

Considering that at low food concentrations quantity of food is more likely to be the limiting factor, at medium concentrations taxonomic food diversity is likely to be high (Irigoien *et al.*, 2004), and at high concentrations copepod feeding selectivity may compensate for low quality, one might wonder whether taxonomic food quality can be a limiting factor for copepods in specific marine ecosystems. Nevertheless, in our results, obtained over a wide gradient of food concentrations, 40–50% of the EPR variability remains unexplained. This could be because of temperature, previous feeding history and experimental error, but also to food quality. Considering the previous arguments on phytoplankton diversity and feeding selectivity, it can also be argued that food quality issues should be addressed in terms of biochemical or stoichiometric composition of the whole nano- and microplankton community rather than in terms of taxonomic groups. Recent work by Jones and Flynn (Jones and Flynn, 2005) has shown the C : N status of a phytoplankton mixture to be more important than its taxonomic composition. Hence, depending on the growth rate and environmental conditions, a biochemical characteristic cannot be automatically attributed to a taxonomic group, and therefore quality issues cannot be addressed from taxonomic analyses alone.

Another question to pose is whether food quality can limit the production of different marine ecosystems, as has

been shown to be the case for freshwater ecosystems (Muller-Navarra *et al.*, 2004). Even assuming that selective feeding could attenuate food quality issues for copepods, one has to consider that (i) although copepods dominate marine zooplankton, not all zooplankters are selective feeders (e.g. Appendicularia and Cladocera); (ii) if the main species of prey in a bloom has a high food quality, it is likely to result in higher production; (iii) stoichiometric quality (C : N : P) is likely to have an effect (Jones and Flynn, 2005; Mitra and Flynn, 2005) and all phytoplankton would have a similar stoichiometric quality at the same time; and (iv) small differences in productivity may accumulate through the trophic chain. Probably, a useful approach to evaluate food quality issues would be a comparison among ecosystems or a seasonal study including periods when nutrients limit phytoplankton growth, and therefore the whole phytoplankton population might be nutritionally insufficient.

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