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Larval growth in the dominant polychaete *Polydora ciliata* is food-limited in a eutrophic Danish estuary (Isefjord)

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ABSTRACT: Food limitation in larval growth of the spionid polychaete *Polydora ciliata* was examined in a typical eutrophic estuary, Isefjord, in Denmark. In the field, food availability and the energetic requirements of the *P. ciliata* larval population were measured during 2 different periods in 2004 and 2007 that together cover the productive part of the year for plankton. In the laboratory, specific growth rates (μ) of larvae reared on natural food suspensions (~0.10 d^{-1}) were always lower than those of larvae reared on phytoplankton-enriched food suspensions (100% retention efficiency for *Rhodomonas salina*; ~0.21 d^{-1}). Total meroplankton biomass (average: 3.72 μg C l^{-1}, range: 0.11 to 26.05 μg C l^{-1}) was frequently similar to or exceeded that of holoplankton (average: 5.70 μg C l^{-1}, range: 0.08 to 29.89 μg C l^{-1}), suggesting a trophic significance of meroplankton in the estuary. *P. ciliata* was commonly the dominant meroplanktonic larvae (average: 0.5 μg C l^{-1}, range: 0.03 to 2.51 μg C l^{-1}). The available food in the optimal prey size fraction (2004, average: <20 μm; range: 99 to 274 μg C l^{-1}; 2007, average: 7 to 18 μm; estimated carbon demand: 119 μg C l^{-1}; range: 19 to 474 μg C l^{-1}) seemed to be sufficient to cover the energetic carbon requirements of the population throughout the study (0.09 to 3.15 μg C l^{-1} d^{-1}), but insufficient to attain the maximum specific growth rates reported in previous laboratory experiments. This suggests that *P. ciliata* larvae probably exhibit a low feeding efficiency and their maximum specific growth rates are consequently attained at food concentrations even higher than those found in this eutrophic environment.

KEY WORDS: Food limitation · Larval growth · *Polydora ciliata* · Larvae

INTRODUCTION

Spawning of benthic invertebrates, including *Polydora ciliata*, results in the release of a large number of planktonic larvae (meroplankton) which spend a variable period of time, from minutes to months, in the water column (Thorson 1950, 1964). Since planktonic food resources are highly patchy, it is likely that larvae are often food limited in natural environments, i.e. natural food concentrations are below those required to support maximum rates of growth and development (Olson & Olson 1989, Fenaux et al. 1994). Food limitation prolongs the planktonic period and increases the exposure of larvae to other sources of mortality such as predation (Thorson 1950). Consequently, food limitation during the planktonic larval stages is suggested to decrease recruitment into benthic populations (Thorson 1950, Vance 1973, Olson & Olson 1989).

Beside food quantity, food quality should be taken into account as well when evaluating potential food-limited growth in nature. Plankton cells may be too large or small for larvae to catch and ingest and can vary considerably in nutritional quality (Pechenik 1987). Bivalve, gastropod and many capitellid polychaete larvae retain

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food particles in the nano-fraction (2 to 20 µm) from a few to 15 µm equivalent spherical diameter (ESD) (Riisgård et al. 1980, Hansen 1993, Raby et al. 1997). In contrast, small spionid polychaete larvae (5 to 10 setigers) are able to ingest large centric diatoms, e.g. *Coscinodiscus* spp. (Anger et al. 1986). Recent experiments indicate that the particle regime which *Polydora ciliata* can exploit increases during the larval ontogeny from 4 to 50 µm ESD, and the optimal food size for small larvae (230 ± 30 µm in length) is around 12 µm (Hansen et al. unpubl.). A similar ontogenetic development of the retention spectra has previously been reported in gastropod veliger larvae (Hansen 1991). Thus, food limitation is not solely due to a general low food concentration; i.e. a match between available food size and the physiological capabilities of the larvae is also crucial for optimal feeding and growth.

Food limitation is thought to be more important for crustacean larvae than for the ciliated larvae of bivalves and echinoderms (Olson & Olson 1989). Furthermore, food limitation is expected to be less important in coastal waters than in oceanic waters (Huntley & Boyd 1984). However, food limitation in coastal waters has been reported for bivalve (Fotel et al. 1999) and echinoderm larvae (Fenaux et al. 1994, Eckert 1995). In case of polychaete larvae, studies are very scarce but the existing research suggests food-limited growth during summer (Paulay et al. 1985, Hansen 1999).

Spionid polychaetes, and very often *Polydora* spp., are among the most common invertebrates in neritic benthic environments. During certain periods, their planktotrophic larvae can be the major component of mesozooplankton (200 to 2000 µm) (Schram 1968, Anger et al. 1986 and references therein, Zajac 1991, Pedersen et al. 2008), suggesting an important role as grazers on primary producers. However, the possible trophic function in the marine carbon flow of polychaete larvae, as well as other meroplanktonic larvae, remains largely unknown. In order to fully understand the function of planktotrophic larvae in the ecosystem, their temporal and spatial abundance as well as their physiological requirements must be assessed.

The objectives of the present study were to first test if larvae of the spionid polychaete *Polydora ciliata* exhibited food-limited growth *in situ* throughout the entire productive part of year in plankton. To address this topic we carried out 2 experimental approaches: (1) we determined the food availability (in terms of quantity and quality [size]) for *P. ciliata* larvae in its habitat and evaluated if it was enough to support the maximum growth rates *in situ* as observed in previous laboratory experiments (Almeda et al. 2009); (2) we assessed the degree of food limitation by comparing growth rates of larvae reared on natural food suspensions with those reared on natural food suspensions enriched with cultivated phytoplankton (*Rhodomonas salina*) in excess in the laboratory. The second objective of the present study was to estimate the energy requirements of *P. ciliata* larval populations and their trophic significance.

