Mar Biol (2008) 154:719–734
DOI 10.1007/s00227-008-0965-4

ORIGINAL PAPER

"Good" and "bad" diatoms: development, growth and juvenile mortality of the copepod *Temora longicornis* on diatom diets

Marja Koski · Thomas Wichard · Sigrun H. Jónasdóttir

Received: 14 January 2008 / Accepted: 17 March 2008 / Published online: 1 April 2008

Abstract We measured development, growth and juvenile mortality of the common copepod *Temora longicornis* on 11 different monospecific diatom diets in order to estimate (1) how common the negative effects of diatoms are on the development of this copepod and (2) whether the arrested development is connected to deleterious polyunsaturated aldehydes (PUA) or food nutritional quality. Four diatom species (*Thalassiosira weissflogii*, *Thalassiosira rotula* CCMP1647, *Leptocylindricus danicus* CCPM469 and *Skeletonema costatum* CCMP1281) supported complete development, whereas development failed in or before metamorphosis on seven diatom species/strains (*Chaetoceros affinis* CCMP158, *C. decipiens* CCMP173, *C. socialis*, *T. rotula* CCMP1018, *Thalassiosira pseudonana* CCMP1010 and CCMP1335). However, four out of these seven species were not ingested by nauplii, either due to morphology (*Chaetoceros* spp.) or large size (*T. pseudonana* CCMP1010). The growth rate did not correlate with the ingestion rate of PUA, neither with ingestion of food mineral (nitrogen) nor with biochemical (polyunsaturated fatty acids, sterols) components. We show that, although some diatoms are of inferior food quality, this is unlikely to be connected to toxicity or due to a direct limitation by a single food nutritional compound.

Communicated by U. Sommer.

M. Koski (✉) · S. H. Jónasdóttir
National Institute of Aquatic Resources,
Technical University of Denmark, Kavalergården 6,
2920 Charlottenlund, Denmark
e-mail: mak@difres.dk

T. Wichard
Princeton Environmental Institute, Guyot Hall,
Princeton University, Princeton, NJ 08544, USA

Introduction

Despite nearly two decades of intensive research, the effect of diatoms on copepod secondary production is still an unresolved question. It seems, however, clear that (1) all or most diatom species induce reduced hatching success after some days of incubation if offered as a monospecific diet (Turner et al. 2001 and references therein; Dutz et al. 2008), (2) diatom effects on other life-history parameters such as egg production, female mortality and juvenile development are more diverse (e.g., Ban et al. 1997; Carotenuto et al. 2002) and (3) egg production and hatching success are often uncoupled and regulated by different factors (Jónasdóttir and Kiørboe 1996). Still, the questions about the mechanisms and relevance of the diatom effects remain unsolved.

Until now, several mechanisms have been suggested to be the reason behind decreased hatching success (and sometimes nauplii development) on diatom diets. Firstly, α,β, γ, δ-polyunsaturated aldehydes (PUA) have been observed to block the embryogenesis of copepod eggs and therefore to reduce the hatching success (Miralto et al. 1999). Secondly, nutritional (either biochemical or mineral) deficiencies of diatoms have been shown to induce poor food quality of pure diatom diets, which, similarly to PUA effects, reduce hatching success (Jónasdóttir and Kiørboe 1996; Jónasdóttir et al. 1998; Jones and Flynn 2005). Recent studies add a physiological explanation concerning low assimilation efficiency of essential compounds in copepod guts (Dutz et al. 2008), a depletion of polyunsaturated fatty acids (especially eicosapentaenoic acid EPA) in certain diatoms caused by the PUA production (Wichard et al. 2007) and compounds inducing oxidative stress (Fontana et al. 2007) to the list of suggested mechanisms behind the negative effects of diatoms. Since it seems, at present, likely

that neither PUA production nor direct nutritional limitations can fully explain the diatom effects (Poulet et al. 2006, 2007; Dutz et al. 2008; Wichard et al. 2008), the actual mechanism(s) remain, however, unknown.

Similarly, field studies on copepod–diatom interactions present controversial results, and the field relevance of diatom effects is under debate. While some studies observed decreased hatching success during diatom blooms (Miralto et al. 2003), other studies found no evidence of negative effects of high diatom concentrations on hatching success in nature (Irigoien et al. 2002). Studies including grazing experiments give similarly contrasting results: while ingestion of PUA-producing diatoms had a negative effect on hatching success and early nauplii survival of *Calanus pacificus* during diatom blooms (Leising et al. 2005; Pierson et al. 2005), high ingestion of diatoms coincided with high hatching success and early nauplii development of *Calanus finmarchicus* (Koski 2007). Although selective omnivorous feeding should lead to diverse (not exclusively diatoms) diets (see Kleppel 1993), some copepod species seem, however, to select for diatoms (Meyer-Harms et al. 1999; Koski 2007), which may or may not lead to harmful effects for reproduction (Pierson et al. 2005; Meyer-Harms et al. 1999, respectively). From an evolutionary perspective, for species, which have annual peak abundance during the spring diatom bloom, it should be essential to be able to feed on diatoms and to produce viable eggs. For recruitment to be successful, the hatched nauplii should in addition be able to develop on diatom-dominated diets. The evolutionary aspects of copepod–diatom interactions, such as shown for copepods developing during dinoflagellates blooms (Colin and Dam 2002a), have, however, not yet been investigated.

Until now, several laboratory studies have shown low development rates and high nauplii mortality of several copepod species on monospecific diatom diets (Poulet et al. 1995; Carotenuto et al. 2002; Ianora et al. 2004), suggesting, similarly to hatching success, that PUA production (Carotenuto et al. 2002; Ianora et al. 2004) or algae mineral (Jones and Flynn 2005) and biochemical (Klein Breteler et al. 2005) composition are the factors determining the quality of diatoms for somatic growth and development of copepods. However, several copepod species have also been successfully grown from early nauplius stages to adults with monospecific diatom diets, without any obvious negative effects (Paffenhöfer 1976; Harris and Paffenhöfer 1976; Vidal 1980a, b). The effect of diatoms on copepod nauplii seems thus to be more diverse than the effect on hatching success: sometimes contrasting results have been obtained with the same diatom species or even between different replicate experiments (Carotenuto et al. 2002).

In diatom studies, maternal diet seems to be equally important in determining the development of the next generation than the actual diet of the extant generation (Poulet et al. 1995, 2003; Ianora et al. 2004), although a pure diatom diet can also be inadequate for development irrespective of the maternal diet (Carotenuto et al. 2002). Potentially both PUA production and nutritional deficiencies could also induce maternal effects. Poulet et al. (1995, 2003) showed that ≥70% of the nauplii produced on maternal diatom diets had serious malformations leading to high mortality, and attributed the effects to PUA production. However, poor egg and nauplii quality can also be induced with low food concentration (Guisande and Harris 1995) or starvation (Poulet et al. 2003), suggesting that the maternal effects of poor nutrition and PUA production on copepod development may be similar. The reports of low juvenile growth (Carotenuto et al. 2002), arrested embryonic development (Ban et al. 1997) and malformations of nauplii (Poulet et al. 1995) on diatoms that are not listed as PUA-producers (Wichard et al. 2005a), as well as the observations of the gradually decreasing development rates with increasing nutrient limitation (Klein Breteler et al. 2005), further stress the role of other factors in determining the food quality of diatoms. Simultaneous measurements of juvenile growth rate and mortality with the algae nutritional quality and production of deleterious PUA are therefore necessary.

Studies that directly link copepod growth or development on diatom diets to either mineral or biochemical limitations are, however, rare (Jones and Flynn 2005; Klein Breteler et al. 2005), as are the studies including actual quantification of PUAs (Carotenuto et al. 2005 for cladocerans). Further, most of the studies concerning copepod development and diatoms have been done with a limited number of diatom species (namely *Thalassiosira rotula*, *Phaeodactylum tricornutum* and *Skeletonema costatum*), excluding, e.g., the genus *Chaetoceros* which is among the dominant spring bloom diatoms in, e.g., North Sea (Riebesell 1991). The present study focuses on these aspects by estimating (1) how common the negative effects of diatoms are on copepod development and nauplii mortality and (2) whether the arrested development is connected to deleterious PUAs or food nutritional quality. For this purpose we measured development, growth and juvenile mortality of a common boreal spring copepod *Temora longicornis* on 11 different monospecific diatom diets and related the growth and development rates to the ingestion of PUA, particulate organic carbon (POC), nitrogen (PON), polyunsaturated fatty acids (PUFA) and sterols. In addition, the suspect algae were tested for toxicity using a bio-assay approach of Jónasdóttir et al. (1998). We show that, although some diatoms are of inferior food quality, this is unlikely to be connected to the PUA content of the species or due to a direct limitation by a single dietary nutritional compound.

Materials and methods

Algae and copepods

Experiments were conducted using a calanoid copepod *T. longicornis*, originating from the central North Sea, but cultured in laboratory for over ten generations. Copepod cultures were kept at 14°C in dark, and fed in excess (>400 µg C l^{-1}) a mixture of *Rhodomonas* sp., *Thalassiosira weissflogii* and *Heterocapsa* sp. Nauplii for the experiments were collected from the stock culture directly at the start of the experiments, using a 140 µm net which separated early naupliar stages (NI–IV) from late naupliar stages, copepodites and adults. To additionally ensure an even quality of nauplii, a treatment with a standard diet (*Rhodomonas* sp.) was included in every series of experiments (see below).

Algae used in experiments were the cryptophyte *Rhodomonas* sp. and the diatoms *T. weissflogii* (grown at the National Institute of Aquatic Resources, Technical University of Denmark; strain unknown), *T. rotula* strains CCMP1647 and 1018, *Thalassiosira pseudonana* strains CCMP1010 and 1335, *S. costatum* CCMP1281, *Leptocylindricus danicus* CCMP469, *P. tricornutum* CCMP630, *Chaetoceros affinis* CCMP158, *Chaetoceros decipiens* CCMP173 and *Chaetoceros socialis* (originating from the Royal Netherlands Institute for Sea Research; strain unknown; Table 1). Algae were cultured in 1–2 l batch cultures with F/2 + Si medium (Guillard 1975), at 18°C, an irradiance of 100 µmol photons m^{-2} s^{-1} and 14:10 h light–dark cycle. The algae were kept in exponential growth phase by diluting 30–50% of the culture 3–4 times a week. The cell concentration in cultures was checked 3–4 times a week using a Coulter Counter (Multisizer 3; Beckman Coulter): a low algae biomass (<20 µg C ml^{-1}) was taken as an indication of an exponential growth phase. All analyses were performed on exponentially growing algae.

