

# Annual cycle of early developmental stage survival and recruitment in the copepods *Temora stylifera* and *Centropages typicus*

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**ABSTRACT:** *In situ* seasonal oscillations in female abundances, fecundity, egg-hatching success and survivorship of the first naupliar stage (N1) of the copepods *Temora stylifera* and *Centropages typicus*, collected from February 2000 to February 2001, were studied at a coastal station located in the Gulf of Naples (Mediterranean Sea). Adult *T. stylifera* and *C. typicus* females reached maximum abundances in different periods of the year, even though both species had similar breeding patterns with maximum egg-production rates (120 eggs female<sup>-1</sup> d<sup>-1</sup>) in spring and early summer. Hatching success was generally >80%, showing weak seasonal trends in both species. In contrast, survivorship of N1 nauplii was much lower in *T. stylifera* (mean = 12%), compared to *C. typicus* (mean = 67%); on nearly 40% of the sampling dates, not a single N1 larva survived to moult to the second larval stage. On average, only 11 *T. stylifera* nauplii m<sup>-3</sup> d<sup>-1</sup> were recruited *in situ* over the year studied, compared to 139 *C. typicus* nauplii m<sup>-3</sup> d<sup>-1</sup>. Only seasonal changes in egg- and pellet-production rates in both species were correlated with total integrated chlorophyll *a* (chl *a*), whereas hatching success and naupliar survival were not correlated with either chl *a* concentrations or with phytoplankton composition. Laboratory experiments showed that maternal feeding on *Isochrysis galbana* (ISO) or *Prorocentrum minimum* (PRO) for 7 d did not enhance naupliar survival in either copepod species, indicating that the negative effects of maternal diet did not disappear after feeding on a high quality food source. Naupliar survivorship was enhanced to >90% with the addition of a K algal culture medium to the natural phytoplankton assemblage, indicating that N1 nauplii were 'drinking' and absorbing dissolved material from the medium. Our results show that high hatching success in copepods can be followed by very low early larval survivorship and that the causes for low survival are not only related to maternal diet but also to the quality of the water in which the nauplii hatch. Scoring maternal and environmental effects on copepod recruitment rates should therefore also consider mortality of early naupliar stages.

**KEY WORDS:** Annual copepod cycle · *Temora stylifera* · *Centropages typicus* · Egg production · Hatching success · Naupliar survival · Maternal diet

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## INTRODUCTION

Sub-temperate and temperate copepod species generally undergo strong seasonal oscillations in population abundances that reflect changes in fecundity, viable egg production and larval fitness. Of these life

history traits, fecundity has been the most investigated, with field studies indicating periods of lower and higher breeding intensity (Ianora & Buttino 1990, Ianora 1998, Halsband-Lenk et al. 2001). The causes of fluctuations in egg-production rates have been related to changes in ambient temperature (Ambler 1986, Uye

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& Shibuno 1992), adult female size (Ban et al. 2000, Halsband & Hirche 2001), and quantity and quality of food (Kjørboe & Nielsen 1994, Saiz et al. 1999, Kleppel & Hazzard 2000, Calbet et al. 2002).

Much less is known about the factors affecting copepod egg quality and larval recruitment rates. Extremely high losses of eggs and/or early naupliar stages have been reported in the past (Uye 1982, Kjørboe & Nielsen 1994, Liang et al. 1994, Peterson & Kimmerer 1994, Liang & Uye 1996, Zaballa & Gaudy 1996, Ohman et al. 2002), suggesting that not only productivity but also mortality of early stages can play an important role in copepod population dynamics. Most of these studies concluded that losses were due to predation and cannibalism of eggs and early nauplii, or advection away from the spawning site. On the other hand, other studies have indicated that seasonal fluctuations in egg mortality (Ianora et al. 1992, Ianora & Poulet 1993, Pond et al. 1996, Laabir et al. 1998, Gómez-Gutiérrez & Peterson 1999, Miralto et al. 1999, Ara 2001) are related to maternal diet, phytoplankton composition and food quality characteristics at the time of feeding. Furthermore, there is recent evidence suggesting that poor maternal diet in the field not only negatively impacts egg hatching success, but also the development and recruitment of hatched larvae, with greatest losses occurring during the naupliar stages (Ianora et al. 2004).

According to the classical view that early naupliar stages of copepods (N1 and/or N2) are non-feeding stages that rely on maternal yolk reserves (Marshall & Orr 1955), their survival should thus be strongly dependent on the past feeding history of females. Laboratory rearing studies have in fact shown that maternal diet is extremely important in promoting high hatching success and larval fitness and survivorship (Frangópoulos et al. 2000, Lacoste et al. 2001, Carotenuto et al. 2002). Similarly, several field studies have reported the occurrence of pre-feeding naupliar stages with low survival and/or morphological aberrations due to the past-feeding history of the female (Guisande & Harris 1995, Laabir et al. 1995, Pond et al. 1996, Ban et al. 2000, Ianora et al. 2004).

Here we investigate *in situ* seasonal changes in fecundity, hatching success and survival of pre-feeding larval stages of the copepods *Temora stylifera* and *Centropages typicus* in order to: (1) estimate their secondary production and potential recruitment rates, and (2) relate these to seasonal changes in phytoplankton biomass and composition. Laboratory experiments were also conducted to better study the effect of maternal and neonate diets on naupliar survivorship. Both *T. stylifera* and *C. typicus* are dominant coastal/neritic copepod species in the Mediterranean Sea (Mazzocchi & Ribera d'Alcalà 1995), reaching 25 and 50% of total

copepod numbers during periods of maximum population densities (Ianora & Buttino 1990, Ragosta et al. 1995). The 2 species differ in their seasonal distribution patterns, with *T. stylifera* dominating in the late summer to early winter, and *C. typicus* from early spring to late summer (Mazzocchi & Ribera d'Alcalà 1995, Ribera d'Alcalà et al. 2004). Although maximum reproductive activity may at times occur in the same period (Halsband-Lenk et al. 2001), other studies have reported that *T. stylifera* produces the highest numbers of eggs in autumn and *C. typicus* in spring (Ianora 1998). Both species have already been shown to undergo strong fluctuations in egg mortality under natural conditions (Ianora et al. 1992, Ianora & Poulet 1993), but no information is available on early naupliar mortality. We discuss our results in the light of recent findings on the impact of maternal and neonate diets on copepod offspring fitness.

## MATERIALS AND METHODS

**Annual cycle experiments.** Sampling was carried out weekly from February 2000 to February 2001 at a coastal station (MC: 40° 48' N, 14° 15' E; depth = 80 m) located in the Gulf of Naples, which has been the subject of an intensive ongoing field study since 1984. Due to instrumental problems, no temperature data could be obtained regularly during the year studied. However, the seasonal cycle of temperature in the Gulf of Naples from 1984 to 1999 has been published elsewhere (Ribera D'Alcalà et al. 2004).