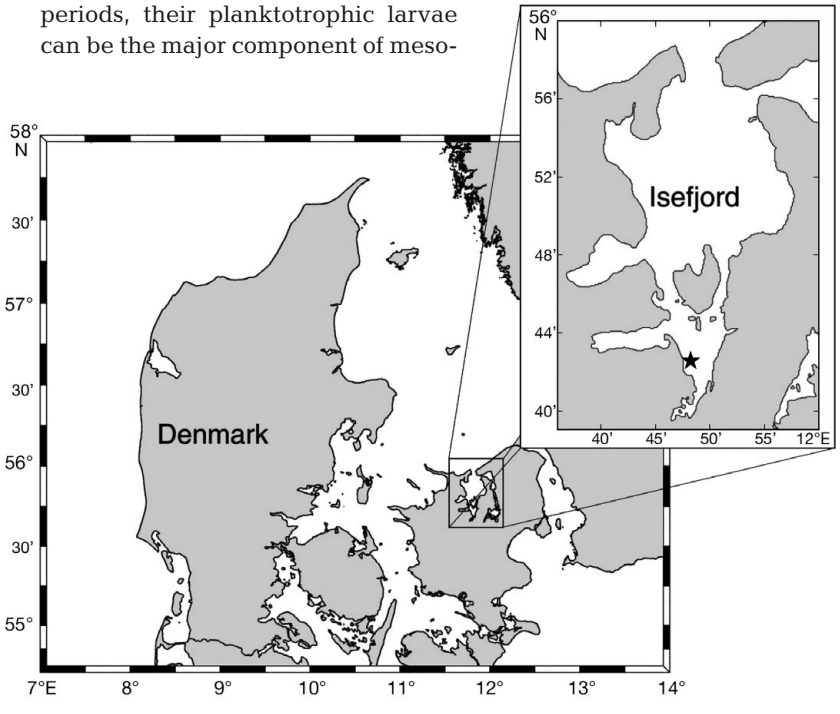

Fig. 1. Søminestationen sample station (★) in the Danish estuarine system, Isefjord (55° 42′ 44.45″ N, 11° 47′ 45.48″ E)

MATERIALS AND METHODS

Field site and sampling. The present study was conducted during 2 periods in 2004 (April to September) and 2007 (September to November), covering the entire productive part of the year, at the field station Søminestationen (Fig. 1) situated in the Isefjord, Denmark. The field site has a depth of 4 m and is characterized by muddy bottom sediments and eutrophic waters (Rasmussen 1973), being a relevant example of a boreal shallow water estuary (Conley et al. 2000).

Quantitative zooplankton samples were collected every 3 to 4 wk by duplicate hauls in 2004 and more frequently (every second day to second week) by single hauls in 2007. The hauls were conducted from bottom to surface with a 45 µm mesh size WP-2 net equipped with a closed cod-end and a digital flow meter (Hydro Bios, model 438 110). Samples were gently concentrated on a

45 µm screen and stored in containers with 80 to 96% ethanol in final concentration.

Live zooplankton for growth experiments were gently collected by trawling the WP-2 net equipped with a 1 l closed cod-end at slow speed through surface water (0 to 2.5 m). Immediately after the net was retrieved, zooplankton were diluted by surface water and brought to the laboratory in a 50 l thermo box.

Seawater samples for food availability studies and growth experiments were collected every second day during growth incubation experiments. The discrete samples were taken at 0, 1, 2, 3 and 4 m depth by a 3 l heart-valve water bottle sampler pooled in a 30 l bucket; these were considered integrated water column samples. Temperature was also measured every second day during growth incubation experiments by a thermometer attached to the sampling bottle, and salinity was determined by a hand-held refractometer (ATAGO) with a resolution of 0.5 salinity units.

Food availability. According to an ongoing study (Hansen et al. unpubl.), the particle regime *Polydora ciliata* can exploit increases during the larval development from 4 to 50 µm ESD, and the optimal food size for small larvae (230 ± 30 µm in length) is around 12 µm (range: 7 to 18 µm). Therefore, large phytoplankton chains or colonies can most likely not be captured by *P. ciliata* larvae. We used 2 experimental approaches to determine the food availability for *P. ciliata* larvae: (1) fractioned chlorophyll *a* (chl *a*) measurements and (2) plankton community analysis.

Fractioned chl a measurements: We fractionated collected seawater through Nitex screens and into different size fractions (<20, 20–50, 50–200 and >200 µm) in order to assess the available phytoplankton biomass for *Polydora ciliata* larvae. Water samples (50 ml) of each fraction were filtered in triplicate through GF/F filters by use of syringes and filter capsules. The filters with retained particles were extracted overnight in 96% ethanol as the extraction solvent (according to Jespersen & Christoffersen 1987). If necessary, the extract was filtered through 0.2 µm filter capsules or centrifuged to get rid of particles. Chl *a* and phaeopigments in the extractions were measured in quartz glass cuvettes on a Turner Designs 700 fluorometer.

Plankton community analysis: We analysed the size structure and morphological composition of the plankton community in order to assess the available food for *Polydora ciliata*. Samples of natural seawater were fixed in acid Lugol's to a final concentration of 5%, stored in brown glass bottles and analysed in an automated microscope (FlowCAM®, www.fluidimaging.com) within 48 h after fixation. Each sample was analysed in triplicate for 20 min. Images of plankton particles were continuously acquired by a video camera. The automated microscope counted and registered the metric dimensions of the particles and assigned a unique identifier to each which was used later for image retrieval and sample analysis (Sieracki et al. 1998). We used the autoimage mode data acquisition in the software package VisualSpreadsheet (VISP, v 1.5.16, www.fluidimaging.com) with a minimal particle size of 5 µm. The procedure allowed us to derive individual particle width (W) and length (L), while particle volume (V) was calculated assuming prolate spheroid particle shapes:

$$V = \frac{\pi \times W^2 \times L}{6} \quad (1)$$

The aspect ratio (W/L) was used to differentiate between chain-formed and spherical particles. The relevant aspect ratio was decided for each sample run, but it was typically around 0.4. The individual particle volumes were converted to cell carbon using the carbon:volume relationship given by Montagnes et al. (1994) for autotrophic flagellates (spherical particles). The chain-formed particles presented a different challenge. Because chains are formed by smaller cells with similar carbon content, we applied a constant carbon:volume relationship. This fixed value was based on inspections of the images generated by the FlowCAM which revealed dominance of diatoms of the species *Skeletonema* cf. *costatum* during periods when chain-formed particles were present. Typically, these cells had a size of 20 µm which corresponds to a carbon:volume ratio of 0.06 using the appropriate isometric carbon:volume relationship for diatoms (Montagnes et al. 1994).