The volumes of the algal cells were measured 3–4 times a week by using the Coulter Counter except for species which formed chains (*Chaetoceros* spp.). For these latter, the volume was evaluated by using an inverted microscope. Carbon and nitrogen content of algae were determined by combustion in a Carlo Erba Analyser, after filtering 5–10 ml of the culture on combusted GF/F filters (2–11 replicates), and kept frozen until analysis. The replicate samples were taken at different time points before and during the experimental period in order to account for the potential variability in the cultures. For *T. pseudonana* CCMP1335, *S. costatum*, *C. decipiens*, and *C. socialis* carbon and nitrogen contents were not measured, but estimated from the average volume:carbon and volume:nitrogen regressions obtained for the other diatom species ($r = 0.90$, $n = 7$, $P < 0.01$). Despite the cell nitrogen content being a

Table 1 Strain number, volume (µm^3), carbon and nitrogen content (pg $cell^{-1}$), C:N ratio (weight), content of polyunsaturated fatty acids EPA, ARA and DHA, total PUFAs (pg $cell^{-1}$) and Δ5 sterols (pg $cell^{-1}$) and the fatty acid *ω3*:*ω6* ratio in the algae used in experiments (mean ± SD)

Algae	Strain	Volume	C	N	C:N	EPA	ARA	DHA	*ω3*: *ω6*	PUFA tot	Sterols
Rhodomonas sp. (Rh)	Unknown	202 ± 44	73 ± 22	13 ± 4.4	6.2 ± 2.0 (11)	0.3 ± 0.1	0.02 ± 0.1	0.3 ± 0.1	259	4.3 ± 2.0 (4)	0.5
T. weissflogii (Tw)	Unknown	1,099 ± 173	112 ± 16	22 ± 3.3	5.0 ± 0.2 (6)	0.4 ± 0.5	0.003 ± 0.005	0.07 ± 0.1	33	0.9 ± 1.5 (2)	0.02
T. rotula (Trt)	CCMP1647	2,199 ± 916	267 ± 35	52 ± 15	5.3 ± 0.9 (2)	0.3	0	0.12	28	0.8	1.0
T. rotula (Tr)	CCMP1018	2,287 ± 756	428 ± 8.6	97 ± 3.7	4.4 ± 0.1 (2)	1.1	0	0.43	57	4.3	0.5
T. pseudonana (Tpb)	CCMP1010	1,912 ± 582	448 ± 102	52 ± 0.4	8.6 ± 2.0 (3)						
T. pseudonana (Tps)	CCMP1335	96 ± 29	*17*	*1.6*	*11.0*						
L. danicus (Ld)	CCMP469	717 ± 267	57 ± 3.2	10 ± 0.9	5.7 ± 0.5 (5)	0.1 ± 0.09	0.4 ± 0.02	0.005 ± 0.003	2	0.4 ± 0.3 (2)	0.2
S. costatum (Sc)	CCMP1281	513 ± 432	*34*	*3.5*	*9.7*	0.01 ± 0.01	0.001 ± 0.002	0.002 ± 0.003	10	0.1 ± 0.06 (2)	0.03
C. affinis (Ca)	CCMP158	1,167 ± 78	137 ± 16	15 ± 5.2	9.2 ± 2.0 (2)	0.9 ± 0.8	0.5 ± 0.5	0.04 ± 0.02	4	2.2 ± 1.9 (3)	0.1
C. decipiens (Cd)	CCMP173	1,204 ± 440	*102*	*12.9*	*7.9*						
C. socialis (Cs)	Unknown	654 ± 30	*42*	*4.5*	*9.3*						
P. tricornutum (Ph)	CCMP630	75 ± 21	9.5 ± 3.0	0.6 ± 0.2	17 ± 5.3 (5)	0.1 ± 0.02	0.01 ± 0.005	0.01 ± 0.00	5	0.1 ± 0.04 (3)	

The number of replicate samples is given in parenthesis. Blank: missing data. Carbon and nitrogen content and C:N ratio of *Thalassiosira pseudonana* CCMP1335, *Skeletonema costatum*, *Chaetoceros decipiens* and *Chaetoceros socialis* (in italics) were estimated based on volume and volume:carbon and volume:nitrogen regressions obtained for the other diatom species (see "Materials and methods"). The abbreviations used for the algae in tables and figures are given in parenthesis; *Trt* refers to toxic *Thalassiosira rotula*, since this strain (CCMP1647) is commonly observed producing PUA (Wichard et al. 2005a), *Tps* and *Tpb* refer to small and large *T. pseudonana* strains, respectively

rather linear function of the cell volume, a high C:N ratio of some species (most notably *P. tricornutum;* Table 1) indicated nitrogen stress. It should thus be noted that, although the algae were kept in an exponential growth state, this did not necessarily exclude a possibility of a nutrient limitation.

Fatty acid and sterol analyses were conducted from 1–4 replicate samples, with a slight adjustment of standard methods (Klein Breteler et al. 1999) as described in Dutz et al. (2008). Here we considered only samples taken at different time points to be replicates; in fact each replicate measurement presented in Table 1 consisted of 2–3 replicate analysis (samples taken at the same day). Extracted FAMEs were analyzed on a GC–MS (Agilent 6890 with PTV inlet and Agilent 5973 mass selective detector) on an Agilent DB23 (60 m × 0.25 mm) column using helium as a carrier gas and subsequently, after silylation on a GC–MS (as above) on a Sil-5 (25 m × 0.32 mm) column using helium as a carrier gas. Retention times were compared to those of known FAME and sterol mixtures (from Matreya and Larodan, respectively). Sterols are presented here as the total concentration of three identified Δ5 sterols, cholesterol (Cholesta-5-en-3β-ol), brassicasterol (24-β-methylcholesta-5,22-dien-3β-ol) and campesterol (24α-methylcholesta-5-en-3β-ol). Total concentration of PUFAs represents a sum of 11 C16-C22 PUFAs. The algal volumes, carbon and nitrogen contents, C:N ratios, and concentrations of PUFAs eicosapentanoic acid (EPA; 20:5 ω3), arachidonic acid (ARA; 20:4ω6) and docosahexaenoic acid (DHA; 22:6ω3), total PUFAs and Δ5 sterols, as well as the fatty acid ω3 to ω6 ratios, are listed in Table 1.

For quantification of PUA, samples were taken once during the experiments and prepared as described in Wichard et al. (2005b), derivatized with *O*-(2,3,4,5,6-pentafluorobenzyl) hydroxylamine hydrochloride, and extracted with hexane. The potential PUA production in the algal diets was quantified by GC–MS in triplicates. Except for *T. rotula* CCMP1018, the measured PUA concentrations (or lack of PUA) were consistent with previous measurements from the same species/strains (Wichard et al. 2005a). However, *T. rotula* CCMP1018, which a few months earlier was producing only trace amounts of PUA (Dutz et al. 2008), produced elevated amounts of PUA at the time of the experiments and formed a distinguishable pattern of PUA compared to the strain *T. rotula* CCMP1647. The total PUA production of CCMP1018 seems thus to be very variable, ranging from zero to ca 6 fmol cell^{-1} (Pohnert et al. 2002; Wichard et al. 2005a). The produced amounts per cell and the distribution of various PUAs are presented in Table 2.

Experiments

Temora longicornis was grown from early naupliar stages on 11 different monospecific diatom diets (Table 1), a control diet of *Rhodomonas* sp., and 1:1, 1:3 and 3:1 mixtures (in carbon) of *Rhodomonas* sp. with *P. tricornutum* and *Rhodomonas* sp. with *T. rotula* CCMP1018. The monospecific diets were offered at a volume concentration of 2.2 (±0.9) ppm, which corresponded to ca 240 (±100) μg C l^{-1}. This concentration corresponds to an average spring bloom concentration in, e.g., the North Sea (Riebesell 1991; Kiørboe and Nielsen 1994). There were, however, two exceptions for this concentration: *Rhodomonas* sp. had a 2–3 times lower volume to carbon ratio than diatoms, resulting in a high carbon concentration of 760 (±22) μg C l^{-1} and *S. costatum* was offered to copepods in a lower concentration of 0.8 ppm (ca 50 μg C l^{-1}). The mixtures had a total food concentration of 1.2 (±0.4) ppm or 250 (±80) μg C l^{-1}.

The set up of the experiments followed the protocol described in Koski et al. (1998): a high number of nauplii (ca 1,000 l^{-1}) were placed in 1.2 l bottles filled with the aimed food suspension, the bottles were sampled three times weekly, and the sample volume was adjusted to keep the copepod biomass in the bottles constant (the removed biomass represented approximately the biomass increase due to growth). Simultaneously with the sampling, >80% of the food suspension was renewed with reverse filtration. The food concentration never decreased by more than 20% during the incubations. The number, length (total length of nauplii, prosome length of copepodites and adults ± 20 μm) and development stage of copepods in samples were analysed using a binocular microscope (at

Table 2 Concentration (fmol cell^{-1} ± SD) and profile (%) of polyunsaturated aldehydes (PUA) in the species with a detectable amount of PUA

Strain	Total PUA	C7:2	C8:2	C8:3	C10:2	C10:3
T. rotula CCMP1647	1.37 ± 0.21	43	13	17	0	27
T.rotula CCMP1018	5.97 ± 0.22	58	27	15	0	0
S. costatum	0.09 ± 0.01	21	72	7	0	0
L. danicus	0.08 ± 0.01	0	0	0	45	55

Rhodomonas sp., *Thalassiosira weissflogii*, *Thalassiosira pseudonana* (CCMP1010 and 1335), any of the *Chaetoceros* species or *Phaeodactylum tricornutum* did not produce PUA. Isomeric mixtures of (C7:2) 2,4-heptadienal, (C8:2) 2,4-octadienal, (C8:3) 2,4,7-octatrienal, (C10:2) 2,4-decadienal and (C10:3) 2,4,7-decatrienal are indicated

least 15, but typically >30 individuals per sample). The experiments were terminated either when all copepods had died, or when the first adults appeared. The experiments were conducted in four sessions during a period of ca 6 months, with each diatom experiment being repeated 1–3 times. Simultaneously with every set of a diatom experiment, a *Rhodomonas* sp. control was run (see Fig. 1a), to ensure that the quality of nauplii remained stable: if nauplii development in *Rhodomonas* sp. control was not optimal, the whole set of experiments were omitted.