Total chl *a* concentrations in water samples collected with 5 l Niskin bottles at selected depths (0.5, 2, 5, 10, 20, 40 and 60 m) were determined with a spectrofluorometer (Neveux & Panouse 1987), and then integrated to obtain a single 0 to 60 m value. Phytoplankton samples were collected at 0.5 m and fixed with neutralized formaldehyde (0.8% final concentration). Cell counts were made using an inverted microscope following Utermöhl (1958) (see Ribera d'Alcalà et al. 2004, for details). Phytoplankton carbon content was calculated from mean cell biovolume using formulae from Strathmann (1967).

On each sampling date, 2 vertical tows were also taken from 50 m to the surface with a 200 µm Nansen net to collect zooplankton samples. One of the samples was preserved in 5% formaldehyde-seawater for later enumeration of adult female numbers using Huntsman's method (van Guelpen et al. 1982). The other live sample was transferred to the laboratory in an insulated box within 1 h of collection. In the laboratory, *Temora stylifera* and *Centropages typicus* females (n = 15) were randomly sorted with the aid of a dissecting microscope and incubated individually in crystallizing

dishes containing 100 ml of 50  $\mu\text{m}$ -filtered surface (0.5 m) seawater so as to remove contaminant eggs but retain the natural phytoplankton assemblage. Containers were incubated at 20°C and on a 12:12 h dark:light cycle. This temperature was chosen to be consistent with previous experimental studies in this area, and also because it is included in the seasonal cycle of temperatures measured at Stn MC over many years (Ribera D'Alcalà et al. 2004). After 24 h, females were removed and egg production and fecal pellet production rates were determined with an inverted microscope. Experimental containers were incubated for an additional 48 h under the same conditions as above (i.e. filtered seawater was not changed). After each 24 h period, the numbers of empty egg membranes and dead nauplii on container bottoms were counted. The percentage of hatched eggs (with respect to the number of eggs produced) and survivorship of N1 nauplii (with respect to the number of hatched eggs) were calculated. Counts were done twice over 48 h to ensure that we were not underestimating the number of dead N1 nauplii (since these tend to decompose very quickly) and to double-check that all of the eggs had hatched.

Values for female abundance (*FA*, females  $\text{m}^{-3}$ ), egg production rate (*EPR*, eggs female $^{-1}$  d $^{-1}$ ) and egg viability (*EV*, % egg viability) were used to calculate the total number of eggs produced (*TEP* = *FA*  $\times$  *EPR*, eggs  $\text{m}^{-3}$  d $^{-1}$ ) and eggs hatched daily (*TEH* = *TEP*  $\times$  *EV*, hatched eggs  $\text{m}^{-3}$  d $^{-1}$ ) by the female population *in situ*, according to the equations reported in Poulet et al. (1995) and Miralto et al. (2003). We then used our new data for naupliar survivorship (*NS*) to calculate an additional parameter, which we have termed total nauplii recruited daily (*TNR* = *TEH*  $\times$  *NS*, nauplii recruited  $\text{m}^{-3}$  d $^{-1}$ ). Mean values for each of these parameters were calculated for both *Temora stylifera* and *Centropages typicus* over the year, and these were used to assess differences between the 2 species using *t*-test analysis.

**Effect of maternal and neonate diet on larval survivorship.** In order to investigate the relative importance of maternal and neonate diets on copepod offspring fitness, laboratory experiments were run in 2004 with *Temora stylifera* and *Centropages typicus*. In a set of experiments run in October 2004 with both copepod species, we tested the effect of maternal past feeding history on naupliar survival. Freshly caught *T. stylifera* and *C. typicus* females were divided into 2 groups: half ( $n = 5$  to 6) were incubated for 24 h in 0.22  $\mu\text{m}$ -filtered seawater with the addition of either the flagellate prymnesiophyte *Isochrysis galbana* (ISO) (cell volume = 65  $\mu\text{m}^3$ ), targeting final concentrations of  $8 \times 10^4$  cell  $\text{ml}^{-1}$  (corresponding to about 1400  $\mu\text{g C l}^{-1}$ ), or the dinoflagellate *Prorocentrum minimum*

(PRO) (cell volume = 1340  $\mu\text{m}^3$ ) at a final concentration of  $7 \times 10^3$  cell  $\text{ml}^{-1}$  (corresponding to about 1200  $\mu\text{g C l}^{-1}$ ). The other half ( $n = 5$  to 6) was incubated in a natural food assemblage (NFA) (50  $\mu\text{m}$ -filtered seawater, control) for the same length of time. After 24 h, newly spawned eggs were counted, collected with a Pasteur pipette and left to hatch in filtered seawater (Day 1). Egg viability and N1 naupliar survival were determined 48 h after egg spawning. Females from each group were then incubated in fresh medium (ISO, PRO or only NFA), which was changed daily for 7 d, after which the eggs from each experimental group were collected and incubated in filtered seawater (Day 7). Hatching success and naupliar survival were determined 48 h after egg laying. All experiments were conducted at 20°C, and on a 12:12 h dark:light cycle.

*Isochrysis galbana* and *Prorocentrum minimum* cultures were chosen since they have already been shown to promote high egg production and egg viability (Lacoste et al. 2001) in other copepods, as well as high naupliar survivorship in *Temora stylifera* (Carotenuto et al. 2002). The algae were grown to the late exponential phase in 2 l glass jars filled with 0.22  $\mu\text{m}$ -filtered seawater enriched with K medium (Keller et al. 1987), at 20°C, and on a 12:12 h dark:light cycle.

In another set of experiments, we tested whether survival of N1 nauplii was affected by the food assemblage nauplii encountered once they hatched. Preliminary experiments with *Temora stylifera*, in fact, had shown that the addition of *Isochrysis galbana* culture enhanced naupliar survivorship. We needed, therefore, to test whether this was due to the presence of particulate material (the algal cells), or to the presence of dissolved organic and inorganic material (e.g. vitamins) normally present into the algal culture medium (K).