Total phytoplankton biomass (µg C l^{-1}) was regressed against the measured chl *a* (µg chl *a* l^{-1}) to obtain a conversion factor. This conversion factor was applied to calculate the available phytoplankton biomass in term of carbon from the chl *a* measurements.

Growth experiments. We carried out 9 growth experiments during the 2 study periods (Table 1). *Polydora ciliata* larvae were concentrated from the container by light attraction using a cold fiber optic light source. Early larvae (<300 µm in length) were sorted with a pipette under a dissecting microscope and incubated within a few hours from collection. Larvae were incubated for 5 d in 2 types of food treatments (3 to 5 replicates per treatment except in September 2004, see 'Results'): (1) natural seawater from the sampling site (screened through 200 µm), i.e. natural food suspensions; and (2) natural seawater (screened through 200 µm) with added culture phytoplankton (*Rhodomonas salina*, ~9 to 10 µm ESD) in excess, i.e. enriched food suspensions.

Excess of food (~40 000 cells ml^{-1}) was obtained from published saturation levels of functional food responses according to Almeda et al. (2009). Incubations were conducted in 70 ml acid-washed glass bottles

Table 1. *Polydora ciliata.* Larval concentration and initial and final larval length in the growth experiments (*converted from number of setigers according to Hansen 1999). Percent cleared in the bottles per day is calculated from the clearance rate versus body length relationship presented in Hansen et al. (unpubl.). The minimum and maximum clearance is based on the initial and final body length, respectively, and the intermediate is an average of the 2, each in natural food suspension. Estimates of the pelagic life span are based upon the growth rates in natural and enriched food suspension. In these estimates the initial length was set to 2.5 setigers and the length at metamorphosis was set to 17 setigers (Anger et al. 1986)

Year	Experiment	Larval concentration (larvae ml^{-1})	Initial larval length (µm)	Final larval length (µm)		Bottle volume cleared (% d^{-1})			Pelagic life span (d)	
				Natural	Enriched	Min.	Intermediate	Max.	Natural	Enriched
2004	May	0.5	388.8 ± 18.5*	645.8 ± 17.2*	801.8 ± 50.7*	37.5	48.8	60.0	42.8	27.4
	Jun	0.5	245.9 ± 16.4*	421.7 ± 33.4*	678.5 ± 42*	21.5	31.0	40.5	47.9	19.7
	Aug	0.5	127.3 ± 0*	342.2 ± 95.9*	663.1 ± 50*	11.5	21.0	30.5	23.9	12.3
	Sep	0.5	241.3 ± 23*	462.1 ± 58.9*	488.1 ± 25.8*	21.0	32.0	43.0	46.4	38.9
2007	Sep (initial)	1.43	229.4 ± 14.5	283.6± 17.2	524.2± 12.1	57.1	64.3	71.4	57.8	15.0
	Sep (terminal 1)	1.43	217.7 ± 25.3	313.4 ± 12.7	435.7 ± 17.6	54.3	68.6	82.9	32.8	17.2
	Sep (terminal 2)	0.86	212.9 ± 60.6	283.7 ± 8.5	350.5 ± 7.8	31.7	37.3	42.9	29.8	17.1
	Oct	0.71	295.2 ± 52.5	452.4 ± 5.6	612.5 ± 33.1	38.6	49.6	60.7	27.0	16.0
	Nov	0.57	244.1 ± 1.8	318 ± 14.6	490.4 ± 7.8	24.0	28.9	33.7	49.5	17.0

placed on a plankton wheel at 0.5 rpm in dim light at 16°C. Larval densities varied depending on the experiment (Table 1). In the September terminal 1 experiment, we conducted an additional growth experiment (September terminal 2) with a different larval density in order to assess possible crowding effects. An initial subsample of larvae from each experiment was taken and fixed for length and setiger number determination. Every second day, 80% of the food suspension was removed by inverse filtration through 45 µm mesh and the suspension renewed from pre-screened integrated *in situ* seawater collected the same day from the sampling area (for natural food suspensions) or by *Rhodomonas salina* in excess (enriched food suspensions). At the end of the incubation, larvae from each experimental bottle were concentrated and fixed with acid Lugol's solution for later size measurements.

Larval specific growth rates were estimated from the increases in somatic biomass according to the expression for logarithmic growth:

$$\mu = (24/T) \times \ln(W_f/W_i) \quad (2)$$

where T is the duration of incubation (h) and W_i and W_f are the initial and final carbon biomasses of the larvae (µg), respectively.

In 2004, larval biomasses were estimated by counting the number of setigers (~30 larvae replicate^{-1}). Average number of setigers (S) was converted to carbon biomass (W, µg) according to the equation (Hansen 1999):

$$W = (6.81 \times 10^{-3})S^{2.03},\ R^2 = 0.990 \quad (3)$$

In 2007, larval biomasses were estimated by measuring body length (~40 larvae replicate^{-1}). We converted average larval length (L, µm) to carbon biomass (W, µg) according to the equation (Hansen 1999):

$$W = (1.58 \times 10^{-4})L^{1.38},\ R^2 = 0.996 \quad (4)$$

Setiger counting was carried out under an inverted microscope (100×). To measure body length, preserved larvae were placed under a dissecting microscope equipped with a digital camera and at least 40 images of random chosen larvae were taken per bottle (40× or inverted microscope [100×], depending on the experiment). These images were used with ImageJ software to measure length.

Zooplankton abundance and energy requirements. Quantitative zooplankton samples were split by a Folsom plankton splitter and a minimum of 300 individuals were counted and identified. Carbon biomass of the individual zooplankton taxa, including *Polydora ciliata* larvae, was estimated using the formulas reported by Nielsen et al. (2007, their Table 5.1).

Energy requirements of the population of *Polydora ciliata* larvae were determined in terms of estimated carbon demand (ECD) according to equation:

$$ECD = (P\mu)/GGE \quad (5)$$

where P is the population biomass of *P. ciliata* larvae (µg C l^{-1}), µ is the specific growth rate (d^{-1}) and GGE (= specific carbon growth/specific carbon ingestion) is the gross growth efficiency assumed equal to 0.29, R^2 = 0.98 (Almeda et al. 2009). The population biomass of *P. ciliata* larvae was calculated as an average for each relevant month and the specific growth rates determined in our growth experiments were used.

