

Feeding and reproduction in a small calanoid copepod: *Acartia clausi* can compensate quality with quantity

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ABSTRACT: We analyzed the feeding, egg production rate (EPR), and the egg hatching success (EHS) of resulting eggs of adult *Acartia clausi* subject to realistic food levels ($100 \mu\text{g C l}^{-1}$) of 7 algae. Feeding was maximum (ca. $20 \text{ ml ind.}^{-1} \text{ d}^{-1}$) with *Thalassiosira weissflogii* and minimum (ca. $0 \text{ ml ind.}^{-1} \text{ d}^{-1}$) with *Dunaliella tertiolecta* and *Prymnesium parvum*. EPR was highest with *T. weissflogii*, *Tetraselmis* sp., *Rhodomonas* sp., and *Ditylum brightwellii* (21 to 26 eggs $\text{ind.}^{-1} \text{ d}^{-1}$) and moderate with *Prorocentrum minimum* (15 eggs $\text{ind.}^{-1} \text{ d}^{-1}$). EHS was highest in *P. minimum* (84%), followed by *Rhodomonas* sp. (80%), *D. brightwellii* (60%), *T. weissflogii* (52%) and *Tetraselmis* sp. (40%). Supplementary nutritional effects (higher EHS and gross growth efficiency) appeared when *A. clausi* fed on mixtures of algae with contrasting effects on EPR and EHS (*T. weissflogii* and *P. minimum*) offered as mixed suspensions, or alternating between unialgal suspensions on a 12:12 h basis. However, realized fecundity (RF) was fairly stable for most single and mixed diets (range 12.3 to 17.3 nauplii $\text{female}^{-1} \text{ d}^{-1}$), with the exception of *Tetraselmis* sp. (8.9 nauplii $\text{female}^{-1} \text{ d}^{-1}$). Such stable RF was attained by compensating low EHS with enhanced feeding and EPR, and consequently lower population growth efficiency. That represents a strategy with a high cost in terms of metabolism and predation risk.

KEY WORDS: *Acartia clausi* · Reproduction · Food quality · Diatoms · Nutrition deficiency

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INTRODUCTION

Interest in copepod–food interactions has notably increased during the last years. Early results suggesting that diatoms could induce embryo mortality (Kleppel et al. 1991, Poulet et al. 1995, Ianora et al. 1996) challenged their role in marine food webs and boosted research into the effects of food quality on copepod production and reproductive success. Field observations (Miralto et al. 1999, Miralto et al. 2003) and laboratory experiments (Chaudron et al. 1996, Kang & Poulet 2000, Turner et al. 2001) suggested that certain diatoms may indeed arrest copepod egg development. Thus, while diatoms may favour high egg production rates (EPR), the reproductive output of copepods feeding on a diatom-dominated diet would be seriously compromised by a very low egg hatching success (EHS).

However, EPR and EHS responses were highly variable among experimental studies involving different copepod–algae combinations (Ban et al. 1997). Furthermore, *in situ* dominance by diatoms in the plankton and in the diet is not necessarily associated with impairment in copepod reproductive output (Irigoiien et al. 2000, 2002). This appears to contradict results reported elsewhere (i.e. Poulet et al. 1994, Miralto et al. 2003), and the debate is still open.

Several possible causes may lay behind apparently inconsistent results. Negative effects of diatoms were typically found in experiments at unrealistically high food levels (i.e. $1000 \mu\text{g C l}^{-1}$); also, the concentration of aldehydes shown to affect embryogenesis was orders of magnitude too high compared with those copepods may experience in the field (Poulet et al. 1994, Ianora et al. 1995). Other potential drawbacks of

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experimental studies are that actual ingestion was seldom measured (i.e. Poulet et al. 1994, Chaudron et al. 1996, Ban et al. 1997), while food levels among treatments were not always comparable. Inadequacies of experimental designs do not allow the detection of whether observed EPR and/or EHS responses were due to nutritional deficiencies or to the presence of toxic compounds in the tested algae.

Feeding experiments considering mixtures of algae with known and contrasting effects on EPR and EHS are a potential tool for discriminating between nutritional deficiency and toxic effects (Jónasdóttir et al. 1998). EPR and EHS responses are measured in animals subject to carefully controlled (and non-saturating) food availability along an experimental mixing gradient between a 100% 'good' alga and a 100% 'suspect' alga. In the simplest case, if the suspect alga is nutritionally deficient, the response will fall on a reference line connecting the extremes of the gradient (i.e. the response is proportional to the amount of food ingested from each alga). If observed responses fall below the reference line, the suspect alga is toxic, while responses above the reference line evidence complementary nutritional effects (and absence of toxicity). Further details in the interpretation of possible outcomes can be found in Jónasdóttir et al. (1998). This approach has been recently employed to test toxic effects of several algal strains to *Acartia tonsa* (Colin & Dam 2002).

The design also provides information that is ecologically relevant with respect to food quality. Diversity of natural phytoplankton assemblages, the patchy distribution of food (i.e. Bjørnsen & Nielsen 1991, Castro et al. 1991) and behavioural traits of copepods (i.e. vertical migration) may add to a wide variability in food quality on a circadian scale. For small copepods the time to produce eggs is close to 24 h (Kiørboe et al. 1985, Stearns et al. 1989, Tester & Turner 1990). It is not obvious which effect temporal variability in food quality would have on EPR and EHS, although responses can be expected to integrate the feeding conditions during that period.