Three to six replicate grazing experiments were conducted with nauplii together with each of the diatom species, *Rhodomonas* sp., and mixtures, to check that any failure in development was not due to low ingestion. At the start of the experiments, 40–60 nauplii of stages IV–VI were collected from the stock culture and placed in 330-ml

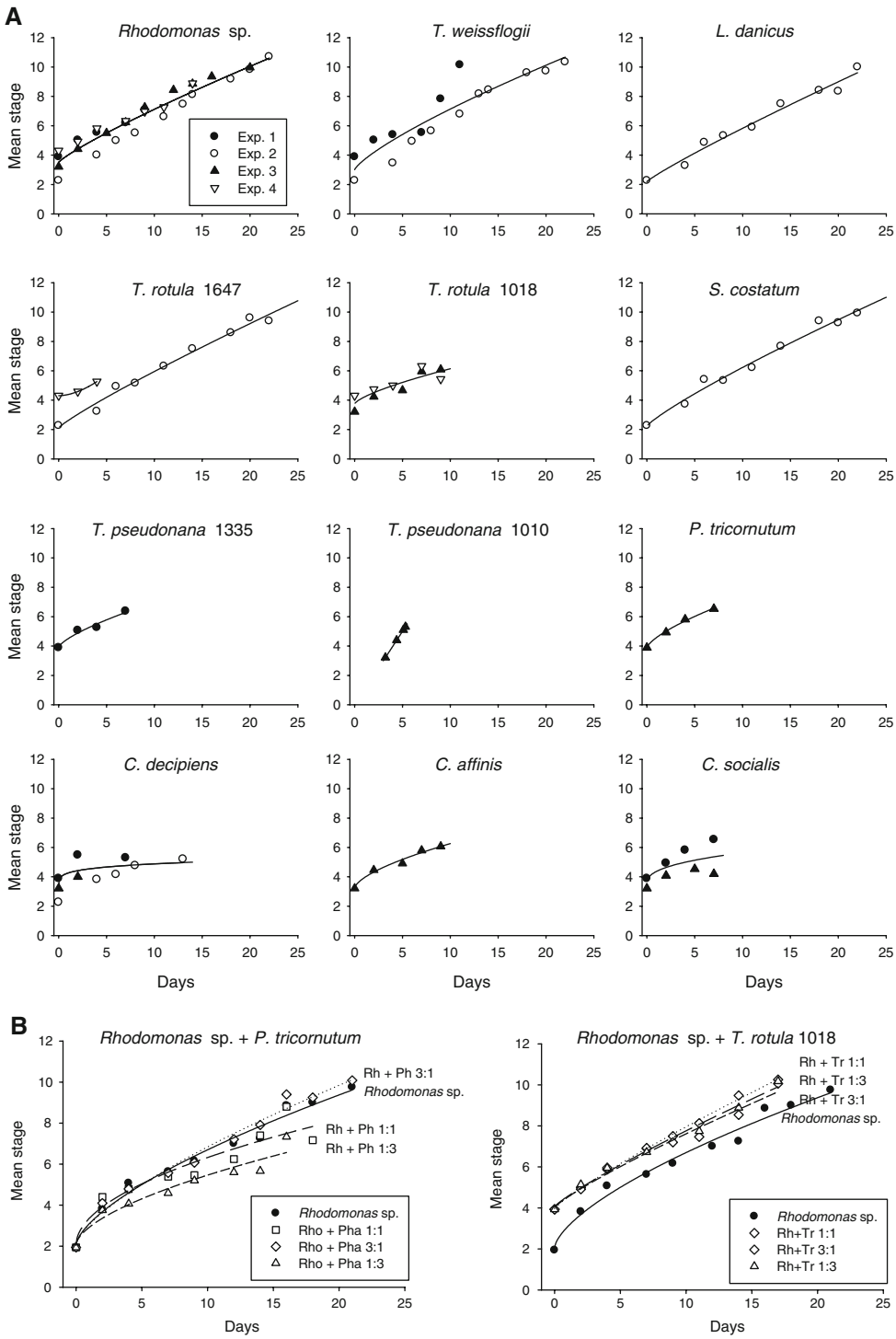

Fig. 1 *Temora longicornis*. Development on **a** monospecific diatom diets and on the control diet *Rhodomonas* sp. and **b** in 1:1, 1:3 and 3:1 mixtures of *Rhodomonas* sp. + *Phaeodactylum tricornutum* and *Rhodomonas* sp. + *Thalassiosira rotula* CCMP1018 (mean stage as a function of time). *Different symbols* indicate different experiments; in **a** the *symbols* in diatom experiments correspond to the simultaneously run *Rhodomonas* sp. controls, in **b** *open symbols* and *dashed* or *dotted lines* indicate mixtures, *closed symbols* and *solid line Rhodomonas* sp. The *line* indicates a three parameter power function. For abbreviations, see Table 1

bottles containing the aimed food suspension. After 24 h of adaptation to food, copepods were carefully transferred into a new food suspension and three replicate control bottles without animals were set up. The concentration of cells in the start suspension was counted from replicate samples using a coulter counter. After 24-h incubation in dark, cell concentration was recounted with the coulter counter, the contents of the bottles were carefully filtered onto a 30 µm net and flushed into a petri-dish, and the number, condition (dead/alive) and development stage of copepods were analysed. All experiments were conducted at 14°C, in a walk-in temperature controlled room, and the bottles were rotated at a speed of ca one round-per-minute.

Development was expressed as an increase in mean stage (calculated from the stage frequency distribution in the samples) against time. Copepod carbon content was estimated based on length measurements and length:dry weight regression of Klein Breteler et al. (1982), assuming carbon content to be 40% of the dry weight (Mullin 1969). Instantaneous growth rates were estimated assuming an exponential increase in carbon content over the course of time (e.g., Huntley and Lopez 1992). Instantaneous rates of mortality during development were calculated after correcting for sampling mortality according to Klein Breteler et al. (2004). To avoid bias due to a low number of individuals in the samples with species inducing high mortality (mean stage based only on few individuals), only samples with $\geq$15 individuals were used to estimate development and growth rate, whereas all samples were used to calculate mortality. This resulted in somewhat different numbers of observations in development/growth rate versus mortality rate (cf. Tables 3, 4; Fig. 1). Filtration and ingestion rates were calculated according to Frost (1972). To calculate weight-specific ingestion, the mean development stage measured in the end of the grazing experiments was converted to carbon, by using a mean stage:carbon (based on length) regression obtained for copepods fed *Rhodomonas* sp. ($r = 0.98$, $n = 25$, $P < 0.0001$). Gross growth efficiency was calculated by dividing the weight-specific ingestion with the weight-specific growth rate.

Growth and mortality between treatments were tested for differences using an analysis of covariance (ANCOVA), while filtration and weight-specific ingestion rates were tested using a one-way analysis of variance (ANOVA). In addition, filtration and ingestion rates were formally tested against zero (insignificant ingestion/filtration) using a one-sample *t* test. Tukey HSD post hoc test was used for pairwise comparisons. To test whether growth or mortality were related to the quantity or quality of ingested food, Spearman rank order correlation analysis was run for weight-specific growth, daily mortality, carbon and nitrogen ingestion, C:N ratio, fatty acid ω3 to ω6 ratio, and ingestion of PUA, total PUFA, linolenic acid (LIN; C18:3ω3), EPA (20:5ω3), ARA (20:4ω6), DHA (22:6ω3) and Δ5 sterols.

Table 3 *Temora longicornis*: parameters of linear model of growth, relating ln mean weight (µg C) to time (days)

Species	Slope	Intercept	*R*	*N*
(A)				
T. weissflogii	0.16 ± 0.02^{1}	−2.4	0.96	10[a]
S. costatum	0.15 ± 0.01^{1}	−2.4	0.99	9
T. rotula CCMP1647 Exp. 1	0.15 ± 0.01^{1}	−2.1	0.95	9
Rhodomonas sp.	0.14 ± 0.007^{1}	−2.0	0.97	26[a]
C. affinis	$0.13 \pm 0.01^{1,2}$	−2.1	0.98	5
T. pseudonana CCMP1010	$0.13 \pm 0.02^{1,2,3}$	−2.3	0.96	4
L. danicus	$0.12 \pm 0.01^{2,3}$	−2.3	0.98	9
P. tricornutum	0.09 ± 0.03^{3}	−2.1	0.88	5
C. decipiens	0.09 ± 0.01^{3}	−2.3	0.98	7[a]
T. rotula CCMP1018	0.07 ± 0.03	−1.8	0.68	10
T. rotula CCMP1647 Exp. 2	Ns			3
C. socialis	Ns			4[a]
(B)				
Rhodomonas sp.	0.14 ± 0.008^{1}	−1.7	0.99	10
Rh + Ph 1:1	$0.13 \pm 0.01^{1,2}$	−1.7	0.97	9
Rh + Ph 3:1	0.14 ± 0.007^{1}	−1.7	0.99	10
Rh + Ph 1:3	$0.11 \pm 0.01^{2,3}$	−1.8	0.97	8
Rh + Tr1018 1:1	0.15 ± 0.02^{1}	−1.4	0.97	8
Rh + Tr1018 3:1	0.14 ± 0.01^{1}	−1.3	0.97	8
Rh + Tr1018 1:3	0.14 ± 0.01^{1}	−1.3	1.0	7

(A) Experiments with single species diet, (B) mixtures. Slope describes the mean (±SE) weight-specific growth rate [µg C (µg C)$^{-1}$ day^{-1}]. If replicate experiments differed substantially (*Thalassiosira rotula* CCMP1647; see Fig. 1a) the growth rate was calculated separately for each experiment (Exp. 1 and 2). Only samples which contained >15 individuals were included to estimate the development (Fig. 1) and growth. The superscript numbers group slopes which were not significantly different from each other (ANCOVA; $P > 0.05$). Abbreviations as in Table 1

Ns slope not significant

[a] Length measurements on diatom diets were not conducted in the first series of experiments (see Fig. 1a) and these experiments are thus not included in the calculations of the growth rates

Results

Temora longicornis was able to complete its development on the control algae *Rhodomonas* sp. and on the diatoms *T. weissflogii*, *T. rotula* strain CCMP1647, *L. danicus*, and *S. costatum*. In contrast, development was arrested in late copepodite stages when copepods were fed *T. rotula* CCMP1018, either of the *T. pseudonana* strains, *P. tricornutum* or any of the *Chaetoceros* species (Fig. 1a). However, with *T. rotula* CCMP1647 there was a large variation between replicate experiments, with complete development

Table 4 *Temora longicornis*: parameters of linear model of mortality, relating ln no. of individuals to time (days)

Species	Slope	Intercept	*R*	*N*
(A)				
Rhodomonas sp.	Ns			33[a]
L. danicus	Ns			9
S. costatum	Ns			9
T. rotula CCMP1647 Exp. 1	Ns			9
T. weissflogii	Ns			16
C. affinis	-0.18 ± 0.04^{2}	6.9	0.92	5
C. decipiens	-0.20 ± 0.07^{2}	6.3	0.62	16[a]
P. tricornutum	-0.23 ± 0.03^{2}	6.9	0.96	7[a]
T. rotula CCMP1018	-0.24 ± 0.03^{2}	7.0	0.94	12[a]
T. pseudonana CCMP1010	-0.29 ± 0.04^{2}	7.1	0.97	5[a]
T. pseudonana CCMP1335	-0.41 ± 0.09^{2}	7.3	0.92	6[a]
T. rotula CCMP1647 Exp. 2	-0.42 ± 0.04^{2}	6.9	0.98	6[a]
C. socialis	-0.47 ± 0.06^{2}	7.3	0.95	10[a]
(B)				
Rhodomonas sp.	Ns			10
Rh + Ph 1:1	Ns			9
Rh + Ph 3:1	Ns			10
Rh + Ph 1:3	-0.17 ± 0.06^{2}	6.3	0.75	8
Rh + Tr1018 1:1	-0.04 ± 0.01^{1}	6.7	0.78	8
Rh + Tr1018 3:1	-0.07 ± 0.01^{1}	6.8	0.97	8
Rh + Tr1018 1:3	-0.04 ± 0.01^{1}	6.9	0.93	8