Ripe *Centropages typicus* and *Temora stylifera* females ( $n = 12$  to 15) were sampled weekly from June to July and from September to October 2004, respectively, and incubated in 50  $\mu\text{m}$  natural-filtered seawater. Egg production was determined after 24 h, as reported above, and the containers were then separated into 5 sub-groups: one group received ISO culture at a concentration of  $4 \times 10^4$  cell  $\text{ml}^{-1}$  (corresponding to about 700  $\mu\text{g C l}^{-1}$ ), so that particulate food was available for the nauplii at the moment of hatching; a second group received a 0.22  $\mu\text{m}$ -filtered ISO culture, in which algal cells were removed and nauplii were supplemented with dissolved organic matter; a second group received 0.22  $\mu\text{m}$ -filtered K culture medium, used to grow the algae, which only contained inorganic salts and vitamins; a fourth group received fresh 0.22  $\mu\text{m}$ -filtered seawater; and the last group was the control group, where nauplii were left in natural-filtered seawater. The same volume (8 ml) of ISO, cell-

free ISO, K culture medium, or filtered seawater was added with a sterile plastic pipette to each sub-group. Egg viability and N1 naupliar survival were determined 48 h later. Three experimental replicates were obtained for each group. To verify whether nauplii-fed ISO had actually ingested the algae, nauplii were examined with a Confocal Laser Scanning Microscope (CLSM) to detect the presence of algal autofluorescent material in the stomach. N1 and N2 nauplii were fixed in 4% formaldehyde and observed under a Zeiss 410 CLSM equipped with a He/Ne laser (543 nm  $\lambda$ ) with a  $\times 20$  water immersion objective.

## RESULTS

*Temora stylifera* and *Centropages typicus* showed similar seasonal breeding patterns. In *T. stylifera*, highest egg production rates occurred from February to July (up to 120 eggs female<sup>-1</sup> d<sup>-1</sup>), followed by a sharp decline from August to mid-October, with <20 eggs produced female<sup>-1</sup> d<sup>-1</sup>. Fecundity was again high during the remaining autumn months, and the following February (up to 70 eggs female<sup>-1</sup> d<sup>-1</sup>). Changes in the seasonal fecundity of *C. typicus* were less dramatic compared to *T. stylifera*. Maximum fecundity in *C. typicus* was restricted to a shorter period (February to April), but was comparable in terms of production (up to 120 eggs female<sup>-1</sup> d<sup>-1</sup>). This was followed by a long period of lower egg production rates (<60 eggs female<sup>-1</sup> d<sup>-1</sup>) until February, when higher fecundity was again recorded (up to 80 eggs female<sup>-1</sup> d<sup>-1</sup>) (Fig. 1). Interestingly, the 2 species had the same annual fecundity, with *T. stylifera* producing 43.9 eggs female<sup>-1</sup> d<sup>-1</sup> and *C. typicus* 54.0 eggs female<sup>-1</sup> d<sup>-1</sup> (*t*-test:  $t_{81} = 1.64$ ,  $p > 0.05$ ).

Faecal pellet production rates were also very similar for both species, with high defecation rates in March to June for *Temora stylifera* (up to a maximum of 100 pellets female<sup>-1</sup> d<sup>-1</sup>) and in March to April for *Centropages typicus* (up to 70 pellets female<sup>-1</sup> d<sup>-1</sup>). Faecal pellet production remained low in both species from then till February (<20 pellets female<sup>-1</sup> d<sup>-1</sup>) (Fig. 1). However, the amplitude of pellet production was different in the 2 species, with a mean annual value that was significantly higher in *T. stylifera* compared to *C. typicus* (27.5 pellets female<sup>-1</sup> d<sup>-1</sup> vs. 15.5 pellets female<sup>-1</sup> d<sup>-1</sup>, respectively) (*t*-test:  $t_{81} = 2.86$ ,  $p < 0.01$ ). Trends in egg and pellet production rates were significantly correlated in both species (Pearson:  $r = 0.48$ ,  $p < 0.05$  for *T. stylifera*, and  $r = 0.35$ ,  $p < 0.05$  for *C. typicus*).

The hatching success of *Temora stylifera* did not change seasonally and remained high throughout the year (>80%), except for September to October (65%) (Fig. 1). Hatching success showed no seasonal trend in

*Centropages typicus* either, remaining high for most of the year (>80%). However, hatching fluctuated widely around this mean value, dropping to <60% on a few sampling occasions from August to December (Fig. 1). The mean annual hatching success in this species (80.1%) was significantly lower than that of *T. stylifera* (87.6%) (*t*-test:  $t_{81} = 2.84$ ,  $p < 0.01$ ). Hatching success in *C. typicus* was positively correlated with egg production rate (Pearson:  $r = 0.39$ ,  $p < 0.05$ ).

Seasonal patterns and absolute values for survivorship of N1 nauplii differed greatly for the 2 species. *Temora stylifera* N1 nauplii showed extremely low survivorship in all months; on nearly 40% of the sampling dates, not a single N1 survived to moult to the second larval stage. Highest survivorships were recorded in March (57%) and August (94%) (Fig. 1), with a mean annual survivorship that represented only 12.3% of the total number of hatched eggs. In contrast, *Centropages typicus* had comparatively higher N1 nauplius survivorship, with an annual mean of 66.6% (*t*-test:  $t_{81} = 12.4$ ,  $p < 0.0001$ ). Naupliar survivorship was high in February and decreased in spring to early summer, to rise once again during the following summer to winter months. Low values were at times recorded in June, August and February (<40%) (Fig. 1). Trends in naupliar survival for both species were not correlated with egg production, faecal pellet production or hatching success.

Both egg and pellet production rates in the 2 species were positively correlated with the annual pattern of integrated chl *a* values (Pearson:  $r = 0.35$ ,  $p < 0.05$ , and  $r = 0.34$ ,  $p < 0.05$  for egg production and pellet production rates in *Temora stylifera*, respectively. Pearson:  $r = 0.56$ ,  $p < 0.001$ , and  $r = 0.58$ ,  $p < 0.001$  for egg production rate and pellet production rates in *Centropages typicus*, respectively).