Here we report the response of *Acartia clausi* feeding, EPR and EHS when subject to monospecific diets of different types of algae offered at controlled and realistic food levels. None of the *A. clausi*-algae combinations reported here had been tested before in terms of their concurrent effects on feeding, EPR, and EHS. One of the algae tested, the diatom *Thalassiosira weissflogii*, has been reported to induce a lower EPR and EHS in *Acartia steueri* and *Temora longicornis*, and decreased EHS in *Calanus helgolandicus* (Ban et al. 1997). We tested the mixture of a diatom (which induced high EPR and low EHS) with a similarly sized dinoflagellate (that induced lower EPR but higher

EHS) offered either as a combined suspension or in an alternating regime between single-alga incubations. Our objectives were to assess whether negative effects observed with single-alga diets resulted from toxicity of the diatom diet or from nutritional inadequacy, and to explore the existence of nutritional complementarities between the combined algae. Given current uncertainty in relation to the proposed action mechanism of toxic compounds, we did not search for specific potentially toxic substances in the tested algae but based our analysis on the response pattern of *A. clausi* to the algal mixing experimental design (sensu Jónasdóttir et al. 1998); for instance, *T. weissflogii* would be considered toxic to *A. clausi* embryonic viability if a Type 2 response was found in the mixing diagram (Jónasdóttir et al. 1998, Colin & Dam 2002). Also, we explored the feeding selectivity of *A. clausi* when offered suspensions of 2 mixed algal types and the ability of this copepod to integrate the food intake when subject to a shifting regime on a 24 h basis.

MATERIALS AND METHODS

We made 2 types of experiments: one series where single algae were tested over several days, and a second approach where a mixture of algae was presented to the copepods either simultaneously (MIX) or by alternating (ALT).

Single-alga experiment. In the first series, we measured the feeding (as filtering rate), EPR, and EHS response of *Acartia clausi* to single-alga suspensions. Copepods were obtained from cultures maintained at 18 to 21°C and routinely fed a mixture of *Thalassiosira weissflogii* and *Rhodomonas* sp., and occasionally *Tetraselmis* sp. Between 10 and 15 recently moulted (<7 d) adult females were transferred to 625 ml bottles and placed on a rotating wheel (0.2 rpm). Algal prey were grown at 18°C in batch cultures using B1 medium + vitamins under a 12:12 h (light:dark) illumination regime provided by cool white fluorescent lamps. Algae for experiments were obtained during exponential growth phase at densities between ca. 10 and 25 $\mu\text{g C ml}^{-1}$. Residual nitrogen concentration in cultures was >900 $\mu\text{mol l}^{-1}$ (between 77 and 90% of initial levels) according to stoichiometric calculations of nutrient input to culture medium and nutrient usage by algae, assuming algal biomass composition follows an approximate Redfield ratio of 106:16:1 (carbon, nitrogen, phosphorus, respectively). Such residual nutrient levels confirmed that algae were never nutrient-limited, and that the overall procedure ensured a consistent quality within species and suitable conditions for comparison between species.

Nominal algal concentration in experimental bottles was 100 $\mu\text{g C l}^{-1}$ (observed range 101 to 109 $\mu\text{g C l}^{-1}$),

a food level below saturation for *Acartia clausi* (Gismervik & Andersen 1997, Dutz 1998). Carbon content of the algae was estimated based on biovolume measurements as equivalent spherical diameter (ESD) with an electronic particle analyzer (Elzone 5380) and equations in Mullin et al. (1966). Tested algae were the diatoms *Thalassiosira weissflogii* (TW, Bacillariophyceae, 11.6 μm ESD) and *Ditylum brightwellii* (DIT, Bacillariophyceae, 18.1 μm ESD), the autotrophic dinoflagellate *Prorocentrum minimum* (PRO, Dinophyceae, 10.4 μm ESD), and the autotrophic flagellates *Rhodomonas* sp. (RHO, Cryptophyceae; 6.9 μm ESD), *Prymnesium parvum* (PRY, Prymnesiophyceae; 5.8 μm ESD), *Tetraselmis* sp. (TETRA, Prasinophyceae; 7.2 μm ESD) and *Dunaliella tertiolecta* (DUN, Chlorophyceae; 5.5 μm ESD). All algal strains were provided by the marine algal culture centre at Göteborg University (GUMACC).

Three replicates and 3 controls (algal suspension without copepods) were incubated for each algal treatment. Response variables were measured at 24 h intervals, and incubations lasted for 3 to 4 d. Every day, incubation bottles were checked for dead animals, which were recorded and picked out.

Algae-mixture experiment. These were designed to provide the copepods with a mixture of 2 algae, either mixed into 1 suspension or kept for 12 h in one and 12 h in the other. Experiments were carried out in 1 l glass beakers with inserted plastic cylinders with a 100 μm mesh net in the bottom, which allowed copepods to move between food suspensions, while eggs and pellets would remain in the beakers. Three replicates and 3 control beakers (algal suspension without copepods) were considered for each algal treatment. The treatments were as follows:

(1) Single alga only (PRO or TW) provided for 12 h each, with copepods incubated in PRO from noon to midnight and in TW from midnight to noon.

(2) Mixtures of equal carbon amounts of PRO and TW, with copepods moved between freshly prepared mixtures every 12 h.

To keep the algae mixed and in suspension, beakers were gently stirred with a plunger every 6 h. Response variables were measured at 12 h intervals, and incubations lasted for 4 d.

Feeding was estimated as filtering rate by measuring the algal concentration or particle volume in the algal suspension on the Elzone (5380) particle analyser at the end of the incubations in experimental and control bottles. Since cell volumes of TW and PRO overlap, separation of algae by electronic particle analyses was not possible in mixed algal suspensions. To determine which alga was ingested in the MIX treatment, subsamples of control and experimental bottles were therefore counted under an inverted microscope. Fil-

tering rates (F , $\text{ml ind.}^{-1} \text{d}^{-1}$) were estimated following Frost (1972), and ingestion (I , cells or particle volume $\text{ind.}^{-1} \text{d}^{-1}$) was calculated as $I = F \times C$, where C is average algal concentration.

Egg production and hatching. EPR ($\text{eggs ind.}^{-1} \text{d}^{-1}$) was estimated by counting the number of eggs at the end of each incubation (single alga 24 h, mixtures 12 h but combined with the next incubation to yield a 24 h result) and transferred to plates with filtered sea water, where the number of nauplii was recorded after 24 h; EHS was expressed as a percentage of the eggs produced over the same period. Cannibalized eggs (evidenced by crumpled egg shells) were considered for EPR calculation but not for estimation of EHS. Temperature during experiments was 18.5 to 20°C.

After the experiments, copepods were measured (prosoma length, PL), and their individual carbon content estimated according to Uye (1982). Size of the animals ranged from 810 to 846 μm PL.