Growth data were not normally distributed because of 2 outliers. Levine's test indicated that variances

were homogeneous, so further analysis was conducted on untransformed data. A 2-way ANOVA (Systat v.11) was conducted to determine the effects of treatment, time of growth experiment and their interaction on the specific growth rates. Multiple comparisons of significant effects were conducted with Bonferroni-corrected probabilities for planned comparisons, in particular comparing treatment for each experiment and seasonal differences within each treatment.

RESULTS

Abiotic factors and food availability

Temperature showed a typical seasonal pattern with spring temperatures of ~10°C, increasing in summer to around 20°C, then declining throughout fall to ~5°C (Fig. 2A). The salinity differed by ~5 units between September 2004 and September 2007 (Fig. 2A). Total

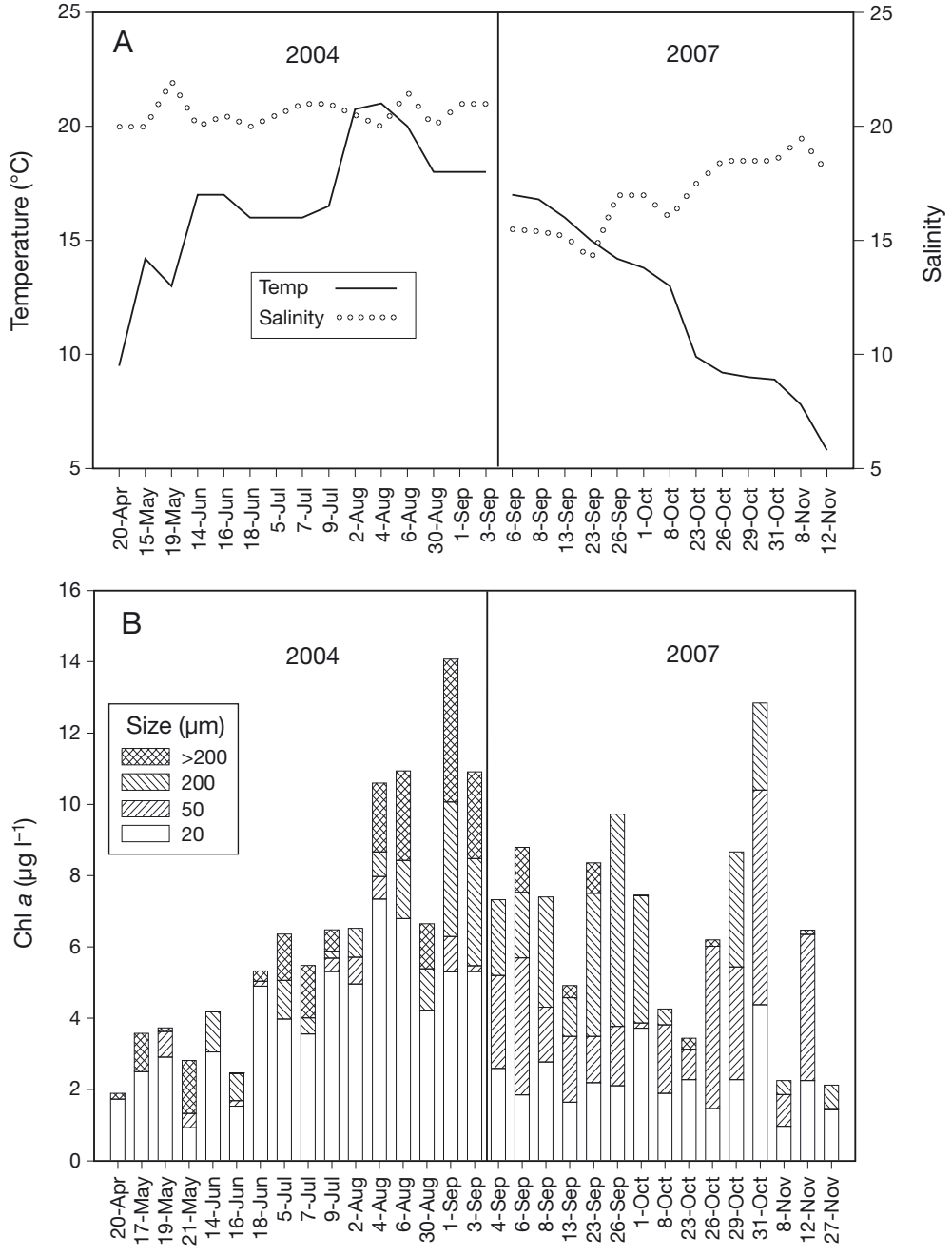

Fig. 2. (A) Integrated temperature and salinity and (B) size composition of the *in situ* phytoplankton community in Isefjord during the experimental periods in 2004 and 2007

chl *a* (Fig. 2B) reflects the bell-shaped pattern of the water temperature generated by the global radiation, with spring values of 2 to 3 µg chl *a* l^{-1}; values increased during summer, reaching ~10 µg chl *a* l^{-1}, and then declined with some fluctuations through the fall. The <20 µm phytoplankton size fraction was dominant most of the time from April to September 2004 (Fig. 2B) except 21 May, when the >200 µm size fraction was prevalent. In September 2007, phytoplankton were more or less equally distributed among 3 size classes (<20 µm, 20–50 µm, 50–200 µm) except on 23 and 26 September, when the 50–200 µm size fraction dominated. During October and November 2007, the 20–50 µm phytoplankton size fraction became more dominant until late November, when the phytoplankton present was either <20 or 50–200 µm. Phytoplankton >200 µm were mostly found in August and the beginning of September 2004.

Plankton biomass dynamics in 2007 estimated by the FlowCAM are shown in Fig. 3. The study site was highly dynamic in terms of plankton abundance and composition (Fig. 3A). Biomass fluctuations showed an initial dominance in chain-formed phytoplankton followed by 2 smaller biomass peaks of chain-formed particles, most likely *Skeletonema* cf. *costatum*, separated by ~20 to 30 d. The phytoplankton biomass outside the *S.* cf. *costatum* peaks was characterized by smaller ellipsoid particles. In September, most of the non-chained particles were dinoflagellates such as *Heterocapsa triquetra*, unidentified spheroid dinoflagellates of unknown trophy, cell doublets of *S.* cf. *costatum* and various unidentified flagellates. After the last pulse of *S.* cf. *costatum*, most of the particles were *H. triquetra* and small unidentified algae, while *S.* cf. *costatum* was present below the detection level. Since small larvae have much narrower prey size spectra than larger larvae (Hansen et al. unpubl.), we isolated the biomass fraction available for newly hatched larvae in the range of 7 to 18 µm (Fig. 3B).