(A) Experiments with single species diet, (B) mixtures. Slope describes the mean (±SE) specific mortality rate (day^{-1}). If replicate experiments differed substantially (*Thalassiosira rotula* CCMP1647; see Fig. 1a) the mortality rates were calculated separately for each experiment (Exp. 1 and 2). The superscript numbers group slopes which were not significantly different from each other (ANCOVA; $P > 0.05$). Abbreviations as in Table 1

[a] All samples, irrespective of the number of individuals, were included in the calculation of mortality (see "Materials and methods"), whereas samples with <15 individuals were omitted from the estimates of development (Fig. 1) and growth (Table 3)

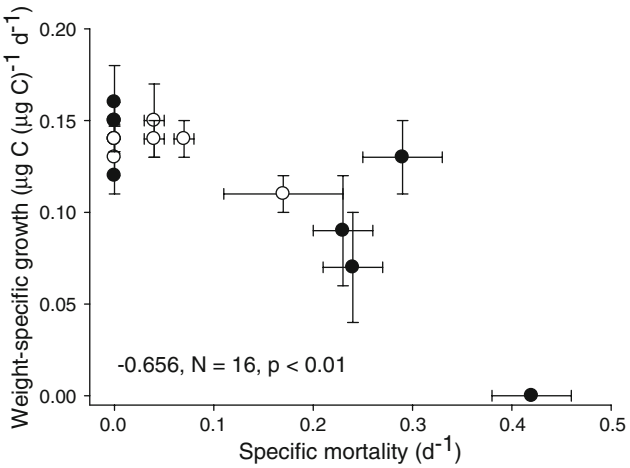

Fig. 2 *Temora longicornis*. Weight-specific growth [µg C (µg C)$^{-1}$ day^{-1}] as a function of specific mortality (day^{-1}) in development experiments (mean ± SE). *Closed symbols* indicate monospecific diets, *open symbols* mixtures. Parameters from the Spearman rank correlation are indicated in the figure; only species which were ingested by *T. longicornis* are included in the figure

in one experiment, but arrested development in the other, although the growth rate of nauplii on *Rhodomonas* sp. in simultaneous incubations was invariably high indicating a stable quality of nauplii (Fig. 1a). In food mixtures, development was faster than on pure *Rhodomonas* sp. diet when *Rhodomonas* sp. was supplied with *T. rotula* CCMP1018, but slower (1:1 and 1:3) or similar (3:1) than on pure *Rhodomonas* sp. when *Rhodomonas* sp. was supplied with *P. tricornutum* (Fig. 1b).

Growth and mortality reflected the development, with highest growth rates (14–16% body carbon day^{-1}) on *T. weissflogii*, *S. costatum*, *T. rotula* CCMP1647, *Rhodomonas* sp., all *Rhodomonas* sp. + *T. rotula* CCMP1018 mixtures and *Rhodomonas* sp. + *P. tricornutum* 3:1 mixture (slope in Table 3), and insignificant or low mortality (≤7% day^{-1}) with the same species/mixtures and *L. danicus* (slope in Table 4). Based on the analysis of covariance, the rest of the species were divided into two groups: with *T. rotula* CCMP1018, *T. pseudonana* CCMP1010, *P. tricornutum*, *C. affinis*, *C. decipiens* and *Rhodomonas* sp. + *P. tricornutum* 1:3 the growth was 7–13% body carbon day^{-1} and the mortality 17–29% day^{-1}, while with *T. pseudonana* CCMP1335 and *C. socialis* growth was insignificant and mortality 44–47% day^{-1} (Tables 3, 4). There were thus significant differences in both growth and mortality between the different species (ANCOVA; $P < 0.0001$). Further, there was a significant negative correlation between growth and mortality (Spearman rank correlation, $P < 0.01$), suggesting that with the species inducing high growth rate mortality was low (Fig. 2).

Of the species, which did not support full development, neither *T. pseudonana* CCMP1010 nor any of the *Chaetoceros* species were ingested by *T. longicornis* nauplii (Fig. 3). Most of the remaining species were filtered with a rate ranging from 0.01 to 0.04 ml ind.$^{-1}$ h^{-1} (Fig. 3a), which resulted in a weight-specific ingestion of 0.1–0.6 µg C (µg C)$^{-1}$ day^{-1} for the nauplii (Fig. 3b). Due to the significantly higher filtration rate on *S. costatum* than on any of the other food species (Tukey HSD; $P < 0.001$), there were significant differences in filtration rate between different food species (1-way ANOVA; $F_7 = 19$, $P < 0.001$), although the differences in ingestion rate were only weakly significant (ANOVA; $F_7 = 3.5$, $P < 0.05$). The high filtration rate on *S. costatum* was likely due to an accidentally low food concentration with this species (see "Materials and methods"). When *P. tricornutum* was mixed with *Rhodomonas* sp., it was never ingested, irrespective of

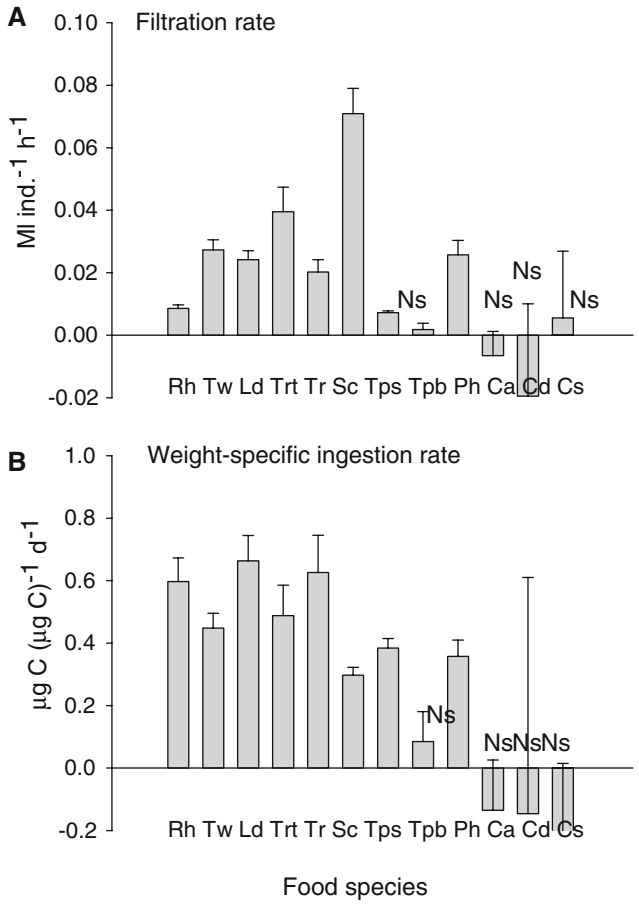

Fig. 3 *Temora longicornis* nauplii. **a** Filtration rate (ml ind.$^{-1}$ h^{-1}) and **b** weight-specific ingestion rate [µg C (µg C)$^{-1}$ day^{-1}] on monospecific diets (mean ± SE). *Ns* Not significantly different from zero. For other abbreviations, see Table 1

the food ratio. In contrast, in *Rhodomonas* sp. + *T. rotula* CCMP1018 mixture, both species contributed to ingestion of nauplii (Fig. 4). There was no correlation between the C:N or ω3: ω6 ratio of the diet, ingestion of carbon, nitrogen, PUFA (either total or LIN, ARA, EPA and DHA separately), Δ5 sterols or PUAs and either nauplii growth or mortality (Spearman; $P > 0.05$; Fig. 5).

The nauplii gross growth efficiency with species where both growth and ingestion were significant varied between 0.11 (*T. rotula* CCMP1018) and 0.49 (*T. rotula* CCMP1647). The average gross growth efficiency on diatoms was 0.30 ± 0.15, which was similar to the gross growth efficiency on *Rhodomonas* sp. (0.23). However, the carbon concentration in *Rhodomonas* sp. experiments was 2–3 times higher than with diatoms (see "Materials and methods"), which likely resulted in over-saturated feeding conditions and thus a lower gross growth efficiency. A generally higher gross growth efficiency of *Rhodomonas* sp. was also suggested by mixture experiments: in mixtures where only *Rhodomonas* sp. was consumed (*Rhodomonas* sp. + *P. tricornutum*) the nauplii gross growth efficiency was high (>0.5), whereas when both *Rhodomonas* sp. and *T. rotula* CCMP1647 contributed to the total ingestion, the gross growth efficiency was substantially lower (0.17; Table 5).

Discussion

The diatom community in the North Sea during the peak development of *T. longicornis* nauplii includes species such as *Chaetoceros* spp., *Thalassiosira* spp., *S. costatum*, *Rhizosolenia* spp., *Coscinodiscus* spp. and *Leptocylindricus* spp., and tends to consist of several species (Riebesell 1991; Maar et al. 2002), with bloom concentrations ranging from ca 140 µg C l^{-1} (Kiørboe and Nielsen 1994) up to 800 µg C l^{-1} (Halsband and Hirche 2001). Our choice of species and food concentrations thus reflected the actual spring bloom conditions.

Temora longicornis was able to complete its development on 4 out of 11 diatom species tested (*T. weissflogii*, *T. rotula* CCMP1647, *L. danicus* and *S. costatum*), whereas the other 7 species proved to be inadequate food for nauplii development (*T. rotula* CCMP1018, two strains of *T. pseudonana*, all three *Chaetoceros* sp. and *P. tricornutum*). Four of the diets, which resulted in low development and high mortality, were not ingested by *T. longicornis* nauplii (all *Chaetoceros* species, *T. pseudonana* CCMP1010). Since the *Chaetoceros* species were both spiny and chain-forming, and the remaining species *T. pseudonana* was among the largest species offered, morphology and/or size were sufficient to explain the lacking ingestion. There was no indication that diatom derived PUAs such as 2,4,7-decatrienal would function as feeding deterrents (Jüttner 2005; Leising et al. 2005), but it rather seemed that copepod nauplii were feeding on diatoms if they were morphologically capable of feeding on them.