In 2000, annual integrated chl *a* concentrations were maximal in late winter to spring (up to 1.3  $\mu\text{g l}^{-1}$ ), followed by smaller peaks alternating with minimum values in summer and autumn; low concentrations were recorded from December 2000 to February 2001 (0.2  $\mu\text{g l}^{-1}$  on average) (Fig. 2a). Phytoplankton concentrations at the surface showed a rather different pattern compared to integrated chl *a* concentrations, with the main difference being the absence of high values in February to March 2000 (Fig. 2b). This discrepancy was due to the seasonal dynamics of the water column, which resulted in the redistribution of phytoplankton biomass over a deeper mixed layer in winter. Maximum phytoplankton abundances (up to  $5.6 \times 10^7$  cells l<sup>-1</sup>) were recorded in spring, which were followed by peaks of different amplitude and length during summer and autumn. Minimum concentrations were observed in winter. Diatoms and small phytoflagellates (<4  $\mu\text{m}$ ) were the dominant groups throughout the year (35 and 58% on average of

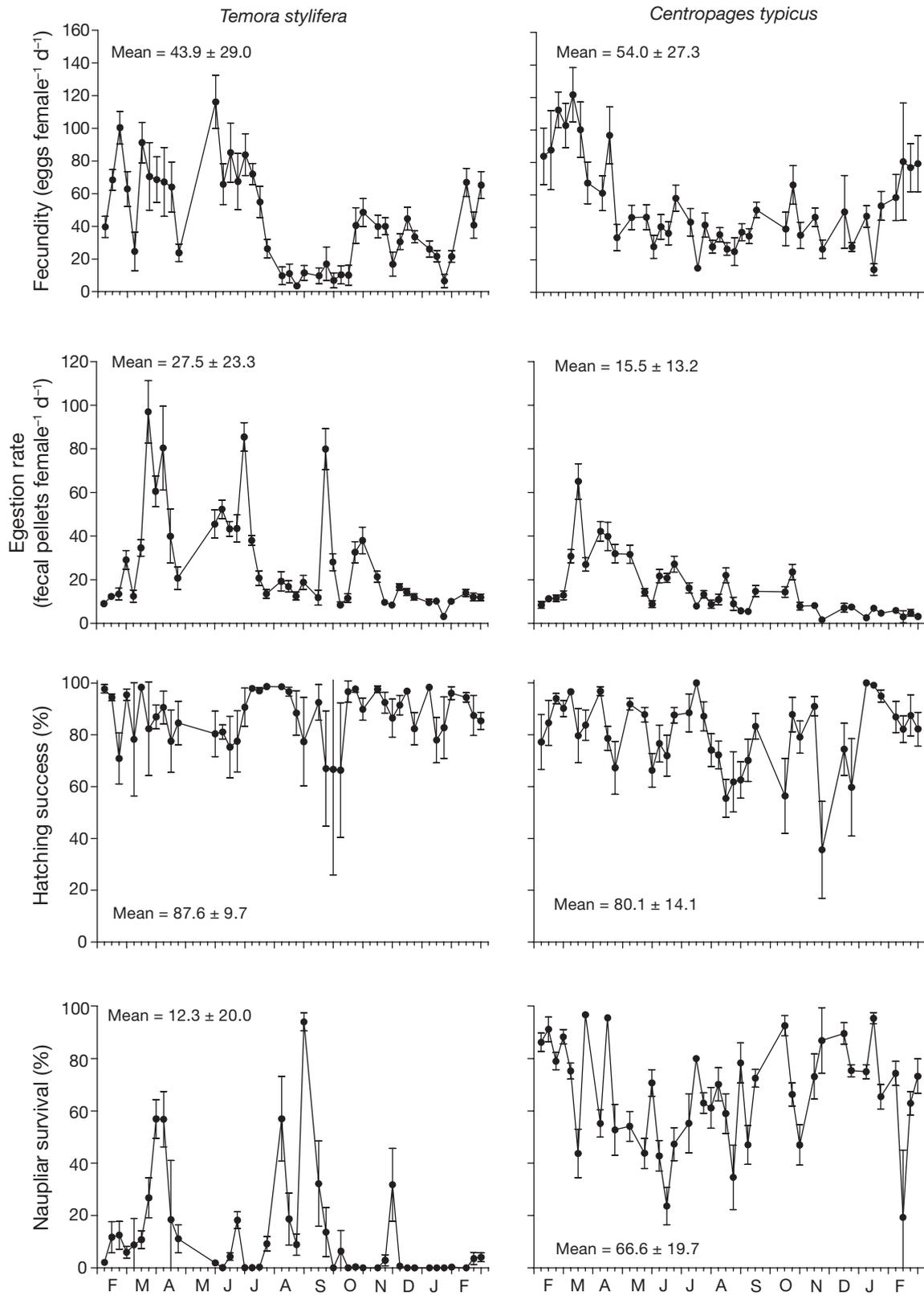


Fig. 1. *Temora stylifera* and *Centropages typicus*. Annual cycle of fecundity (eggs female<sup>-1</sup> d<sup>-1</sup>), egestion rate (fecal pellets female<sup>-1</sup> d<sup>-1</sup>), hatching success (%) and naupliar survival (%), at Stn MC from February 2000 to February 2001. Each point: mean ± SE, Mean: annual mean ± SD

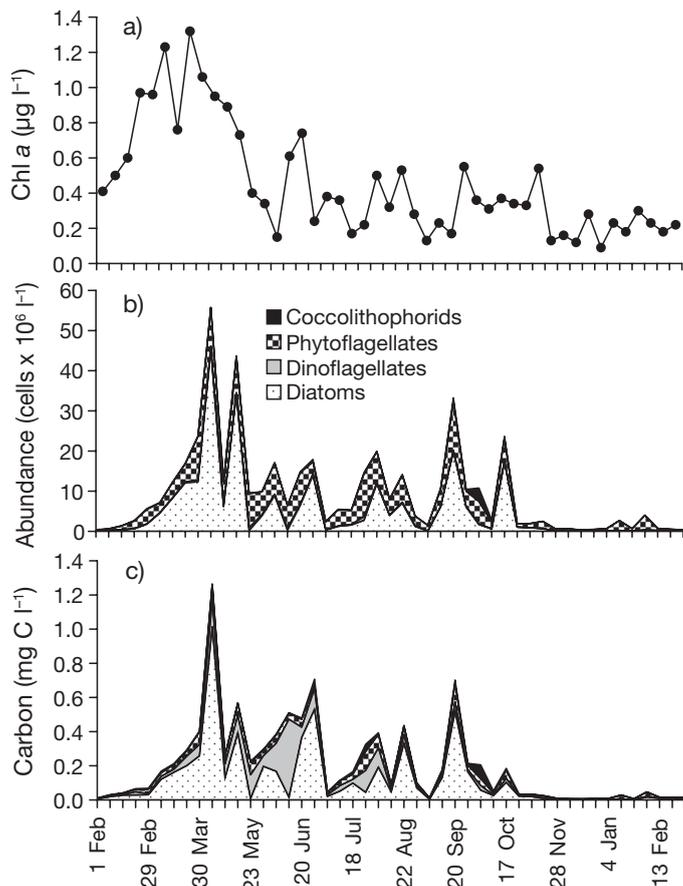


Fig. 2. Annual cycle of integrated values (0 to 60 m) of chl *a* and surface (0.5 m) phytoplankton at Stn MC from February 2000 to February 2001. (a) Chl *a*, (b) total abundance and (c) total carbon content of different taxonomic groups of phytoplankton

the total phytoplankton, respectively). Peaks in phytoplankton concentrations were mainly due to diatom blooms, which were intermittent and formed by several different species succeeding and overlapping each other. Large colonial species (e.g. *Pseudo-nitzschia delicatissima*, *Chaetoceros diadema*, *Thalassiosira mediterranea*) characterized diatom assemblages in winter to early spring, whereas small species, often in a non-colonial stage (e.g. *C. socialis*, *C. tenuissimus*, *Skeletonema pseudocostatum*, *S. menzeli*), were mainly responsible for blooms from spring to autumn. Compared to cell abundances, carbon content revealed a reduced contribution of phytoflagellates to the total biomass (Fig. 2c). Dinoflagellates, which never attained high concentrations, in spring to summer may contribute significantly to phytoplankton biomass due to their larger size (20.5% of the total biomass, on average).