Chl *a* was plotted against carbon, and a linear regression (±SE) rendered a carbon:chl *a* conversion factor of 47 (C = 47(±11)chl *a* – 81(±78); R^2 = 0.52, p = 0.0054).

Available carbon biomass for the prey size ranges that can be captured by *Polydora ciliata* larvae in the study area, corresponding to the date of the growth experiment, is shown in Table 2.

Zooplankton community and biomass of *Polydora ciliata* larvae

The density and biomass of both holo- and meroplanktonic organisms are depicted in Fig. 4. Generally, both density and biomass of holo- and meroplanktonic organisms were higher in May to August 2004 than in September to November 2007. High densities of Rotifera were observed in October 2007. The holoplankton was dominated, both in density and biomass, by Calanoida (Copepoda) throughout the sampling periods, with the exception of 30 August 2004 and October 2007, when Rotifera showed high biomass and densities. The meroplankton was dominated both in density and biomass by Polychaeta in May 2004. From June to August 2004, biomass was mostly dominated by Gastropoda, whereas density was dominated by Bivalvia. Throughout the rest of the sampling period (September to November 2007), Polychaeta dominated the meroplankton.

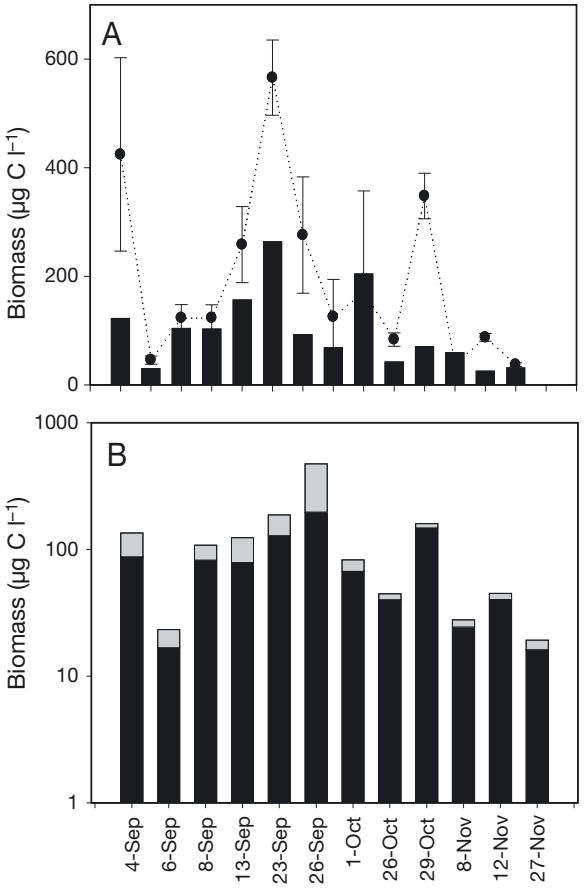

Fig. 3. Food availability in Isefjord during fall 2007 estimated by the FlowCAM. (A) Structural diversity of the standing plankton biomass. Closed circles are the total plankton biomass and the black bars are biomass of round particles (particles with aspect ratio >0.4). The difference between total carbon and round particles is mostly due to the biomass of the chain-formed diatom *Skeletonema* cf. *costatum* (B) Biomass of plankton particles in the 7–18 µm size range representing the optimal prey size spectrum of *Polydora ciliata* small larvae, according to Hansen et al. (unpubl.). Black bars are round particles (flagellates) and gray bars are elongated particles (chain-formed particles)

Table 2. *Polydora ciliata*. Available carbon biomass for the prey size ranges that can be captured by *P. ciliata* larvae in the study area corresponding to date of the growth experiments. Phytoplankton biomass was calculated by fractioned chlorophyll *a* measurements and plankton community by FlowCam analysis. na: not available

Year	Experiment	Phytoplankton biomass (µg C l^{-1})		Plankton community (µg C l^{-1}, 7–18 µm)
		0–50 µm	<20 µm	
2004	May	117	99	na
2004	Jun	153	149	na
2004	Aug	290	274	na
2004	Sep	276	249	na
2007	Sep (initial)	238	113	89
2007	Sep (terminal 1)	169	93	262
2007	Oct	256	119	95
2007	Nov	152	73	31

The relative proportion of holo- and meroplankton biomass is shown in Fig. 5. The meroplankton represented more than 50% of the planktonic biomass on 7 out of 18 sampling days, more than 80% on 3 sampling days and more than 90% of the total biomass on 26 September 2007. Hence, meroplankton indeed episodically dominated the zooplankton community.

Polydora ciliata was the most dominant polychaete larvae; it constituted 20 to 100% (average: 71%; Fig. 6) of the total polychaete larval population and was found in densities from 0.03 larvae l^{-1} in late October (31 Oct) to 8.05 larvae l^{-1} in May (17 May). On average, *P. ciliata* represented 38.5% of the total meroplanktonic biomass.

Growth experiments

The 2-way ANOVA showed that there was interaction between time of the growth experiments and treatment (F = 12.69, p << 0.005). This indicates that the effect of added food on larval growth differed during the course of the year. Specific growth rates of *Polydora ciliata* larvae were higher when offered enriched food suspensions (Fig. 7). Based on multiple comparisons, larval growth was significantly different between treatments (p < 0.05) in all experiments except in May and September 2004 (p ≤ 0.14 and p ≤ 1,