An estimate of gross growth efficiency (GGE_{eggs}) was obtained as the ratio of carbon produced as eggs to carbon ingested. Production was calculated by converting EPR to carbon units following Kiørboe & Sabatini (1995). We also estimated the population growth efficiency (PGE , $\mu\text{g viable egg C } \mu\text{g C ingested}^{-1}$) according to Jones et al. (2002).

Pellet production. Pellets were collected in the mixed-algae experiment by sieving the remains from a beaker through a 25 μm sieve. A subsample of the pellets was counted and measured (length and width) under a stereo microscope and volume (μm^3) estimated assuming an ellipsoid shape and volume-to-carbon conversion factor taken from González et al. (1994).

Differences in feeding, EPR and EHS among treatments were tested by 1-way ANOVA and Tukey HSD post hoc test. Homoscedasticity was verified using the Brown and Forsythe test, and variables transformed when necessary. Comparison between responses in single-alga and mixed-algae experiments were performed by ANCOVA where experimental treatment (single TW, single PRO, TW-PRO alternation and TW-PRO mix) was the categorical factor, and ingestion rate of TW was the continuous predictor (covariate). Comparison of EPR and EHS between treatments (TW-PRO alt vs TW-PRO mix) was performed using Mann-Whitney U -test.

RESULTS

Survival of animals was always high, average number of animals dead during the experiments was 3% (max. 7% during DUN experiment). Egg cannibalism was negligible, and ranged between 0 and 2.9% (average 0.5%).

Table 1. Results of 1-way ANOVA to assess differences in response variables among tested single algal diets. Response variables are filtering rate ($\text{ml ind.}^{-1} \text{d}^{-1}$), specific egg production rate (EPR, $\mu\text{g egg C } \mu\text{g body C}^{-1}$), egg hatching success (EHS, %), realized fecundity (RF, viable eggs $\text{ind.}^{-1} \text{d}^{-1}$) and population growth efficiency (PGE, $\mu\text{g viable egg C } \mu\text{g C ingested}^{-1}$). Letters indicate homogeneous groups according to Turkey HSD post-hoc test

	Filtering	EPR	EHS	GGE _{eggs}	RF	PGE
	$F_{6,71} = 30$ $p < 0.01$	$F_{6,14} = 71$ $p < 0.01$	$F_{4,10} = 25$ $p < 0.01$	$F_{4,10} = 6$ $p < 0.05$	$F_{4,10} = 6$ $p < 0.05$	$F_{4,10} = 8$ $p < 0.01$
TW	A	A	A, B	A, B	A, B	A, B
TETRA	A, B	A, B	A	A	A	A
DUN	D	D				
PRO	C	C	C	B	A, B	C
RHO	B, C	A, B	C	B	B	B, C
PRY	D	D				
DIT	A, B	B, C	B	A, B	A, B	A, B, C

Single-algae experiments

Filtering, EPR, and EHS were significantly different among algae (1-way ANOVA, Table 1). TW, TETRA, and DIT induced higher filtering rates ($>12 \text{ ml ind.}^{-1} \text{d}^{-1}$), PRO induced lower filtering rates ($4 \text{ to } 9 \text{ ml ind.}^{-1} \text{d}^{-1}$), while DUN and PRY were not fed upon (rates between 2 and 3 $\text{ml ind.}^{-1} \text{d}^{-1}$); RHO did not seem to be readily ingested during the first 24 h of incubation (filtering rate ca. $3 \text{ ml ind.}^{-1} \text{d}^{-1}$), but then became increasingly cleared from the suspension ($9 \text{ to } 15 \text{ ml ind.}^{-1} \text{d}^{-1}$) (Fig. 1).

EPR ranged between 27 (TW) and 0.8 eggs $\text{ind.}^{-1} \text{d}^{-1}$ (PRY). EPR tended to stabilize after 2 to 3 d. Exceptions were DUN and PRY, where copepods did not feed during the experiments and their EPR decayed over time. In the RHO treatment, EPR decreased first (Day 2) and increased afterwards (Days 3 and 4), reflecting the shift in feeding behaviour with a 24 h delay (Fig. 2).

EHS ranged from rather low (TETRA, 40%; TW, 52%) to moderately high values (RHO, 80%; PRO, 84%, Fig. 3). GGE_{eggs} varied widely from 0.18 (TETRA) to 0.45 (PRO). RF also differed among algae, with TETRA producing the lowest ($8.9 \text{ eggs ind.}^{-1} \text{d}^{-1}$) and RHO the highest RF ($17.3 \text{ eggs ind.}^{-1} \text{d}^{-1}$) (Table 1). PGE was affected by algal type, with highest values ($0.30 \text{ to } 0.36 \mu\text{g viable egg C } \mu\text{g C ingested}^{-1}$) for PRO and RHO, and lowest ($0.08 \text{ to } 0.17 \mu\text{g viable egg C } \mu\text{g C ingested}^{-1}$) for TETRA, TW and DIT diets.

Mixed-algae experiments

Filtering rates estimated from particle volume for 12 h incubations ranged from 16.2 to 19.9 $\text{ml ind.}^{-1} \text{d}^{-1}$ for TW and from 4.7 to 8.8 $\text{ml ind.}^{-1} \text{d}^{-1}$ for PRO, similar to those measured in the corresponding single-algae experiments ($16 \text{ to } 25 \text{ ml ind.}^{-1} \text{d}^{-1}$ in single TW, and 4 to 9 $\text{ml ind.}^{-1} \text{d}^{-1}$ in single PRO). Feeding in the mixed

suspensions ranged from 11 to 20 $\text{ml ind.}^{-1} \text{d}^{-1}$ (Fig. 4). Filtering rates did not differ between TW and the mixtures, but feeding on PRO was significantly lower (ANOVA, $F_{3,32} = 20$, $p < 0.01$). Feeding on the mixture incubated from noon to midnight was not different from feeding in the mixture incubated from midnight to noon (post hoc Tukey HSD test, $p = 0.98$, $df = 41$). Lowered feeding rates on PRO were thus only a response to the diet, not to the time of incubation.