None of the phytoplankton parameters measured (total integrated chl *a*, total surface phytoplankton concentrations and carbon biomass, and surface abun-

dance of each taxonomic group), was correlated with hatching success and naupliar survivorship in the 2 species.

In terms of annual adult female abundance, *Temora stylifera* had a first small peak in July and reached a maximum population density in autumn (up to 80 females  $m^{-3}$  in November). Very low numbers were recorded in winter and spring (Fig. 3). Conversely, *Centropages typicus* adult females were mostly found from February to August, with high densities (up to 20 females  $m^{-3}$ ) followed by a decline in population numbers ( $<5$  females  $m^{-3}$ ). Population numbers were low during autumn and winter. On an annual basis, the average number of *C. typicus* females (4.6 females  $m^{-3}$ ) was not significantly different from that of *T. stylifera* (9.1 females  $m^{-3}$ ) (*t*-test:  $t_{81} = 1.75$ ,  $p > 0.05$ ). Density values were not correlated with egg-production rates, and in both species, maximum fecundity generally corresponded to minimum female abundances.

Seasonal fluctuations in the number of eggs produced  $m^{-3} d^{-1}$ , as well as the number of eggs hatched  $m^{-3} d^{-1}$ , basically reflected changes in female abundances for both species (Fig. 3). Mean annual egg-production rates (up to 300 eggs  $m^{-3} d^{-1}$ ) and number of hatched eggs (up to 280 eggs hatched  $m^{-3} d^{-1}$ ) were of the same order of magnitude for both species. Naupliar recruitment differed for both species, both in terms of absolute numbers and in seasonal patterns. In *Temora stylifera*, the seasonal trend of recruited nauplii basically matched that of hatched eggs except from July to September, when low numbers of hatched eggs corresponded to high naupliar recruitment, and in November, when peaks in naupliar recruitment occurred 1 to 2 wk later with respect to the number of hatched eggs. On average, the annual naupliar recruitment rate of *T. stylifera* was only 11 nauplii  $m^{-3} d^{-1}$ . *Centropages typicus* had more than an order of magnitude higher naupliar recruitment rates compared to *T. stylifera*, with an annual average of 139 nauplii  $m^{-3} d^{-1}$ . The seasonal dynamics of nauplii recruited basically followed that of female abundance, as well as that of hatched eggs, with the highest naupliar recruitment in February and April, and lower recruitment from May onwards (Fig. 3).

Laboratory experiments indicated that feeding female *Temora stylifera* and *Centropages typicus* for 1 d on ISO or PRO immediately after collection did not enhance naupliar N1 survival compared to control females fed on the NFA. Naupliar survival in ISO-fed and PRO-fed females was, in fact, 25 and 26% for *T. stylifera*, and 52 and 38% for *C. typicus*, respectively. These values were similar to naupliar survival in NFA-fed females; 21% for *T. stylifera* and 39% for *C. typicus* (Fig. 4a). These values did not change even when females were continuously fed on ISO or PRO for