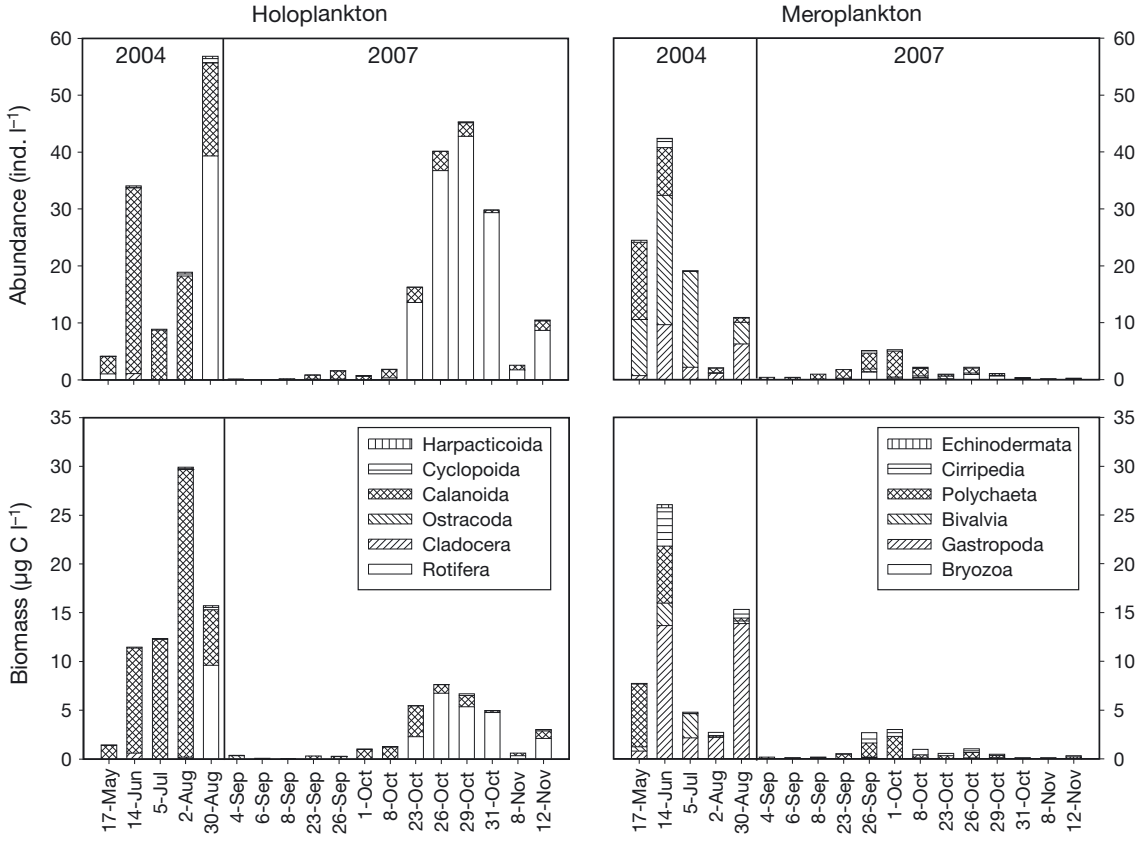

Fig. 4. Composition of the holoplankton and meroplankton in Isefjord, based on both abundance and biomass, for the 2 sampling periods in 2004 and 2007

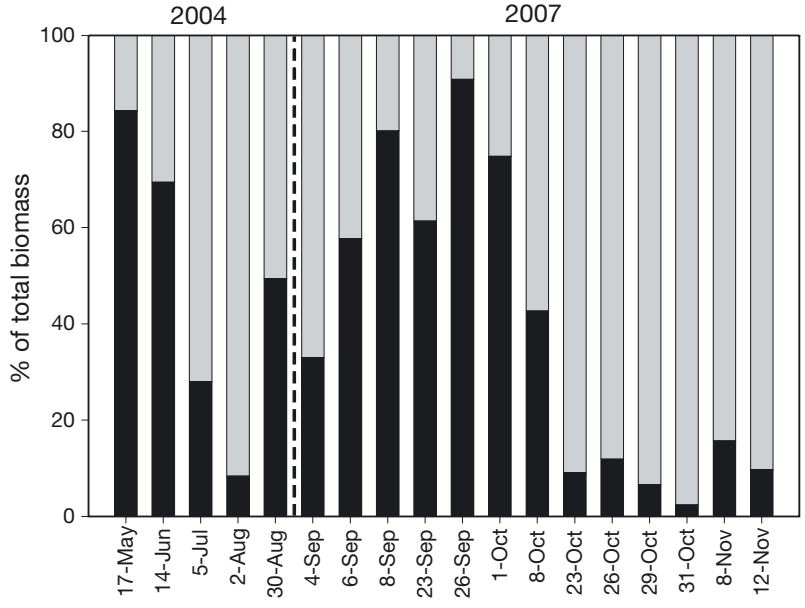

Fig. 5. Fraction of holoplankton (gray bars) and meroplankton (black bars) of the total zoo planktonic biomass in Isefjord for the 2 sampling periods in 2004 and 2007

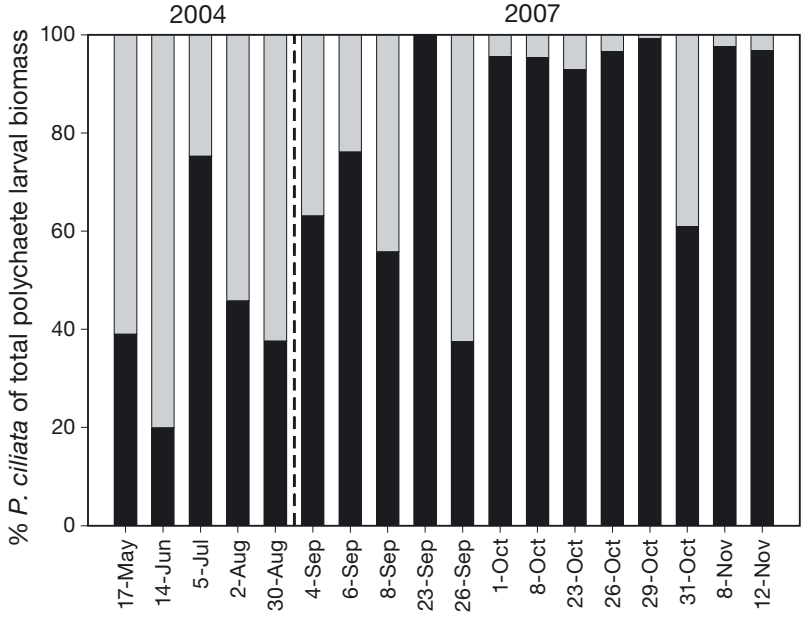

Fig. 6. *Polydora ciliata*. Percentage *P. ciliata* (black bars) of the total polychaete larval biomass (grey bars) in Isefjord for the 2 sampling periods in 2004 and 2007