Filtration rates in mixed-algae experiments (estimated from microscope counts measured on Day 3) for PRO was

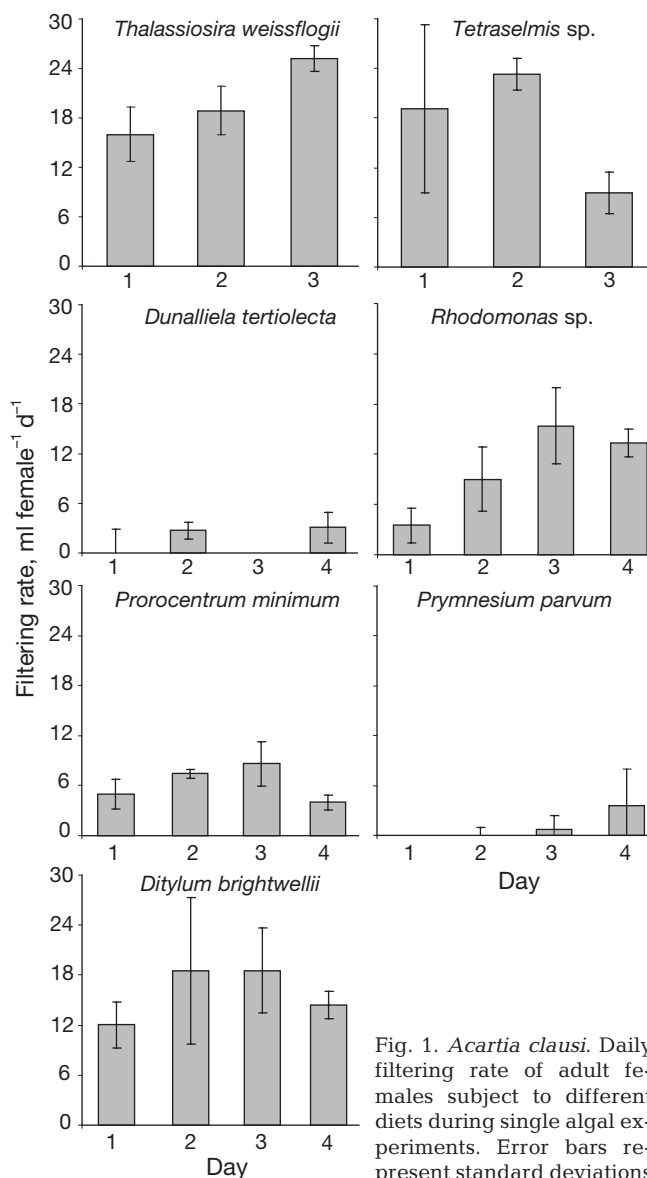


Fig. 1. *Acartia clausi*. Daily filtering rate of adult females subject to different diets during single algal experiments. Error bars represent standard deviations

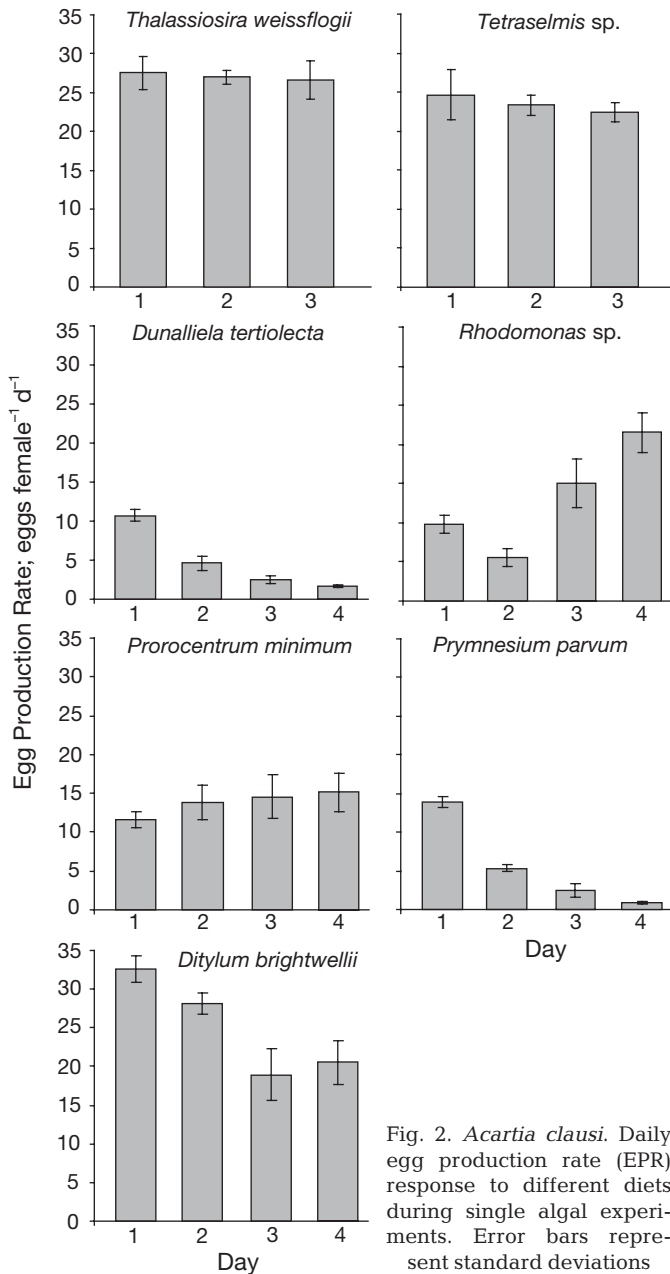


Fig. 2. *Acartia clausi*. Daily egg production rate (EPR) response to different diets during single algal experiments. Error bars represent standard deviations

7.5 ± 2.3 ml ind.⁻¹ d⁻¹ and for TW 22.6 ± 7.6 ml ind.⁻¹ d⁻¹, i.e. the copepods strongly selected TW in the mixture with PRO. Filtration rates for each algal type in the mix were thus very similar to the corresponding single-alga experiments, suggesting a consistent selection for TW irrespective of complimentary food items. Ingestion rates reflected filtering rates with highest ingestion in TW (1.7 to 2.1 $\mu\text{g C ind.}^{-1}$ d⁻¹), lowest in PRO (0.4 to 0.8 $\mu\text{g C ind.}^{-1}$ d⁻¹), and 1.1 to 2 $\mu\text{g C ind.}^{-1}$ d⁻¹ in the mix (Fig. 4).