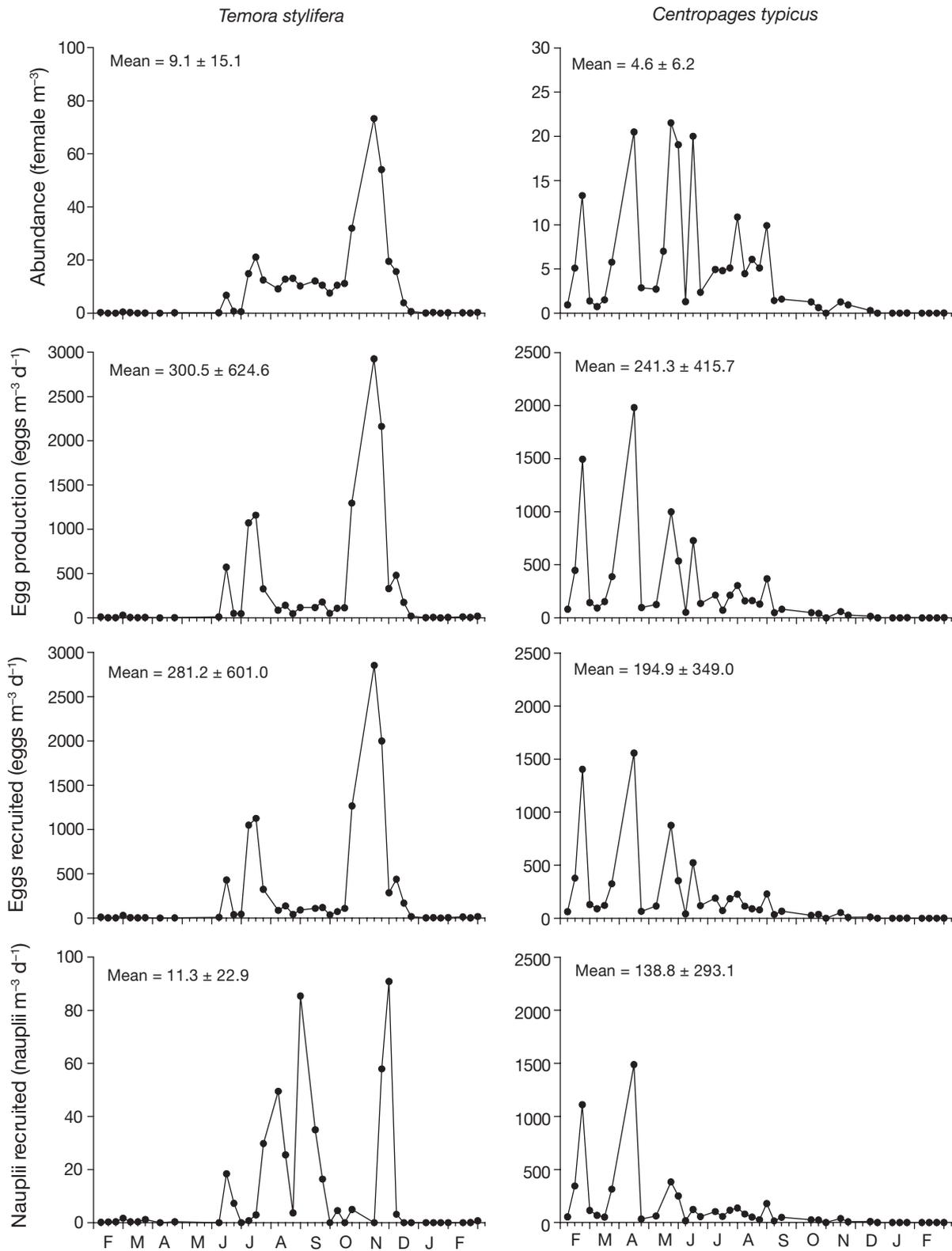


Fig. 3. *Temora stylifera* and *Centropages typicus*. Annual cycle of female abundance (female  $m^{-3}$ ), total egg production (eggs  $m^{-3} d^{-1}$ ), total egg recruitment (eggs recruited  $m^{-3} d^{-1}$ ), and total naupliar N1 recruitment (nauplii recruited  $m^{-3} d^{-1}$ ) rates, at Stn MC from February 2000 to February 2001. Annual mean  $\pm$  SD

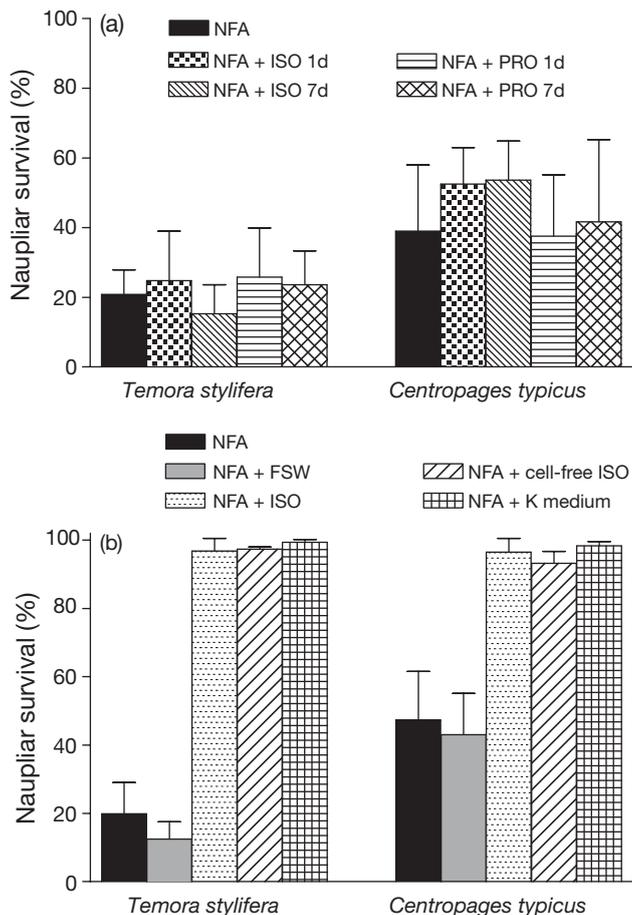


Fig. 4. *Temora stylifera* and *Centropages typicus*. (a) Naupliar N1 survival (%) in seawater containing a natural food assemblage (NFA) and when females are fed on NFA, NFA + *Isochrysis galbana* or NFA + *Prorocentrum minimum* for 1 d and 7 d. (b) Naupliar N1 survival (%) in NFA, NFA + filtered seawater (FSW), NFA + *I. galbana*, NFA + cell-free *I. galbana*, or NFA + K algal medium, when females are fed on NFA. Means  $\pm$  SD

7 d, resulting in a naupliar survival of 15 and 24% in *T. stylifera*, and 54 and 42% in *C. typicus* ISO-fed and PRO-fed females, respectively (Fig. 4a).

In contrast, the addition of an ISO cell culture, or supplementing with an ISO cell-free culture or K medium (inorganic salts and vitamins), to the natural phytoplankton assemblage, enhanced naupliar survivorship in *Temora stylifera* and *Centropages typicus* to >96 and >94%, respectively (Fig. 4b). Naupliar survivorship in parallel containers without the addition of food or inorganic material was 15 and 50%, respectively, similar to values recorded in September and October 2000 for *T. stylifera*, and in June and July 2000 for *C. typicus* (Fig. 1). Similarly low values of naupliar survivorship were observed in containers that received fresh filtered seawater (12% in *T. stylifera* and 43% in *C. typicus*), indicating that the beneficial effect of

adding ISO or K medium was not due to diluting a potential noxious effect of the original seawater (such as low oxygen content, bacteria, etc). Although the 2 species had different naupliar survival under natural conditions ( $t$ -test:  $t_6 = 3.65$ ,  $p < 0.01$ ), the addition of either algae, filtered algal culture, or only K medium, promoted the same high survival in both species (ANOVA:  $F_{5,12} = 1.78$ ,  $p > 0.05$ ), indicating similar requirements once the nauplii had hatched.

A CLSM image in transmitted light of N1 nauplii of ISO-fed *Temora stylifera* is shown in Fig. 5a. Corresponding fluorescent images showed no discernible gut fluorescence linked to ingested algal cells, and only a weak autofluorescence of the chitinous skeleton (Fig. 5b). In contrast, N2 nauplii fed on the same diet (Fig. 5c) presented a bright fluorescent material in the stomach (Fig. 5d), corresponding to the ISO cells ingested by the larvae. These results confirm that *T. stylifera* and *Centropages typicus* (images not shown) begin feeding at the N2 stage, in agreement with the findings that very few copepods feed at the N1 stage (Mauchline 1998).

## DISCUSSION

Our field data indicate that although there was little annual variation in the hatching success of *Temora stylifera* and *Centropages typicus* eggs, very strong fluctuations occurred in naupliar N1 percentage survival. Expressed in terms of percentage of mortality, the annual mortality of N1 nauplii in *T. stylifera* (87.7%) and *C. typicus* (33.4%) was much higher than egg mortality alone (12.4 and 19.9% for *T. stylifera* and *C. typicus*, respectively), and the sum of the 2 mortalities was >50% for both species. Interestingly, for similar numbers of females (9.1 in *T. stylifera* and 4.6 in *C. typicus*), eggs produced (43.9 in *T. stylifera* and 54 in *C. typicus*) and eggs hatched  $m^{-3} d^{-1}$  (281.2 in *T. stylifera* and 194.9 in *C. typicus*), there was 1 order of magnitude difference in the number of nauplii recruited  $m^{-3} d^{-1}$  (11.3 in *T. stylifera* and 138.8 in *C. typicus*), indicating that death rates were much greater for *T. stylifera*.