respectively). The September 2004 growth experiment was only based on 2 replicates for each treatment, which put some constrains on the interpretation of the statistical output. Average specific growth rates on natural food suspensions were ~0.10 d^{-1}, 2-fold lower than that in enriched suspensions (average: ~0.21 d^{-1}). Larval survival was visually checked at the end of the incubation and we did not observe evident mortality in any treatment. The multiple comparisons also showed that there was no consistent effect of enrichment. For example, in 2007, there were no significant differences between the specific growth rates from the enriched experiments, while there were significant differences between many of the specific growth rates in natural food suspensions. Furthermore, there was no significant difference between the specific growth rate in natural food suspension in May 2004 and November 2007, but there was a significant effect of treatment in November 2007 in contrast to May 2004. The overall conclusion is that enrichment stimulated the specific growth rate of *P. ciliata* larvae.

Estimates of *Polydora ciliata* clearance in the experimental containers under natural food conditions were based upon the relationship between body length and maximum clearance rate from Hansen et al. (unpubl.). Maximum clearance ranged between 30.5 and 82.9% of bottle volume per day (Table 1). Using initial larvae length, the minimum clearance ranged between 11.5 and 57.1% of bottle volume per day (Table 1). The intermediate clearance was an average of the minimum and the maximum volume cleared and ranged from 21 to 68.6% (Table 1).

The number of setigers of newly hatched larvae (2 to 3 setigers) and the number of setigers of larvae ready for metamorphosis (17 setigers, according to Anger et al. 1986) were used to estimate the pelagic life span (Table 1) under natural and enriched food conditions. An average reduction of pelagic lifespan under optimal food conditions (i.e. enriched) as compared to natural food conditions was estimated to be as much as 19.6 d.

Estimated carbon demand

The ECD of *Polydora ciliata* larval population for the different growth experiments is shown in Table 3, and ranged from 0.07 to 1.23 µg C l^{-1}. During the entire study period, the available carbon biomass (in relevant size fractions, Table 2) exceeded by several orders of magnitude the food requirements to support the maximum growth of the population (Table 3).

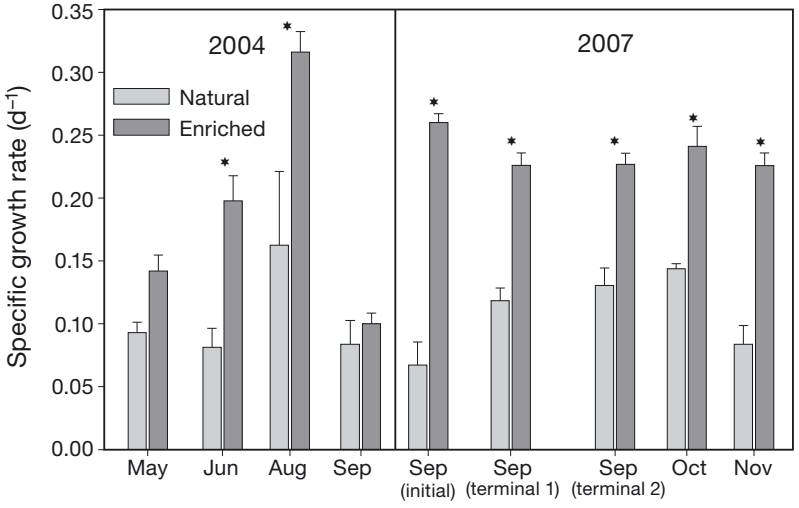

Fig. 7. *Polydora ciliata*. Specific growth rate (d^{-1}, ±SD) of *P. ciliata* larvae in natural and enriched food suspensions in Isefjord for the 2 sampling periods in 2004 and 2007. N = 35 to 100 larvae per incubation bottle. *Significant ($p < 0.05$) difference between treatments

Table 3. *Polydora ciliata*. Estimated carbon demand (ECD) of *P. ciliata* based upon biomass of larval population and the specific growth rates in natural and enriched food suspensions (see Eq. 5). Trophic impact equals ECD (natural) as % of the plankton community biomass in the size fraction that can be grazed by larvae (0–50 µm)

Year	Experiment	ECD (µg C l^{-1})		Trophic impact (%)
		Natural	Enriched	Natural
2004	May	0.81	1.23	0.69
2004	Jun	0.33	0.79	0.21
2004	Aug	0.04	0.07	0.01
2004	Sep	0.03	0.04	0.01
2007	Sep (initial)	0.02	0.07	0.01
2007	Sep (terminal 1)	0.21	0.39	0.12
2007	Sep (terminal 2)	0.23	0.40	0.14
2007	Oct	0.30	0.50	0.13
2007	Nov	0.03	0.08	0.02

DISCUSSION

Food availability and growth rate

According to recent laboratory studies (Almeda et al. 2009), maximum growth rates of *Polydora ciliata* larvae are reached, with optimal food size, at food concentrations ranging from 1410 to 2510 µg C l^{-1} depending on larval size. During the present study periods, the phytoplankton concentrations were always lower than these critical food levels. Moreover, if we consider the size fraction of plankton that can be effectively captured by larvae, the available carbon biomass is one order of magnitude lower than the critical food levels observed in the laboratory. Therefore, *P. ciliata* may not grow at their maximum rates at the Isefjord study site, which is a typical example of a Danish estuary. Considering the typical food concentration of Danish estuaries around the year (Conley et al. 2000), one can extrapolate these findings to other Danish coastal environments and to the remaining part of the year. Since newly hatched larvae only ingest a rather narrow prey size between 7 and 18 µm ESD (Hansen et al. unpubl.), recruitment of larger individuals is limited to the availability of particles within this size range. That is, at periods such as 26 September 2007, when we recorded the highest phytoplankton biomass, the particle window between 7 and 18 µm ESD only reached 480 µg C l^{-1}, which is close to one-fifth of the required biomass for maximum growth by the early larval stage.