Pellet production expressed as carbon (Fig. 4) was significantly higher when the copepods were feeding on TW and mixed diets compared to feeding on PRO (ANOVA, $F_{3,44} = 5$, $p < 0.01$).

Egg production was stable over the 4 d experiments (15 to 21 eggs ind.⁻¹ d⁻¹) and did not differ between TW-PRO consecutive treatments and mixtures (Mann-Whitney test, $U = 71$, $n = 24$, $p \gg 0.05$; Fig. 5). EPR was intermediate compared with the single-alga experiments and significant differences existed between single TW and the other 3 treatments (ANCOVA, $F_{3,8} = 51$ and $F_{1,8} = 770$, for treatment and covariate, respectively, $p < 0.01$ in both cases). EHS (Fig. 5) did not differ between TW-PRO consecutive treatments and mixtures (Mann-Whitney test, $U = 3$, $n = 6$, $p \gg 0.05$) and remained stable around 76 to 90% , similar to the single PRO experiment but significantly higher than the single TW experiment (ANCOVA, $F_{3,8} = 158$, and $F_{1,8} = 845$, for treatment and covariate, respectively, $p < 0.01$ in both cases). RF was $15.6 (\pm 2.3)$ and $12.5 (\pm 3.1)$ nauplii fem.⁻¹ d⁻¹ in alternating and mixing incubations, respectively; no significant differences existed among single TW, PRO, alternating and mixing treatments (ANOVA, $F_{3,8} = 1.4$, $p > 0.05$). PGE differed among treatments (ANOVA, $F_{3,8} = 27$, $p < 0.01$), and values for the single TW experiment (0.13 $\mu\text{g C } \mu\text{g C ingested}^{-1}$) were lower than PGE for the other 3 diets (range 0.29 to 0.39 $\mu\text{g C } \mu\text{g C ingested}^{-1}$).

DISCUSSION

None of the *Acartia clausi*-algae combinations tested in the present study had been previously reported in terms of joint ingestion, EPR and EHS responses. Results reported here indicate that feeding, EPR, EHS and GGE_{eggs} of *A. clausi* were modulated by the type of food offered. Furthermore, in several cases those processes were affected in opposite fashion by a given algae (i.e. enhanced EPR and diminished EHS).

Feeding

Filtering rates varied between moderate and high, except for the flagellates DUN and PRY, which were not ingested. Feeding effort measured as filtration rate was very different in the PRO and TW treatments (Fig. 1). *Acartia clausi* filtered TW at 20 ml ind.⁻¹ d⁻¹ but PRO was only filtered at 6.3 ml ind.⁻¹ d⁻¹, despite their similar sizes. Interestingly, there was a consistent difference also in the mixed-algae experiment, 22.6 and 7.5 ml ind.⁻¹ d⁻¹ for TW and PRO, respectively. Both species are captured through filtration, and the result implies that the retention is still different between the 2 similarly sized species. The dinoflagellate *Prorocentrum minimum* is laterally compressed, which means that the effective size may be smaller than the ESD. The diatom *Thalassiosira weissflogii*, on the other

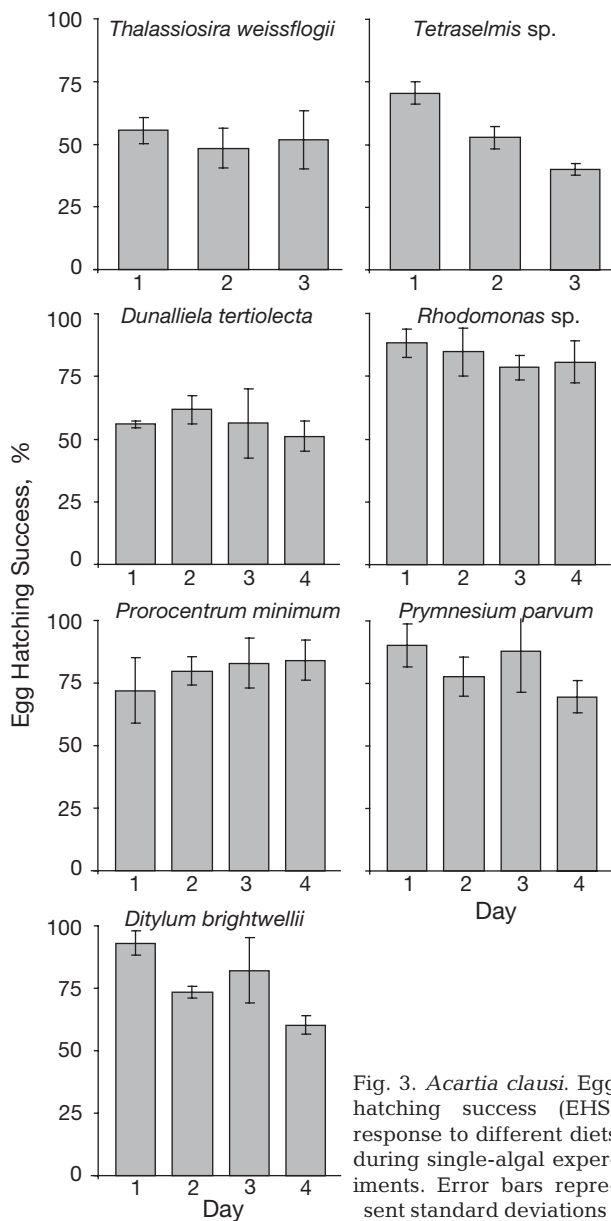


Fig. 3. *Acartia clausi*. Egg hatching success (EHS) response to different diets during single-algal experiments. Error bars represent standard deviations

hand, have small spines that may increase the apparent size without changing the ESD as measured on the particle counter. However, whether the preference for diatoms displayed by *A. clausi* in our experiments is a consequence of capture mechanisms or a deliberate choice by the animal remains an open question.