Both species had maximum egg-production rates in spring (up to 120 eggs female $^{-1} d^{-1}$ ), which differs somewhat from a previous study on *Temora stylifera* in the Gulf of Naples when the same fecundity for this species was recorded in autumn (Ianora & Poulet 1993). The same authors also recorded lower annual hatching success in *T. stylifera* (77.3%) compared to the present study (86.6%) indicating inter-annual variability in both egg production and hatching success for this species. In the case of *Centropages typicus*, Ianora et al. (1992) recorded 56 eggs female $^{-1} d^{-1}$  in 1989 and

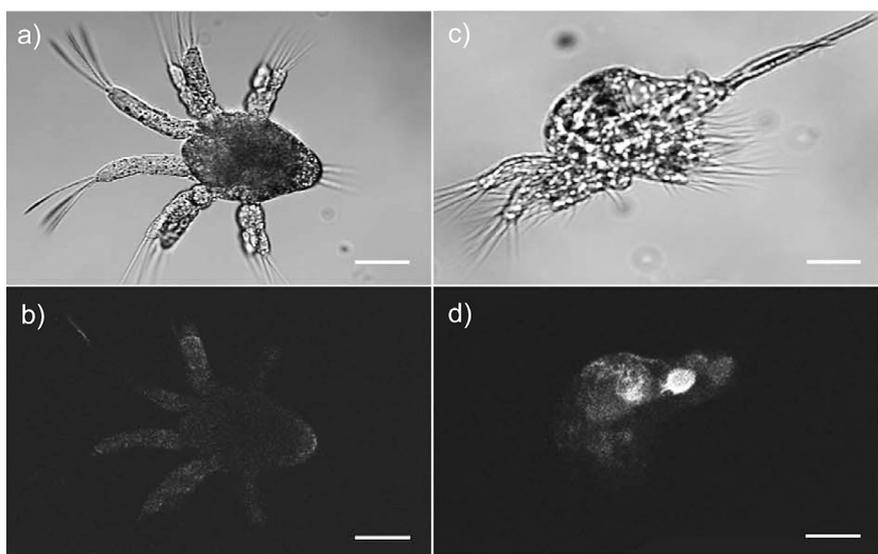


Fig. 5. *Temora stylifera*. Confocal Laser Scanning Microscope (CLSM) images of N1 nauplii fed on *Isochrysis galbana* in (a) transmitted and (b) fluorescent light. The same images of N2 nauplii fed *I. galbana* in (c) transmitted and (d) fluorescent light. The bright area in (d) corresponds to the autofluorescence of ingested algal cells. Scale bar = 40  $\mu\text{m}$

61 eggs female<sup>-1</sup> d<sup>-1</sup> in 1990, very similar to the values reported here (54 eggs female<sup>-1</sup> d<sup>-1</sup>). Corresponding values for hatching success were 73% in 1989 and 84% in 1990, compared to 80% in 2000, indicating that fecundity and hatching were more stable inter-annually in this species.

Even though egg production rates were maximal for both species in spring, only in *Centropages typicus* did this give rise to an increase in adult female numbers in the same period, whereas in the case of *Temora stylifera* all of this production was 'lost' and only the eggs produced after July, when egg-production rates were at an annual low, were recruited to adulthood. These results differ somewhat from those reported by Di Capua & Mazzocchi (2004) who investigated the population structure of *C. typicus* and *T. stylifera* over an annual cycle in the same area the previous year (1999–2000). These authors found peak values of 1724 *T. stylifera* nauplii (N1–N6) m<sup>-3</sup> compared to 100 *C. typicus* nauplii (N1–N6) m<sup>-3</sup>, but the number of copepodites (C1–C5) sampled (350 ind. m<sup>-3</sup> in *C. typicus* and 275 ind. m<sup>-3</sup> in *T. stylifera*) was more or less the same. Hence, much higher initial naupliar recruitment rates were found in *T. stylifera* in their study, even though mortality increased in the later developmental stages.

Seasonal changes in egg-production rates of both species were positively correlated with corresponding egestion rates. In turn, both rates were positively correlated with annual integrated chl *a*. This suggests that faecal pellet production rate in *Temora stylifera* and

*Centropages typicus* is a good proxy for their ingestion rate, as already observed in other copepod species (Nejstgaard et al. 2001, Besiktepe & Dam 2002). The data also show that the fecundity of *T. stylifera* and *C. typicus* was related to *in situ* chl *a* concentrations, whereas hatching success and naupliar survivorship were not, suggesting that the viability of eggs and N1 nauplii was not related to food availability, but more likely, to food quality.

To date, most studies dealing with food quality effects of maternal diets on copepod reproduction have focused on copepod egg-hatching success. Our data indicate that this may be insufficient to account for all early copepod stage mortality. Since both eggs and pre-feeding nauplii rely on maternal yolk reserves, the causes of egg and N1 mortality should likely be the same, and related to the past feeding history of females.

Poor phytoplankton diets have been shown to induce low copepod hatching success. For example, some diatom diets have been shown to potentially reduce copepod egg viability by up to 100% (reviewed by Ianora et al. 2003). This is due to the production of polyunsaturated aldehydes (Miralto et al. 1999) that are cleaved from fatty acid precursors activated by enzymes within seconds after crushing of the cells (Pohnert 2002). Also, field studies have shown that hatching success of copepod eggs can plummet to as low as 15% during certain diatom blooms (Miralto et al. 1999, 2003). Maternal diatom diets in the field may also negatively affect the development of hatched nauplii. Ianora et al. (2004) found that up to 90% of the N1 nauplii of *Calanus helgolandicus*, spawned during a *Skeletonema costatum* bloom, were unable to moult to the N2 stage. In other cases, diatom cell densities may be insufficient to inhibit hatching success, but high enough to reduce early larval development. Ban et al. (2000), for example, found that although egg viability remained high during a diatom bloom, 20 to 40% of the hatched nauplii were abnormal and therefore non-viable. On the other hand, in a worldwide survey across several coastal and oceanic regions, investigators failed to find a link between diatom density and copepod hatching success *in situ* (Irigoien et al. 2002). This can be due to species-specific or strain-specific differences in diatom aldehyde production (Wichard et al. 2005), differing sensitivity of copepod species to diatom aldehydes (Ianora et al. 2003), copepod feed-

ing behavior and food selection, or intensity and duration of diatom blooms in the field.