Of the ingested species, three induced low development and high mortality (*T. rotula* CCMP1018, *T. pseudonana* CCMP1335 and *P. tricornutum*), whereas the development was completed with four species (see above). The diatom species which are reported to have deleterious effects or to induce complete development are often the same throughout the literature: e.g., *T. rotula* has been reported to have both positive (Paffenhöfer and Harris 1976; Harris and Paffenhöfer 1976) and negative (Carotenuto et al. 2002; Poulet et al. 2003) effects on juvenile development, as has *S. costatum* [respectively, Verity and Smayda (1989) and Ianora et al. (2004) for positive and negative effects; Table 6]. It seems that the only intensively studied diatom species which consistently fails to support complete development is *P. tricornutum*, while with the other more frequently used diatoms the results are not consistent

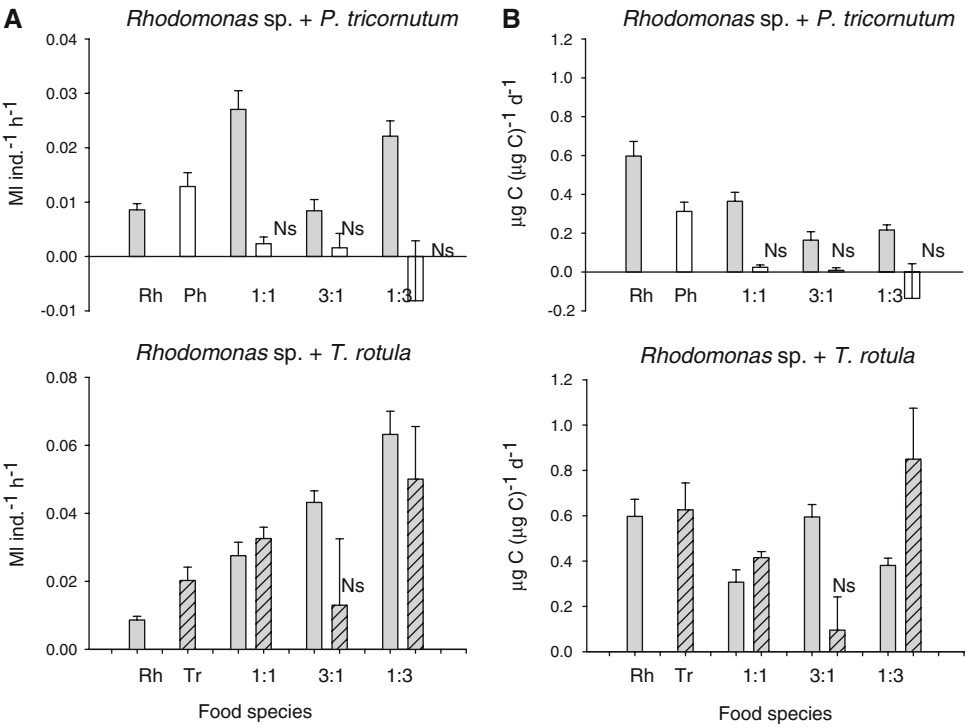

Fig. 4 *Temora longicornis* nauplii. **a** Filtration (ml ind.$^{-1}$ h^{-1}) and **b** weight-specific ingestion rates [µg C (µg C)$^{-1}$ day^{-1}] in food mixtures (mean ± SE). *Grey columns*: *Rhodomonas* sp., *open columns*: *Phaeodactylum tricornutum*, *striped columns*: *Thalassiosira rotula* CCMP1018. *Ns* Not significantly different from zero. Other abbreviations as in Table 1

(Table 6), and variation even between replicate experiments of the same series is high (Carotenuto et al. 2002, this study). However, due to, e.g., its lack of absolute silica requirements and unusual Si uptake kinetics (Del Amo and Brzezinski 1999), *P. tricornutum* neither represents an average diatom, nor is a relevant species during the spring bloom (see, e.g., Riebesell 1991). This makes it of somewhat limited interest when considering copepod–diatom interactions.

We suggest that diatom effects on juvenile development and growth can be divided into four groups similarly to Ban et al. (1997): (a) species which induce fast development (high growth) and low mortality, (b) species which induce arrested development and high mortality, (c) species with which development is completed but mortality high, and (d) species which do not support complete development but promote low mortality. Since juvenile growth and mortality generally seem to be coupled (nauplii die if they can not moult into the next stage; Lopez 1991), a response following the first two groups is most common. A few exceptions have, however, been recorded: *Calanus helgolandicus* and *T. longicornis* were able to complete their development on *S. costatum* and *T. rotula*, respectively, although the mortality was high (Ianora et al. 2004; Carotenuto et al. 2002, respectively), while the development of *T. longicornis* and *Pseudocalanus elongatus* on nutrient-limited *T. weissflogii* was significantly slowed down, without any evident effect on mortality (Klein Breteler et al. 2005; Table 6). The diatoms in the present study fall in the first two categories, with the observed negative correlation between growth and mortality suggesting a coupling between these two processes. Our results therefore show that some diatoms are inadequate food for nauplii development, whereas with some species the development can be completed. With low ingestion excluded, we considered (1) diatom PUA production, (2) presence of other diatom toxins, (3) nutritional quality and (4) nutritional limitation following PUA production as possible reasons behind the observed response of copepod nauplii on diatoms diets.

Diatom toxicity

The potential adverse effects of diatoms result from certain polyunsaturated aldehydes (PUA), which are generated from PUFAs upon a cell disruption during grazing (Pohnert et al. 2002). Adding these PUAs to the maternal diet of *Calanus helgolandicus* induced arrested development and increased rates of nauplii mortality (Ianora et al. 2004). The poor development, high mortality and occurrence of birth defects of copepods associated with deleterious PUAs have also been observed with monospecific diets of *T. rotula* and *S. costatum* (Miralto et al. 1999; Pohnert et al. 2002; Ianora et al. 2004). Although the present study adds *T. pseudonana* (strain CCMP1335) to the list of "bad" diatom species, this strain did not produce any PUAs. Moreover, we did not find any evidence of an adverse effect of PUAs on growth or mortality of *T. longicornis* nauplii (Fig. 5). The growth and development with three diatoms which have a potential for PUA production was comparable to that on *Rhodomonas* sp. In contrast, two species, which were

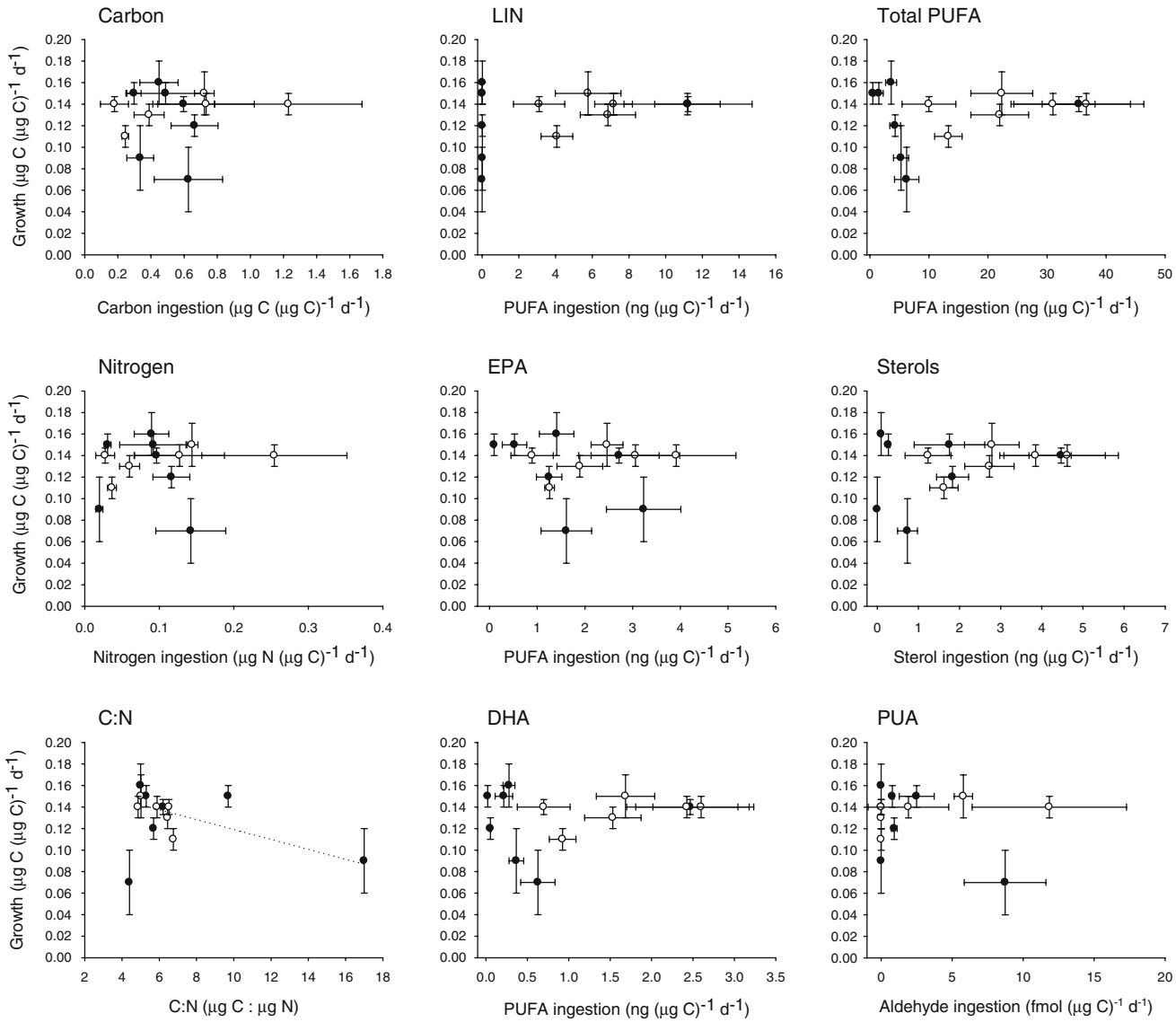

Fig. 5 *Temora longicornis*. Weight-specific growth [µg C (µg C)$^{-1}$ day^{-1}] as a function of weight-specific ingestion of carbon and nitrogen [µg (µg C)$^{-1}$ day^{-1}], PUFAs LIN (18:3ω3), EPA (20:5ω3) and DHA (22:6ω3), total PUFA and Δ5 sterols [ng (µg C)$^{-1}$ day^{-1}] or PUA [fmol (µg C)$^{-1}$ day^{-1}], as well as the C:N ratio (µg C:µg N) of the diet (mean ± SE). Only species which induced significant ingestion rates were included. *Closed symbols* indicate monospecific diets, *open symbols* mixtures. The *dotted line* indicates a significant linear regression between growth and C:N ratio of the diet, obtained when the aldehyde containing diets were removed ($y = 0.17 - 0.005x$; $r = 0.83$, $P < 0.05$; see "Summary")

ingested but did not support a complete development, did not have a potential to produce any PUAs.