Egg production and production efficiency

TW, TETRA, RHO, and DIT induced high EPR. Observed EPR were in the range reported for *Acartia clausi* (Dutz 1998, Mauchline 1998), and are consistent with studies that report high EPR when copepods are subject to experimental diatom diets (*Calanus helgo-*

landicus–Thalassiosira rotula, Chaudron et al. 1996; *Temora stylifera–T. rotula*, Turner et al. 2001) and to a RHO diet (*Acartia tonsa–Rhodomonas baltica*, Dutz 1998). EPR correlates to specific polyunsaturated fatty acids (PUFAs) present in the seston (Jónasdóttir et al. 1995, Müller-Navarra et al. 2003), and in the ingested food (Jónasdóttir 1994). PUFAs were reported to be present in high levels in actively growing TW and *Rhodomonas lens* (Jónasdóttir 1994).

Copepods fed PRO developed significantly lower EPR, which differs from Colin & Dam (2002), who found no differences in EPR by *Acartia tonsa* when fed either *Thalassiosira rotula* or *Prorocentrum minimum*. In turn, the response of *A. clausi* in our experiments was opposite to the findings of decreased EPR by *Calanus helgolandicus* (Poulet et al. 1994, Kang & Poulet 2000), and *A. clausi* fed *T. rotula* (Ban et al. 1997). However, decreased EPR in *A. clausi* fed *T. rotula* cannot be unambiguously attributed to diatom diets given quite similar EPR reductions in the controls and the lack of data regarding food ingestion during experiments.

GGE_{eggs} for *Acartia clausi* (18 to 45%) was generally in the range of previous estimates for *A. tonsa* (e.g. Kjørboe et al. 1985, Berggreen et al. 1988), but lower than the high efficiencies reported for *A. clausi* by Saiz et al. (1992). The latter found GGE under non-turbulent condition ranging from 61 to 70%. However, egg cannibalism was high (up to 40%), and if the re-ingestion had been accounted for, their GGEs may have been lower. PRO and RHO were the diets that rendered highest GGE_{eggs} . These results indicate that, while PRO was the alga that induced the lowest EPR, it was the one that most efficiently converted ingestion into egg carbon ($GGE = 45\%$). The preferred food, TW, only produced eggs with a $GGE_{eggs} = 26\%$.

Egg-hatching success

Results support the idea that monocultures of different types of algae reduce the viability of eggs, in this case the green flagellate TETRA, and the diatoms TW and DIT to a lesser extent. This result is partially in accordance with earlier reports of enhanced embryonic mortality when females are subject to diatom diets, and higher embryonic viability with *Prorocentrum minimum* as food (i.e. Turner et al. 2001). For instance, Ban et al. (1997) found *Acartia clausi* EHS to diminish to 38% (*Skeletonema costatum*), 17% (*Phaeodactylum tricorutum*), and 25% (*Thalassiosira rotula*), but to remain stable when fed *Cylindrotheca closterium*. However, embryonic mortality in our experiments was never as high as that reported elsewhere for other copepod species (i.e. Poulet et al. 1994,

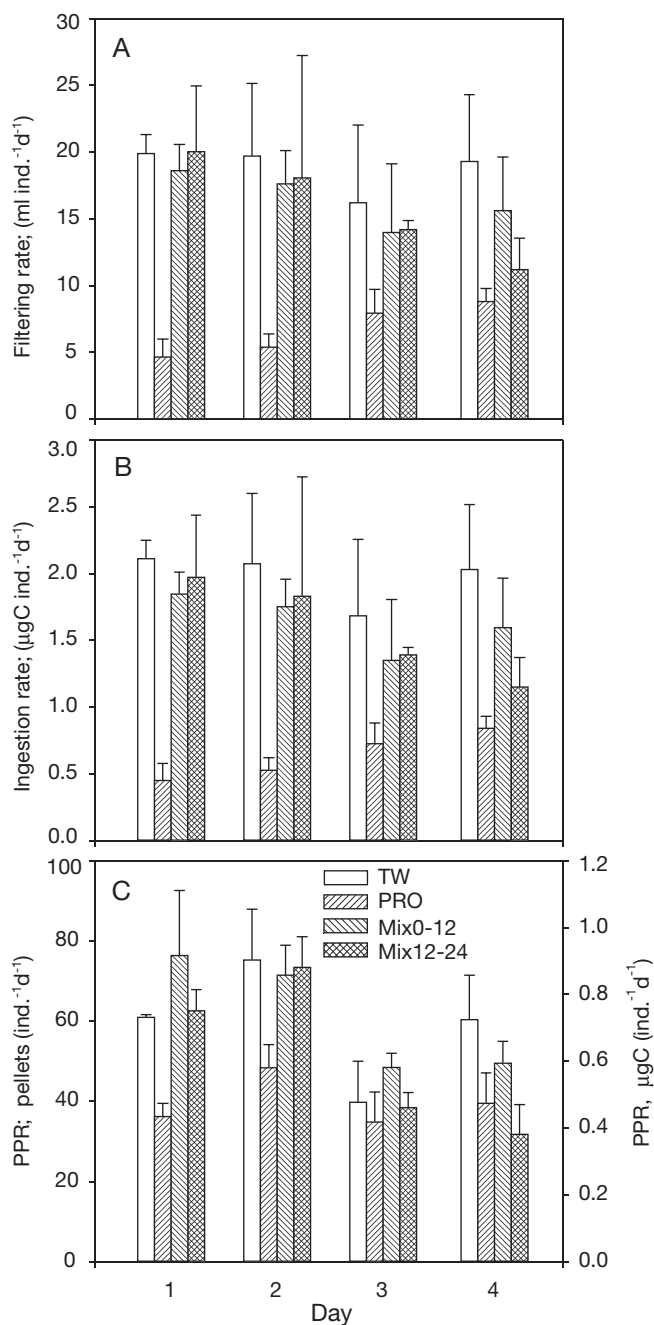


Fig. 4. *Acartia clausi*. Results from 12 h incubations in mixed-algae experiments. (A) Filtering rates; (B) ingestion rates; (C) pellet production rates d⁻¹. TW = *Thalassiosira weissflogii*; PRO = *Prorocentrum minimum*; Mix 0–12 = mixed suspension of 50%–50% of *T. weissflogii* and *P. minimum* in terms of carbon – incubation from midnight to midday; Mix 12–24 = mixed suspension of 50%–50% of *T. weissflogii* and *P. minimum* in terms of carbon—incubation from midday to midnight. Total food concentration was 100 µg C l⁻¹ in all treatments. Error bars represent standard deviations

Kang & Poulet 2000, Turner et al. 2001). Interestingly, our results show that not only diatoms may reduce EHS, but also the flagellate *Tetraselmis* sp.