Growth with natural vs. enriched food suspensions

The larval specific growth patterns between the 2 years were obviously different (Fig. 7). In 2004, specific growth in natural food suspensions fluctuated more than it did in 2007, and growth in the enriched food suspensions steadily increased during 2004, whereas it was more similar and high throughout 2007. Environmental characteristics varied between the 2 years: temperature development was very different, salinity was much lower in 2007 and available food steadily increased during 2004 and was more constant during 2007. Since growth incubations were performed at constant temperature in the laboratory both years, temperature most likely cannot explain the observed larval growth differences. Differences in salinity were presumably not the cause either, since *Polydora ciliata* is a well-adapted euryhaline species. The effect of food availability, however, may be reflected in the basic physiological condition of the larval populations. A gradual change in food concentration was reflected in a steady growth increase during 2004 for both natural and enriched food suspensions (except for the September experiment). It is more difficult, based on food availability, to explain the growth pattern observed in 2007. Therefore, the different growth patterns in both 2004 and 2007 could be due to a combination of the effects

of these environmental variables, or another factor altogether.

Specific growth rates of larvae reared on natural and enriched food suspensions indicated that larvae were not growing at their maximum rates in nature. Maximum growth rates observed in the enrichment treatments were similar to maximum growth rates of larvae observed under food satiating conditions in the laboratory (Almeda et al. 2009). However, artifacts from laboratory bottle incubation conditions are possible, e.g. the decline in food concentration during the 2 d between the water changes. The estimated clearances shown in Table 1 indicate that the larvae in 2 of the growth experiments (September initial and terminal 1 2007) could have experienced starvation through the whole experiment since the percent bottle volume cleared based upon the initial length was above 50% (57.1 and 54.3%, respectively). Furthermore, some larvae in 4 of the 9 growth experiments (May 2004 and September initial, September terminal 1 and October 2007) may have experienced starvation at the end of the experiment. But if one compares September terminal 1 and 2 (Fig. 7), there was only a slight difference in growth rates in natural food suspension. Moreover, growth rates in natural food suspensions in experiments with high larval densities (e.g. September initial and terminal 1 2007) were not different to those in experiments with low larval density (e.g. November 2007). Therefore, an eventual crowding effect cannot explain the differences in growth between treatments, and the depletion of food in the experimental containers was expected to be minimally important. Hence we conclude that the observed differences in growth between treatments are not due to the depletion of food between the water changes.

In addition to food quantity, food nutritional quality may influence larval growth rates of *Polydora ciliata*. The copepod *Temora longicornis* fed on *Skeletonema* cf. *costatum* at rates comparable to feeding rates on *Rhodomonas salina* in the laboratory. However, the copepod egg production and somatic growth was significantly reduced compared to controls fed with *R. salina* (Dutz et al. 2008). There is growing evidence that diatoms including *S.* cf. *costatum* contain biomolecules aimed at defending the algae against predation (Pohnert et al. 2002, Pohnert 2005) and it is very likely that the increased growth in the *R. salina* treatments by *P. ciliata* larvae is a result of improved food quality. But to answer such question we need a thorough analysis of phytoplankton growth and nutritional status as well as other factors influencing the functional food limitation and potential active food selection of meroplanktonic organisms.

Comparing the specific growth rates in both natural and enriched food suspensions, the time required to reach metamorphosis was greatly reduced when excess food was offered (Table 1). Enriching the available food resulted in an average reduction of the pelagic life span of 19.6 d, corresponding to a 47.6% reduction on average. On the one hand, this reduces the dispersal ratio of the propagules; on the other hand, it potentially reduces larval mortality and thereby increases benthic recruitment of *Polydora ciliata* larvae.

Energetic carbon demand and trophic significance

The available carbon was enough to support the food demand considering the food requirements (ECD) of *Polydora ciliata*. However, these larvae were not able to grow at maximum growth rates at *in situ* food concentrations. This suggests a low feeding efficiency and, hence, that the food limitation of larvae is functional. The food requirements of *Polydora ciliata* larvae constituted only negligible fractions of the standing stock of primary producers; therefore, the *P. ciliata* population had a very slight effect on the plankton community (low trophic impact, <1%, on total primary producers in energetic requirements, see Table 3). The daily food requirements (ECD) of the *Polydora ciliata* population corresponds well with the finding of Hansen et al. (1999) in a Greenlandic study, where meroplankton (mainly bivalves and gastropods) daily ingested just 0.12 µg C l^{-1}, equivalent to 0.32% of the average phytoplankton biomass.

The trophic impacts as well as the bottom-up control exerted by microzooplankton are more important factors controlling phytoplankton communities. However, larval grazing pressure on protozoans (Hansen et al. unpubl.) and associated cascading trophic effects could be a key factor structuring the planktonic microbial assemblage in coastal areas during periods with high larval abundances. This trophic interaction remains to be studied quantitatively.

Meroplankton, including polychaete larvae, is an important fraction of mesozooplankton, even periodically exceeding that of holoplankton in boreal estuaries (Blanner 1982, Hansen et al. 2002, present study). The trophic impact of other meroplanktonic larvae has been reported in Isefjord previously by Jørgensen (1981), where, based on ECD, a cohort of bivalve larvae (*Mytilus edulis*) daily cleared 40 to 50% of the surrounding water mass for small particles (probably flagellates). Although more information about the trophic impact of meroplankton on microbial assemblages is required, meroplanktonic larvae potentially play an important trophic role in terms of both their direct control of the microbial community and as a source of cascading effects in the microbial plankton food web

(Martin et al. 1996). Hence we propose that meroplanktonic larvae need more attention in terms of future research and as key components structuring the planktonic microbial assemblage in boreal coastal waters.

CONCLUSIONS

The planktotrophic larvae of *Polydora ciliata* are functionally food limited regardless of the time of year (i.e. even during the productive part of the year), even though they inhabit a heavily eutrophic estuary. Food-limited growth appears to be a general premise for boreal planktotrophic meroplankton (Burckhardt et al. 1997, Hansen 1999, Hansen et al. 2002, Petersen et al. 2002). The ecological consequences of growth-limited larvae could lead to a less fit juvenile population and poor post-metamorphic performance (Pechenik et al. 1996, McEdwards & Qian 2001). The juvenile stage for benthic marine invertebrates is a vulnerable stage in the life cycle, and high mortality rates are often observed (Gosselin & Qian 1997, Pedersen et al. 2008). This could be due to carry-over effects from the nutritional condition of the recruits, the larvae.

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