To check if the negative effect could be due to unknown toxins, we used the toxicity bioassay approach as suggested by Jónasdóttir et al. (1998) and further developed by Colin and Dam (2002b). In this toxicity bioassay the suspect species is mixed in different ratios with the control diet, and if the species has a toxic effect, the copepod response should fall below the reference line connecting growth rate (or egg production or hatching) at 0 and 100% of the control diet (Fig. 6a) or gross growth efficiency on different concentrations of the control diet (Fig. 6b). The two species chosen for the bioassay were *P. tricornutum* and *T. rotula* CCMP1018: *P. tricornutum* because it is supposed to be a potential producer of the unsaturated 12-oxo-dodeca-5,8,10-trienoic acid, which is shown to have adverse effects in sea urchin bioassays (Pohnert et al. 2002), and *T. rotula* CCMP1018 because of its elevated and potentially variable production of PUA at the onset of the development experiments. With neither of the approaches had *P. tricornutum* or *T. rotula* CCMP1018 a toxic effect. In *Rhodomonas* sp. + *P. tricornutum* mixture, *P. tricornutum* did not have any effect on growth, obviously due to the strong selection of nauplii for *Rhodomonas* sp. when the two species were

Table 5 *Temora longicornis*: carbon gross growth efficiency of nauplii on *Rhodomonas* sp., on diatom diets and in mixtures

Algae species	GGE (C)
Rhodomonas sp.	0.23
T. weissflogii	0.36
T. rotula CCMP1648; Exp. 1	0.49
T. rotula CCMP1018	0.11
L. danicus	0.18
S. costatum	0.50
P. tricornutum	0.25
Diatoms; average (±SD)	0.30 ± 0.15
Rh + Ph 1:1	0.33
Rh + Ph 3:1	0.78
Rh + Ph 1:3	0.45
Rh + Ph; average (±SD)	0.52 ± 0.23
Rh + Tr1018 1:1	0.21
Rh + Tr1018 3:1	0.19
Rh + Tr1018 1:3	0.11
Rh + Tr; average (±SD)	0.17 ± 0.05

Only species with significant growth (Table 3) and ingestion (Figs. 2b, 3b) rates were included. Abbreviations as in Table 1

mixed (Fig. 4). In contrast, *T. longicornis* nauplii benefited from *T. rotula* 1018 if it was mixed with *Rhodomonas* sp. (Fig. 6), suggesting either a beneficial effect of pure food quantity (increased ingestion) or possibly supplement of some nutritional component by the diatom. Thus, the results of the toxicity bioassay, together with the lacking correlation between ingestion of deleterious PUAs and growth or mortality, strongly indicated that other factors than the parameters connected to diatom toxicity are behind the failed development on certain diatom diets.

Nutritional deficiencies

Alternatively, arrested development may be due to nutritional deficiencies. Jones and Flynn (2005) argued that single diatom diets are insufficient in terms of mineral nutrients. This is supported by the observations of the detrimental effects of N- or P-limited *T. weissflogii* on juvenile development, because in similar incubations, nutrient replete *T. weissflogii* induced high growth rates and low mortality (Koski et al. 1998; Klein Breteler et al. 2005). Klein Breteler et al. (2005) further showed changes in PUFAs and sterols of nutrient limited algae and suggested that these biochemical limitations, induced by mineral limitations, could be the reason behind the failed development on nutrient limited diatoms. According to a recent study of Wichard et al. (2007), also the production of PUAs can change the fatty acid content of diatoms during ingestion: a rapid depletion in PUFAs (especially EPA, the precursor of

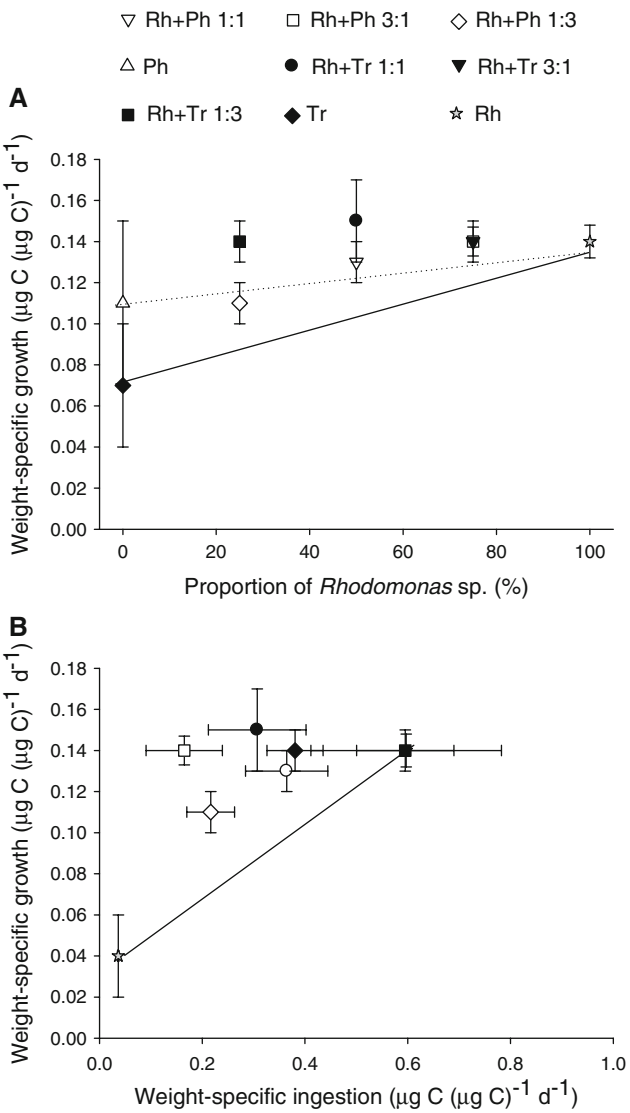

Fig. 6 *Temora longicornis*. Weight-specific growth [µg C (µg C)$^{-1}$ day^{-1}] as a function of **a** percentage of control food *Rhodomonas* sp. (%) in the diet and **b** weight-specific ingestion [µg C (µg C)$^{-1}$ day^{-1}] of *Rhodomonas* sp. as a single species diet (mean ± SE). *Closed symbols*: *Rhodomonas* sp. + *Thalassiosira rotula* CCMP1018, *open symbols*: *Rhodomonas* sp. + *Phaeodactylum tricornutum*, *grey symbols*: *Rhodomonas* sp. as a monospecific diet. The *lines* connect the weight-specific growth in **a** 0 and 100% of *Rhodomonas* sp. or **b** in the *Rhodomonas* sp. ingestion at a concentration of ca 50 and 200 µg C l^{-1}, thus indicating a growth rate which is only dependent on the *Rhodomonas* sp. concentration (**a**) or ingestion (**b**). The *symbols on the line* indicate that food species is neither toxic nor beneficial, *below the line* that the food species is toxic and *above the line* that the food species is beneficial (Jónasdóttir et al. 1998). In **a** the *dotted line* indicates Rh + Ph mixture, the *solid line* Rh + Tr mixture. The ingestion and weight-specific growth of *Rhodomonas* sp. at 50 µg C l^{-1} from M. Koski (unpublished data) and Koski et al. (2006), respectively

decatrienal and heptadienal) occurs during the production of PUAs, which suggests a PUFA limitation in diatoms which have a potential for PUA production. Evidence from

Table 6 Summary of the literature on development, juvenile mortality and/or malformations of early naupliar stages of copepods fed either different monospecific diatom diets or diets dominated by diatoms (in situ and mesocosm experiments)

Diatom	Copepod	PUA	Maternal effects	Mortality	Reference
(a)					
Chaetoceros spp.	*C. finmarchicus, C. helgolandicus*	No	? (field)	No	Diel and Klein Breteler (1986), Koski (2007)[a]
Lauderia borealis	*C. helgolandicus*	?	Yes		Paffenhöfer (1976)
Leptocylindricus danicus	*T. longicornis*	Yes	No	No	This study
Skeletonema costatum	*A. hudsonica*	?	Yes	No	Verity and Smayda (1989)
S. costatum	*T. longicornis*	Yes	No	No	This study
Thalassiosira angstii	*C. pacificus*, *Pseudocalanus* sp.	No?	Yes		Vidal (1980a, b)
Thalassiosira eccentrica	*C. pacificus*, *Pseudocalanus* sp.	No?	Yes		Vidal (1980a, b)
Thalassiosira rotula	*P. elongatus*, *T. longicornis*	?	Yes	No	Paffenhöfer and Harris (1976), Harris and Paffenhöfer (1976)
T. rotula CCMP1647	*T. longicornis*	Yes	No	No	This study
Thalassiosira weissflogii	*C. helgolandicus*, *P. elongatus*, *T. longicornis*, *C. typicus*	No	No	No	Koski et al. (1998), Bonnet and Carlotti (2001)[b], Rey et al. (2001)[a], Klein Breteler et al. (2005), this study
Thalassionema spp.	*C. finmarchicus*	?	? (mesocosm)	No	Hygum et al. (2000)
Thalassiosira spp.	*C. finmarchicus*	?	? (mesocosm/field)	No	Hygum et al. (2000), Koski (2007)[a]
(b)					
Phaeodactylum tricornutum	*C. helgolandicus*, *T. stylifera*	No	Yes/no	Yes	Poulet et al. (1995)[a], Carotenuto et al. (2002), Ianora et al. (2004)
P. tricornutum	*T. longicornis*	No	No	No	This study
S. costatum	*C. helgolandicus*	Yes	Yes	Yes	Ianora et al. (2004)
S. costatum	*T. stylifera*	Yes?	Yes/no	No	Carotenuto et al. (2002)
Thalassiosira conferta	*C. finmarchicus*/*C. helgolandicus*	No	? (field)		Diel and Klein Breteler (1986)
Thalassiosira pseudonana	*T. longicornis*	No	No	No	This study
T. rotula CCMP1647	*C. helgolandicus*	Yes	Yes	Yes	Poulet et al. (2003)[a]
T. rotula	*T. stylifera*	Yes?	Yes	Yes	Carotenuto et al. (2002)
T. rotula CCMP1018	*T. longicornis*	Yes	No	No	This study
N-limited *T. weissflogii*	*P. elongatus*	No	No	No	Koski et al. (1998)
(c)					
S. costatum	*C. helgolandicus*	Yes	Yes/no	Yes	Ianora et al. (2004)
T. rotula	*T. stylifera*	Yes?	No	No	Carotenuto et al. (2002)
(d)					
N/P-limited *T. weissflogii*	*T. longicornis*, *P. elongatus*	No	No	?	Klein Breteler et al. (2005)

Diatom–copepod combinations inducing (a) high development/growth rate and low mortality, (b) arrested development/low growth rate and high mortality, (c) complete development but high mortality and (d) arrested development but low mortality. PUA production is assumed to be as listed in Wichard et al. (2005a); if the strain number was not given in the original article and if the species/genus is a potential PUA-producer, a question mark is added. Maternal effects are assumed to be included if nauplii originated from wild females during a diatom bloom or cultured females fed mainly diatoms. Only studies where diatoms were likely to be ingested are included

[a] Only nauplii development

[b] Only copepodite development

the freshwater literature further indicates the role of diverse PUFAs in crustacean growth (Müller-Navarra et al. 2000), showing increased growth rates of *Daphnia* with supplementation of algal diet with single PUFAs (von Elert 2002).

Besides PUFAs, also Δ5 sterols are suggested to be essential for the growth of juvenile copepods (Klein Breteler et al. 1999, 2004, 2005). Similarly to PUFAs, sterols can not be synthesised de novo by crustaceans, and

must thus be derived from the algal food (Klein Breteler et al. 2005 and references therein). A role of sterols in moulting of crustaceans (D'Abramo et al. 1984) further suggests sterols as limiting factors for juvenile growth, although direct evidence from supplement experiments is still missing.