In our field study, the presence of diatoms was not correlated with low hatching success or naupliar survivorship of *Temora stylifera* and *Centropages typicus*. In 2000, 2 main diatom peaks occurred in spring and several smaller peaks occurred in summer and autumn, whereas low naupliar survivorship in *T. stylifera* and *C. typicus* was not confined to these events but randomly distributed over the year. Phytoplankton communities of the coastal area of the Gulf of Naples are generally well diversified, as a result of the high temporal and spatial variability of physical and chemical parameters that characterize the area. A large number of species belonging to different microalgal classes coexist and succeed throughout the year (our study and Ribera d'Alcalà et al. 2004). This situation differs from the intense, long-lasting (3 mo) and almost monospecific diatom blooms that characterize the Adriatic Sea, and that have been shown to affect both copepod hatching success (Miralto et al. 1999, 2003) and naupliar survivorship (Ianora et al. 2004). The occurrence of a mixed and highly variable phytoplankton assemblage, together with the difficulties of establishing *in situ* copepod diets, makes it difficult to relate the copepod recruitment reported in our study to diatom biomass. Since high diatom densities and long exposure times are required to induce low hatching success/naupliar survivorship in copepods (Ianora et al. 2003).

Other aspects of food quality may have affected our present results. For example, investigations on the nutritional effects of a balanced diet on copepod reproduction have shown that copepods feeding on polyunsaturated fatty acid-rich diets result in higher egg viability (Jónasdóttir 1994, Jónasdóttir & Kiørboe 1996), and that the addition of cholesterol to a diatom diet improves both egg production and hatching viability in some copepods (Hassett 2004). However, high egg viability may be uncoupled to high naupliar fitness. Even though high hatching success (74 to 98%) of the copepod *Calanus helgolandicus* at a coastal station in the western English Channel was correlated with (n-6) fatty acid levels in the surrounding environment and in the eggs, there was a high incidence of deformed nauplii (up to 18% of hatched eggs) (Pond et al. 1996).

Our laboratory experiments showed no enhancement of naupliar survival after freshly caught females were fed for up to 7 d on ISO or PRO, both diets that support high egg viability (Ianora & Poulet 1993, Ianora et al. 1995, Miralto et al. 1995) and successful larval development (Bonnet & Carlotti 2001, Carotenuto et al. 2002) in both species. These results may be due to the strong negative effects of female diets at sea during the time of

sampling, which did not disappear even after 7 d of feeding on a high quality food. *Temora stylifera*, for example, has already been found to be extremely sensitive to certain algal diets, and switching females from a poor to a good diet does not reverse the negative effects on hatching success (Ianora et al. 2003). On the other hand, they indicate that maternal history at any temporal scale may have little effect on naupliar survival, and that the quality of the water in which the larvae hatch is essential for their survival. Our results indicate that although *T. stylifera* and *Centropages typicus* N1 nauplii are non-feeding stages (no algal cells were observed in their gut when fed ISO soon after hatching), they seemed to be capable of taking up dissolved material from the medium that increased their survival. Copepod nauplii have already been shown to be capable of 'drinking' (Tester & Turner 1991, R. Strickler unpubl. data), even though the advantages of doing so are unclear. Although we do not know which component of the culture medium may have enhanced N1 survival, we speculated that vitamins such as biotin, B<sub>12</sub>, or thiamine might have played a role. However, since vitamin concentrations supplied in our experiments were between 10 and 100 times higher than those measured in seawater (Okbamichael & Sañudo-Wilhelmy 2004), it is unlikely that vitamins have a beneficial effect on larval survival at sea.

Whether ambient water conditions affected *in situ* naupliar survivorship in our study is an open question but if this were true, one would expect co-variation of naupliar survivorship in the 2 species, since they were exposed to the same water during sampling. However, not only was survivorship uncorrelated between the 2 species (Pearson:  $r = 0.15$ ,  $p > 0.05$ ), but both species also had strongly different naupliar survival. Higher naupliar survivorship in *Centropages typicus* compared to *Temora stylifera* may be explained with a differential effect of maternal diets in these 2 species. *C. typicus*, in fact, is considered to be a more omnivorous species (Bonnet & Carlotti 2001), and shows opportunistic feeding behavior (Saiz et al. 1992), being able to switch from herbivory to raptorial feeding. A wider food spectrum would, therefore, allow the dilution of possible deleterious effects, as well as compensate for potentially missing nutrients, due to monospecific diets.

Our laboratory estimates of egg- and pellet-production rates, hatching success and naupliar survivorship of *Temora stylifera* and *Centropages typicus* were not affected by differences between ambient temperatures and the temperature at which we conducted our experiments (20°C). In the Gulf of Naples, ambient temperatures in the 0 to 10 m layer generally follow a sinusoidal pattern from 14 to 26°C, with lower monthly averages from December to April (average  $15 \pm 2^\circ\text{C}$ ) and higher

monthly averages from May to November (average  $22 \pm 4^\circ\text{C}$ ) (Ribera D'Alcalà et al. 2004). Saiz et al. (1999) have shown that differences in egg production rates of *C. typicus* incubated at 13, 16 or  $20^\circ\text{C}$ , were not significant and concluded that the procedure of conducting incubation experiments for 24 h in surface waters with a temperature of ca.  $20^\circ\text{C}$  did not affect estimated egg-production rates. Changes in incubation temperature require a longer period (at least 2 d) to be reflected in the egg-production rates. Similarly, Devreker et al. (2005) showed that a difference up to  $\pm 8^\circ\text{C}$  between *in situ* and experimental temperature, did not affect the hatching success of *Temora longicornis*.

We also rule out the possibility that our laboratory incubation of hatched nauplii for an additional 24 h somehow increased naupliar mortality due to some noxious effect of the original medium (low oxygen content, bacteria, etc.). Our results with the addition of fresh filtered seawater to the natural food assemblage, in fact, showed no enhancement of naupliar survival and confirmed that the original incubation medium was not harmful.

In conclusion, our results show that high hatching rates in *Temora stylifera* and *Centropages typicus* can be followed by very low early larval survival, with serious implications for population *in situ* recruitment rates. Finding the causal agent of this 'natural mortality', which is not due to predation, cannibalism or lateral advection, is not easy and is hampered by several difficulties, including scarce knowledge of copepod diets *in situ* and little information on the effects of maternal food quality on offspring fitness, etc. Moreover, since nauplii seem capable of drinking, high naupliar mortality may not only be related to poor maternal diets but also to the quality of the water in which the nauplii hatch. Future studies scoring maternal and environmental effects on the recruitment of copepod populations at sea should therefore also address the issue of compromised larval fitness.

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