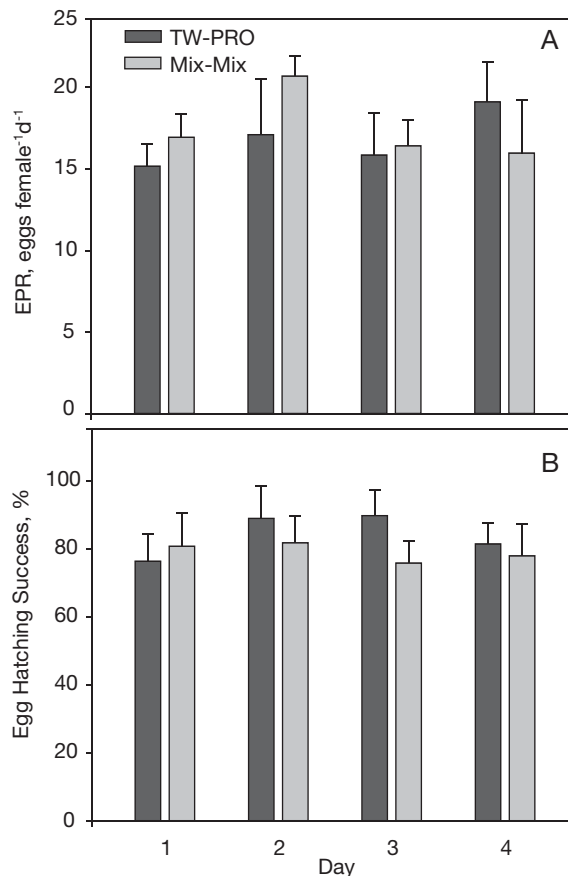


Fig. 5. *Acartia clausi*. Results from 24 h incubations in algal mixing experiments. (A) Egg production rate; (B) egg hatching success (% of eggs laid). TW-PRO = shifting incubations between single *Thalassiosira weissflogii* and *Prorocentrum minimum* algal suspensions; Mix-Mix = shifting incubations between 50% *T. weissflogii* and 50% *P. minimum* algal suspensions. Shift between algal suspensions were made on a 12 h basis. Total food concentration was set to 100 µg C l⁻¹ in all suspensions. Error bars represent standard deviations

There is an on-going dispute over negative effects of diatoms. Hatching success *in situ* is generally high (Irigoiien et al. 2002), but exceptions are found (e.g. Miralto et al. 2003). The low hatching success reported by the latter may be due to negative effects of a large temperature difference between EPR and EHS incubations (12 to 20°C). When *in situ* temperatures were used for both EPR and EHS, low EHS was rare (Irigoiien et al. 2002). Results presented here do not support the contention that embryo development was affected algal toxicity of TW diets. In experiments where copepods were subject to mixed diets with differing effects on EHS (Fig. 5), TW and PRO produced complementary nutritional effects for egg hatchability: mixed diets fall above the reference line connecting 100% TW and 100% PRO diets (Jónasdóttir et al. 1998, Colin & Dam 2002).

Integrating the food intake

Acartia clausi was able to integrate the food intake over 24 h as evidenced by the ALT treatment, which produced EPR, EHS and GGE_{eggs} similar to the MIX or PRO treatment, but different from the single TW treatment. Furthermore, it seems that even a low quantity of PRO allowed higher EHS than the single TW treatment. In turn, TW favoured higher EPR, but when scaled to the amount of food ingested (GGE_{eggs}), it becomes clear that the outcome is less efficient in relation to PRO and mixed diets. So, *A. clausi* also benefited from mixed diets in terms of production (Fig. 6), with significantly higher GGE_{eggs} compared to the single TW treatment. These results indicate that TW is nutritionally sub-optimal, while still promoting significantly higher ingestion rates.

Overall, results showed that none of the algae tested could be considered optimal: PRO yielded highest EHS and GGE_{eggs} , but filtering rates on PRO were rather low and EPR response to PRO was lower than average; TW promoted high feeding and EPR, but it was rather sub-optimal for egg viability. Thus, *Acartia clausi* seems to produce a relatively constant number of viable eggs irrespective of the type of food ingested. The most efficient production occurred with PRO as the single food, as indicated by a GGE_{eggs} of 45%. Alternating PRO and TW did not increase EPR (compared to a single TW treatment) or GGE_{eggs} (compared to a single PRO treatment) and apparently the copepods are able to integrate different feeding intervals over 24 h. In the MIX treatment, ingestion increased slightly due to a selection for TW over PRO, and this was reflected in a slightly decreased GGE_{eggs} while RF

remained constant. The single TW diet caused a significantly higher ingestion accompanied by a drastic decrease in EHS and GGE_{eggs} , and RF remained constant. Thus, it is clear that *A. clausi* can compensate an insufficient diet leading to a low EHS by producing more eggs. This must have strong implications for the survival of the females. Observed PGE outputs strongly suggested qualitative differences among the diverse diets tested, and the action of compensatory behaviour and physiological mechanisms to attain a reasonably balanced RF: compensating low egg survival requires increased feeding, metabolism and predation risk (Tiselius et al. 1997). This is probably not a long-lived strategy, but neither are diatom blooms. Diets in the field are highly variable, and copepod behaviour adds to variability by moving quickly between layers of food. As pointed out by Irigoien et al. (2002), the very low EHS associated with diatom food are generally laboratory findings, and very few negative effects are found in the field. Our results show that a small copepod like *Acartia clausi* is able to modify its reproductive machinery in response to the type of food ingested on a time scale of 1 to 3 d, and that choosing a 'high-quality' food or mixed diets is very beneficial.