However, our results did not show any straightforward relationship between food nutritional components and growth or mortality of *T. longicornis* nauplii, neither for C:N ratio nor for the ingestion of any of the essential PUFAs or the Δ5 sterols tested (Fig. 5). Similarly, since the development was arrested also in species which do not have a potential for PUA production, a PUFA limitation following from PUA production is unlikely. The development was typically arrested in the last naupliar–first copepodite stages. It appears that if the food species is not ingested or is directly toxic for copepods, the development ceases in the first feeding stages (see Huntley et al. 1987), while if the food species is nutritionally poor, the development tends to be arrested at metamorphosis (see, e.g., Klein Breteler et al. 1999; Koski et al. 2006). After passing the first feeding stage, the transition from nauplii to copepodites seems therefore to be a critical phase in nauplii growth (Lopez 1996). Carrillo et al. (2001) suggested that metamorphosis might need substantial amounts of phosphorus, while Epp and Lewis (1980) measured increased metabolic rate at the nauplii–copepodite transition. In contrast to freshwater cladocerans, copepod life-cycle may be too complicated to obtain a correlation between a single limiting nutritional factor and growth or mortality, but different life-stages may have different nutritional needs and limitations. Although growth in general might be limited by PUFAs, sterols or nitrogen, these correlations will disappear if specifically metamorphosis fails in the lack of, e.g., phosphorus, or is more food quantity limited due to increased metabolic demands.

Intra-experimental variability

Similar to Carotenuto et al. (2002), there was a large variability between replicate experiments when copepods were feeding on *T. rotula* strain CCMP1647 (Fig. 1a). Similar intra-experimental variability is also presented in Ianora et al. (2004) and results obtained in more frequently used algae (Table 6) seem to confirm the large variability in response of even the same copepod species on diverse diatom diets. Copepod–diatom interactions are both species and strain-specific (Pohnert et al. 2002), and changes in algae growth conditions can have large consequences for nutritional quality (Klein Breteler et al. 2005) and also change the PUA production (Ribalet et al. 2007). In addition, maternal diets have potentially a large effect on the development of the new generation, which has been shown thoroughly with diatom diets (Ianora et al. 2004; Table 6) and starvation (Poulet et al. 2003, 2007).

The possible explanations for the large variability between experiments could therefore be (1) use of different algae strains with different properties, (2) changes in algae condition affecting PUA production, nutritional quality or both or (3) maternal effects complicating the nauplii response to the diet. However, most studies have used exponentially growing algae, and conflicting effects occur even between replicate experiments with the same copepod species and algae strain. In the present study we used 11 different species and strains, always during an exponential growth state, and paid extra attention to maintain constant growth conditions of the algae. In addition, when possible, replicate samples were taken for algae mineral and biochemical composition during different time points of the experimental period (see "Materials and methods"; Table 1), to account for a potential variability in the algae condition. The effect of maternal condition was minimised by using copepods originating from a standard culture and by monitoring the development on a standard diet. Thus, besides potential changes in algae condition and maternal effects as complicating factors, additional factors seem to be involved in determining juvenile's response on diatom diets.

Summary

Our results show that whereas some diatom species are inadequate food for *T. longicornis* nauplii, the development can be completed with several other diatom species, irrespective of their production of deleterious aldehydes. For some of the common diatom species, lacking ingestion was sufficient to explain the low development and growth. However, we failed to identify the reason behind copepod's response on the ingested diatom diets, but neither toxicity nor nutritional quality (represented by C:N, PUFA and sterols) could directly explain the observed growth. It thus appears that either the nauplii–diatom interactions are affected by something else, such as, e.g., assimilation of nutritional compounds during the gut passage (Dutz et al. 2008), or that the nutritional needs of development stages are too complex to be described by a single nutritional compound.

Additionally, since different algal species can lack different nutritional elements (or contain different harmful substances), it is difficult to assess limitations by single compounds without direct supplement experiments. For instance, in the present study, removing the aldehyde containing diets (both single species and mixtures) would result in a significant negative correlation between growth and C:N ratio of diet (Spearman -0.899, $n = 6$, $P < 0.05$),

suggesting nitrogen limitation of growth, although the relationship is driven by a single data point, *P. tricornutum* (Fig. 5). To estimate limitation by, for instance, PUFAs, one should thus remove both the aldehyde producers and the nitrogen-deplete diets, while PUA effects should ideally be studied after excluding the species deficient in PUFAs and/or nitrogen. However, removing PUFA- and N-deficient species would involve subjective estimates of the threshold levels of limitation, and would not remove the PUFA-deficiencies resulting from N-limitation (Klein Breteler et al. 2005) or PUA production (Wichard et al. 2007). Besides taking into account that specific limitations can occur during different stages of growth, the future studies aiming to identify a single limiting and/or harmful substance behind the diatom effect on growth should thus consider using single algal species, either manipulated by, e.g., nutrient stress, or supplemented by the missing nutritional element or harmful substance.

Whatever the reason behind the observed responses, our results suggest that a diatom spring bloom consisting mainly of species which nauplii are morphologically incapable of ingesting (*Chaetoceros* spp.), with only a low concentration of alternative species, will not support a complete development of *T. longicornis*, whereas a bloom dominated by, e.g., *Thalassiosira* spp. and *Leptocylindricus* spp. will. Although the egg production of *T. longicornis* seems to be dependent on the spring bloom (Kiørboe and Nielsen 1994; Peterson and Kimmerer 1994), its in situ mortality and growth/development rates are, however, relatively unknown, as are their effects on population dynamics. In general, similar negative effects of diatoms for cohort development, as reported for *Calanus helgolandicus* in the Adriatic Sea (Ianora et al. 2004), have not been described for *T. longicornis*. Instead, the few available measurements on the development and/or growth rate in situ seem to suggest fast development during the spring bloom (Bakker and van Rijswijk 1987; Peterson and Kimmerer 1994; M. Koski unpublished data).

The generally high in situ food diversity would suggest that the food quality of monospecific diatom diets would be of limited importance for the cohort development in nature. However, *T. longicornis* nauplii seem to have a limited capacity to utilise food mixtures (Koski et al. 2006) and the juvenile development is regularly limited by food quality and/or quantity on an annual basis (M. Koski unpublished data). Further, the few studies dealing with nauplii selectivity show a preference for diatoms and mainly size-selective grazing (Irigoien et al. 2003), suggesting that nauplii diet would mainly consist of the most available and largest cells (thus diatoms). It can therefore not be excluded that the nauplii–diatom interactions could influence population dynamics and, e.g., result in inter-annual variations in the copepod abundance, such as shown by Durbin et al. (2003) or Halsband-Lenk et al. (2004). Until further field studies have been conducted, the relevance of diatom effects for the annual dynamics of *T. longicornis* in nature, however, remains speculative.

Acknowledgments The authors wish to thank B. Hansen for the C:N analysis, J. Dutz and H. Dam for their constructive comments to the previous version of this manuscript, G. Pohnert for his support and useful discussions concerning the diatom aldehydes, A. Noordeloos for some of the diatom cultures and T. Kiørboe for his sometimes useful comments. M.K. was financed by Carlsberg Foundation. Authors acknowledge the financial support of the VELUX Foundation (Coulter Counter) and the SNF-21-03-0487 grant for S.J. The experiments comply with the current laws of Denmark.

References

Bakker C, van Rijswijk P (1987) Development time and growth rate of the marine calanoid copepod *Temora longicornis* as related to food conditions in the Oosterschelde Estuary (southern North Sea). Neth J Sea Res 21:125–141

Ban S, Burns C, Castel J, Chaudron Y, Christou E, Escribano R., Umani SF, Gasparini S, Ruiz FG, Hoffmeyer M, Ianora A, Kang HK, Laabir M, Lacoste A, Miralto A, Ning X, Poulet S, Rodriguez V, Runge J, Shi J, Starr M, Uye SI, Wang Y (1997) The paradox of diatom–copepod interactions. Mar Ecol Prog Ser 157:287–293

Bonnet D, Carlotti F (2001) Development and egg production in *Centropages typicus* (Copepoda: Calanoida) fed different food types: a laboratory study. Mar Ecol Prog Ser 224:133–148

Carotenuto Y, Ianora A, Buttino I, Romano G, Miralto A (2002) Is postembryonic development in the copepod *Temora stylifera* negatively affected by diatom diets? J Exp Mar Biol Ecol 276:49–66

Carotenuto Y, Wichard T, Pohnert G, Lampert W (2005) Life-history responses of *Daphnia pulicaria* to diets containing freshwater diatoms: effects of nutritional quality versus polyunsaturated fatty acids. Limnol Oceanogr 50:449–454

Carrillo P, Villar-Argaiz M, Medina-Sánchez JM (2001) Relationship between N:P ratio and growth rate during the life-cycle of calanoid copepods: an *in situ* measurement. J Plankton Res 23:537–547

Colin SP, Dam HG (2002a) Latitudinal differentiation in the effects of the toxic dinoflagellate *Alexandrium* spp. on the feeding and reproduction of populations of the copepod Acartia hudsonica. Harmful Algae 1:113–125

Colin SP, Dam HG (2002b) Testing for toxic effects of prey on zooplankton using sole versus mixed diets. Limnol Oceanogr 47:1430–1437

D'Abramo LR, Bordner CE, Conklin DE, Baum NA (1984) Sterol requirement of juvenile lobsters, *Homarus* sp. Aquaculture 42:13–25

Del Amo Y, Brzezinski MA (1999) The chemical form of dissolved Si taken up by marine diatoms. J Phycol 35:1162–1170

Diel S, Klein Breteler WCM (1986) Growth and development of *Calanus* spp. (Copepoda) during spring phytoplankton succession in the North Sea. Mar Biol 91:85–92

Durbin EG, Campbell RC, Casas MC, Ohman MD, Niehoff B, Runge J, Wagner M (2003) Interannual variation in phytoplankton blooms and zooplankton productivity and abundance in the Gulf of Maine during winter. Mar Ecol Prog Ser 254:81–100

Dutz J, Koski M, Jónasdóttir S (2008) Copepod reproduction is unaffected by diatom aldehydes or lipid composition. Limnol Oceanogr 53:225–235

Epp RW, Lewis WM Jr (1980) The nature and ecological significance of metabolic changes during the life history of copepods. Ecology 6:259–264

Fontana A, d'Ippolito G, Cutignano A, Romano G, Lamari N, Massa Gallucci A, Cimino G, Miralto A, Ianora A (2007) LOX-Induced lipid peroxidation mechanism responsible for the detrimental effect of marine diatoms on zooplankton grazers. Chembiochem 8:1810–1818

Frost BW (1972) Effects of size and concentration of food particles on the feeding behaviour of the marine planktonic copepod *Calanus pacificus*. Limnol Oceanogr 17:805–815

Guillard RRL (1975) Culture of phytoplankton for feeding marine invertebrates. In: Smith WL, Chanley MH (eds) Culture of marine invertebrate animals. Plenum Press, London, pp 29–60

Guisande C, Harris R (1995) Effect of total organic content of eggs on hatching success and naupliar survival in the copepod *Calanus helgolandicus*. Limnol Oceanogr 40:476–482

Halsband C, Hirche HJ (2001) Reproductive cycles of dominant calanoid copepods in the North Sea. Mar Ecol Prog Ser 209:219–229