A cautionary note should be introduced regarding the time scale of our experiments, considering the possibility of significantly delayed EHS responses reported even for small copepods (<7 d,

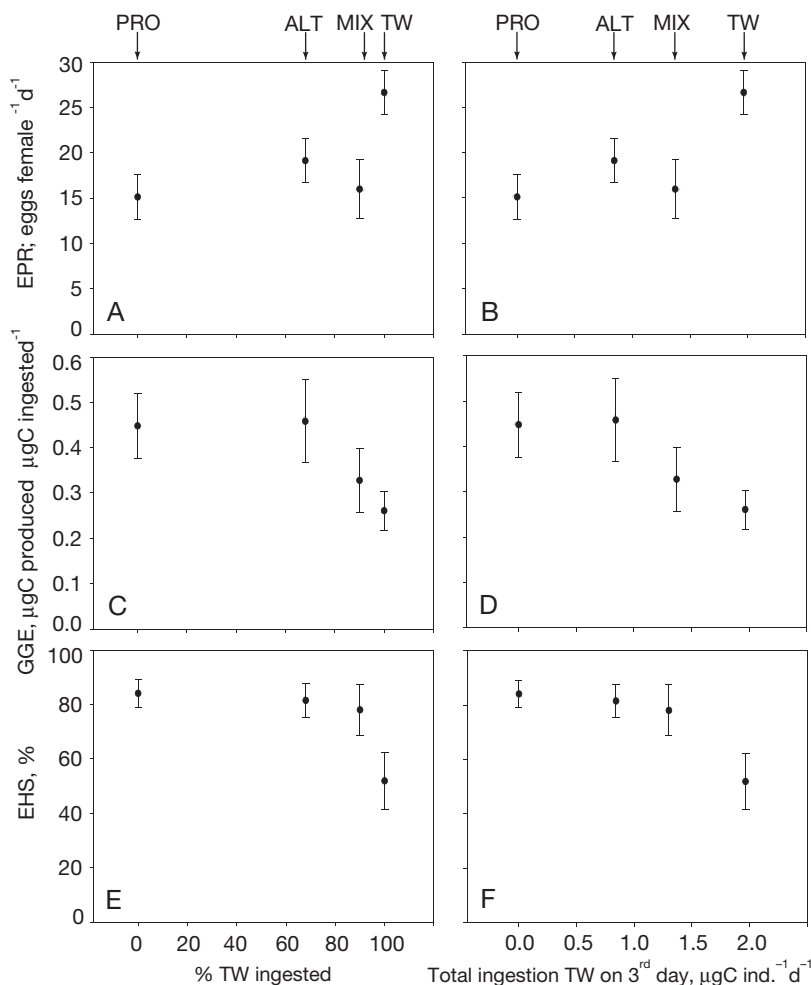


Fig. 6. *Acartia clausi*. Mixing diagrams resulting from assembling data from single-alga and mixed-algae experiments. From top to bottom: egg production rate, gross growth efficiency and egg-hatching success (% of eggs laid); to the left results are plotted vs. the percentage of *Thalassiosira weissflogii* ingested, and to the right results are plotted vs. the amount of *T. weissflogii* ingested on Day 3. Error bars represent standard deviations

Turner et al. 2001, Shin et al. 2003). If delayed responses existed in the present experiments, our EHS and RF data in single TW and mixed diets could be overestimated by an unquantified amount. However, such a case seems very unlikely here, given the rather stable responses observed in single TW and mixed-diet experiments.

In spite of lower filtration and ingestion rates for PRO, RF was as high as for other food sources with higher filtration rates. The total carbon available to *Acartia clausi* in our experiments was always $100 \mu\text{g C l}^{-1}$, and RF was relatively similar, 12.3 to 17.3 viable eggs $\text{ind.}^{-1} \text{d}^{-1}$. This points to a fairly constant transfer efficiency from available food to recruitment, where the modulating factor is the feeding behaviour. Variable filtering rate has implications for survival (Tiselius et al. 1993, 1997), and this is a strong selective force. Recruitment is, however, prioritized, since *A. clausi* modifies its feeding behaviour to produce as many eggs as possible and still keep a high viable naupliar production. The costs of producing many eggs may be of shorter longevity, but in nature the periods of superfluous food are a small fraction of the life of a copepod.

Could there be any advantage to produce more eggs with a low EHS compared to few with a high viability? Mortality of eggs is very high for broadcast spawners (Kiørboe et al. 1988) and an order of magnitude higher than for nauplii. Egg predation is higher at low food concentration (Saiz et al. 1992), which means that in situations with low food concentration, EHS must be high in order for any EPR to result in recruitment. Copepod recruitment is, however, often coupled to phytoplankton blooms (Kiørboe & Nielsen 1994), and during these periods with high food concentrations, predation on eggs may decrease. Indeed, the recruitment during blooms may in fact be caused by this decreased mortality. In our experiments, *Acartia clausi* responded to single diatom food with a high EPR, albeit with a low EHS. This may be a good strategy if egg predation is decreased, since the surviving fraction is still substantial. Most predators on copepod eggs have peak abundances during summer. The peak in predation risk for copepod eggs therefore occurs when water temperature is highest and hatching time shortest. In response to ephemeral diatom blooms in these situations, a high output of eggs with slightly lower EHS and with short hatching times may be a good strategy.

The mixed- and alternating-algae treatments in our study clearly showed that even a modest ingestion of PRO had a significant effect on EHS. It would therefore be rewarding for a copepod to supplement its diet with a non-diatom food source even in times of high EPR. The available food in the field is probably diverse also during blooms, and the capability to search for a mixture should be advantageous. The excellent sensory talents of calanoid copepods are a clear sign of this.

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