Halsband-Lenk C, Carlotti F, Greve W (2004) Life-history strategies of calanoid congeners under two different climate regimes: a comparison. ICES J Mar Sci 61:709–720

Harris RP, Paffenhöfer G-A (1976) Feeding, growth and reproduction of the marine planktonic copepod *Temora longicornis* Müller. J Mar Biol Assoc UK 56:675–690

Huntley M, Lopez M (1992) Temperature dependent production of marine copepods: a global synthesis. Am Nat 140:201–242

Huntley ME, Ciminiello P, Lopez MDG (1987) Importance of food quality in determining development and survival of *Calanus pacificus* (Copepoda: Calanoida). Mar Biol 95:103–113

Hygum BH, Rey C, Hansen BW (2000) Growth and development rates of *Calanus finmarchicus* nauplii during a diatom spring bloom. Mar Biol 136:1075–1085

Ianora A, Miralto A, Poulet SA, Carotenuto Y, Buttino I, Romano G, Casotti R, Pohnert G, Wichard T, Colucci-D'Amato L, Terrazzano G, Smetacek V (2004) Aldehyde suppression of copepod recruitment in blooms of a ubiquitous planktonic diatom. Nature 429:403–407

Irigoien X, Harris RP, Verheye HM, Joly P, Runge J, Starr M, Pond D, Cambell R, Shreeve R, Ward P, Smith AN, Dam HG, Peterson W, Tirelli V, Koski M, Smith T, Harbour D, Davidson R (2002) Copepod hatching success in marine ecosystems with high diatom concentrations. Nature 419:387–389

Irigoien X, Titelman J, Harris RP, Harbour D, Castellani C (2003) Feeding of *Calanus finmarchicus* nauplii in the Irminger Sea. Mar Ecol Prog Ser 262:193–200

Jónasdóttir SH, Kiørboe T (1996) Copepod recruitment and food composition: do diatoms affect hatching success? Mar Biol 125:743–750

Jónasdóttir SH, Kiørboe T, Tang KW, St. John M, Visser AW, Saiz E, Dam HG (1998) Role of diatoms in copepod production: good, harmless or toxic? Mar Ecol Prog Ser 172:305–308

Jones RH, Flynn KJ (2005) Nutritional status and diet composition affect the value of diatoms as copepod prey. Science 307:1457–1459

Jüttner F (2005) Evidence that polyunsaturated aldehydes of diatoms are repellents for pelagic crustacean grazers. Aquat Ecol 39:271–282

Kiørboe T, Nielsen TG (1994) Regulation of zooplankton biomass and production in a temperate, coastal ecosystem. Limnol Oceanogr 39:493–507

Klein Breteler WCM, Fransz HG, Gonzalez SR (1982) Growth and development of four calanoid copepod species under experimental and natural conditions. J Sea Res 16:195–207

Klein Breteler WCM, Schogt N, Baas M, Schouten S, Kraay GW (1999) Trophic upgrading of food quality by protozoans enhancing copepod growth: role of essential lipids. Mar Biol 135:191–198

Klein Breteler WCM, Koski M, Rampen S (2004) Role of essential lipids in copepod nutrition: no evidence for trophic upgrading of food quality by a marine ciliate. Mar Ecol Prog Ser 274:199–208

Klein Breteler WCM, Schogt N, Rampen S (2005) Effect of diatom nutrient limitation on copepod development: role of essential lipids. Mar Ecol Prog Ser 291:125–133

Kleppel GS (1993) On the diets of calanoid copepods. Mar Ecol Prog Ser 99:183–195

Koski M (2007) High reproduction of *Calanus finmarchicus* during a diatom-dominated spring bloom. Mar Biol 151:1785–1798

Koski M, Klein Breteler WCM, Schogt N (1998) Effect of food quality on rate of growth and development of the pelagic copepod *Pseudocalanus elongatus* (Copepoda, Calanoida). Mar Ecol Prog Ser 170:169–187

Koski M, Klein Breteler WCM, Schogt N, Gonzalez S (2006) Role of food biochemistry in development and egg production of two copepods *Temora longicornis* and *Pseudocalanus elongatus*. J Plankton Res 28:919–936

Leising AW, Pierson JJ, Halsband-Lenk C, Horner R, Postel J (2005) Copepod grazing during spring blooms: does *Calanus pacificus* avoid harmful diatoms? Prog Oceanogr 67:384–405

Lopez MDG (1991) Molting and mortality depend on age and stage in naupliar *Calanus pacificus*: implication for development time of field cohorts. Mar Ecol Prog Ser 75:79–89

Lopez MDG (1996) Effects of starvation on development and survivorship of naupliar *Calanus pacificus* (Brodsky). J Exp Mar Biol Ecol 203:133–146

Maar M, Nielsen TG, Richardson K, Christaki U, Hansen OS, Zervoudaki S, Christou EDS (2002) Spatial and temporal variability of food web structure during the spring bloom in the Skagerrak. Mar Ecol Prog Ser 239:11–29

Meyer-Harms B, Irigoien X, Head R, Harris R (1999) Selective feeding on natural phytoplankton by *Calanus finmarchicus* before, during, and after the 1997 spring bloom in the Norwegian Sea. Limnol Oceanogr 44:154–165

Miralto A, Barone B, Romano G, Poulet SA, Ianora A, Russo GL, Buttino I, Mazzarella G, Laabir M, Cabrini M, Giacobbe MG (1999) The insidious effect of diatoms on copepod reproduction. Nature 402:171–176

Miralto A, Guglielmo L, Zagami G, Buttino I, Granata A, Ianora A (2003) Inhibition of population growth in the copepods *Acartia clausi* and *Calanus helgolandicus* during diatom blooms. Mar Ecol Prog Ser 254:253–268

Müller-Navarra D, Brett MT, Liston AM, Goldman CR (2000) A highly unsaturated fatty acid predicts carbon transfer between primary producers and consumers. Nature 403:74–77

Mullin MM (1969) Production of zooplankton in the ocean: the present status and problems. Oceanogr Mar Biol Annu Rev 7:293–314

Paffenhöfer GA (1976) Feeding, growth, and food conversion of the marine planktonic copepod *Calanus helgolandicus*. Limnol Oceanogr 21:39–50

Paffenhöfer GA, Harris RP (1976) Feeding, growth and reproduction of the marine planktonic copepod *Pseudocalanus elongatus* Boeck. J Mar Biol Assoc UK 56:327–344

Peterson WT, Kimmerer WJ (1994) Processes controlling recruitment of the marine calanoid copepod *Temora longicornis* in Long Island Sound: egg production, egg mortality, and cohort survival rates. Limnol Oceanogr 39:1594–1605

Pierson JJ, Halsband-Lenk C, Leising AW (2005) Reproductive success of *Calanus pacificus* during diatom blooms in Dabob Bay, Washington. Prog Oceanogr 67:314–331

Pohnert G, Lumineau O, Cueff A, Adolph S, Cordevant C, Lange M, Poulet S (2002) Are volatile unsaturated aldehydes from diatoms the main line of chemical defence against copepods? Mar Ecol Prog Ser 245:33–45

Poulet SA, Laabir M, Ianora A, Miralto A (1995) Reproductive response of *Calanus helgolandicus.* I. Abnormal embryonic and naupliar development. Mar Ecol Prog Ser 129:85–95

Poulet SA, Richer de Forges M, Cueff A, Lennon J-F (2003) Double-labelling methods used to diagnose apoptic and necrotic cell degradations in copepod nauplii. Mar Biol 143:889–895

Poulet SA, Wichard T, Ledoux JB, Lebreton B, Marchetti J, Dancie C, Bonnet D, Cueff A, Morin P, Pohnert G (2006) Influence of diatoms on copepod reproduction. I. Field and laboratory observations related to *Calanus helgolandicus* egg production. Mar Ecol Prog Ser 308:129–142

Poulet SA, Cueff A, Wichard T, Marchetti J, Dancie C, Pohnert G (2007) Influence of diatoms on copepod reproduction. III. Consequences of abnormal oocyte maturation on reproductive factors in *Calanus helgolandicus*. Mar Biol 152:415–428

Rey C, Harris R, Irigoien X, Head R, Carlotti F (2001) Influence of algal diet on growth and ingestion of *Calanus helgolandicus* nauplii. Mar Ecol Prog Ser 216:151–165

Ribalet F, Wichard T, Pohnert G, Ianora A, Miralto A, Casotti R (2007) Age and nutrient limitation enhance polyunsaturated aldehyde production in marine diatoms. Phytochemistry 68:2059–2067

Riebesell U (1991) Particle aggregation during a diatom bloom. I. Physical aspects. Mar Ecol Prog Ser 69:273–280

Turner JT, Ianora A, Miralto A, Laabir M, Esposito F (2001) Decoupling of copepod grazing rates, fecundity and egg-hatching on mixed and alternating diatom and dinoflagellates diets. Mar Ecol Prog Ser 220:187–199

Verity PG, Smayda TJ (1989) Nutritional value of *Phaeocystis pouchetii* (Prymnesiophyceae) and other phytoplankton for *Acartia* spp. (Copepoda): ingestion, egg production, and growth of nauplii. Mar Biol 100:161–171

Vidal J (1980a) Physioecology of zooplankton. I. Effects of phytoplankton concentration, temperature, and body size on the growth rate of *Calanus pacificus* and *Pseudocalanus* sp. Mar Biol 56:111–134

Vidal J (1980b) Physioecology of zooplankton. II. Effects of phytoplankton concentration, temperature, and body size on the development and molting rates of *Calanus pacificus* and *Pseudocalanus* sp. Mar Biol 56:135–146

Von Elert E (2002) Determination of limiting polyunsaturated fatty acids in *Daphnia galeata* using a new method to enrich food algae with single fatty acids. Limnol Oceanogr 47:1764–1773

Wichard T, Poulet SA, Halsband-Lenk C, Albaina A, Harris R, Liu D, Pohnert G (2005a) Survey of the chemical defence potential of diatoms: Screening of fifty one species for $\alpha,\beta,\gamma,\delta$-unsaturated aldehydes. J Chem Ecol 31:949–958

Wichard T, Poulet SA, Pohnert G (2005b) Determination and quantification of $\alpha,\beta,\gamma,\delta$-unsaturated aldehydes as pentafluorobenzyloxime derivates in diatom cultures and natural phytoplankton populations: application in marine field studies. J Chromatogr B 814:155–161

Wichard T, Gerecht A, Boersma M, Poulet SA, Wiltshire K, Pohnert G (2007) Lipid and fatty acid composition of diatoms revisited: rapid wound-activated change of food quality parameters influences herbivorous copepod reproductive success. Chembiochem 8:1146–1153

Wichard T, Poulet SA, Boulesteix A-L, Ledoux JB, Lebreton B, Marchetti J, Pohnert G (2008) Influence of diatoms on copepod reproduction. II. Uncorrelated effects of diatom-derived alpha,beta,gamma,delta-unsaturated aldehydes and polyunsaturated fatty acids on *Calanus helgolandicus* in the field. Prog Oceanogr (in press)