Feeding rates and prey selectivity of planktonic decapod larvae in the Western English Channel

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**Elaine S. Fileman · Penelope K. Lindeque ·
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Abstract Meroplankton are seasonally important contributors to the zooplankton, particularly at inshore sites, yet their feeding ecology is poorly known relative to holoplankton. While several studies have measured feeding in decapod larvae, few studies have examined the feeding rates of decapod larvae on natural prey assemblages throughout the reproductive season. We conducted 8 feeding experiments with *Necora puber*, *Liocarcinus* spp. and *Upogebia* spp. zoea larvae collected from the L4 monitoring site off Plymouth (50°15.00′N, 4°13.02′W) during spring–summer 2009 and 2010. This period spanned moderate-to-high food availability (0.5–1.6 µg chl-*a* L^{-1}), but a great range in food composition with small cells <20 µm dominating in 2010. Daily rations averaged 17, 60 and 22 % of body C for the 3 respective decapod species. Clearance rates differed according to prey type, and all 3 decapod genera showed evidence of selection of dinoflagellates. Importantly, small cells including nano- and pico-plankton were ingested, this being demonstrated independently by flow cytometric analysis of the feeding experiments and molecular analysis. PCR-based analysis of the haptophyte portion of the diet revealed ingestion of *Isochrysis galbana* by decapod larvae in the bottle incubations and *Isochrysis galbana* and *Phaeocystis globosa* by decapod larvae collected directly from the field. This study has shown that pico- and nano-sized plankton form an important supplement to the diverse and variable diet of decapod larvae.

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Introduction

Meroplankton, which include the planktonic larvae of marine benthic invertebrates, spend only part of their life cycle in the plankton but can form an important component of the zooplankton community in coastal waters during the reproductive season of benthic invertebrates (e.g. Thorson 1946). Decapod larvae, a common component of the coastal meroplankton assemblage, are planktotrophic and can be an important link in the provision of food sources for a variety of fish larvae and other organisms (Lindley et al. 1994).

One of the main factors controlling decapod larval survival and successful development is sufficient quantity and quality of food in their early stages (Boidron-Métairon 1995). Initial feeding is of paramount importance in the early development of most decapod larvae, and any food deprivation at this time could affect ongoing development, long-term survival (e.g. Anger et al. 1981; Anger and Dawirs 1981) and settling success (Olson and Olson 1989). Although once thought to be totally carnivorous (Thorson 1946), it has since been shown that these larvae can ingest diatoms (Hartman and Letterman 1978, Incze and Paul 1983; Harms et al. 1994), autotrophic dinoflagellates (Perez and Sulkin 2005; Burnet and Sulkin 2007; Shaber and Sulkin 2007) and heterotrophic prey including other zooplankton (Incze and Paul 1983; Sulkin et al. 1998; Hinz et al. 2001). Consuming such a range of prey types could compensate for low prey densities and reduce the risk of larval starvation (McConaugha 2002). However, much of the information on decapod diet has arisen

from studies which have involved rearing organisms in the laboratory and offering them a cultured phytoplankton/zooplankton diet (e.g. Harms and Seeger 1989; Welch and Epifanio 1995). Very few studies have characterised decapod feeding preferences and rates under natural conditions and those that have concentrate on larger cells (e.g. Schwamborn et al. 2006). Given the range of food quantity and quality that these larvae will experience in situ, quantifying their feeding on natural assemblages is important for establishing a relationship between food supply and settlement success.

Station L4 in the Western English Channel (http://www. westernchannelobservatory.org.uk/) is a long-term monitoring station which has been sampled for zooplankton on a weekly basis since 1988 (Smyth et al. 2010). It is a seasonally stratified coastal station situated 12 km off Plymouth (Pingree and Griffiths 1978). Meroplanktonic larvae comprise 30–40 % of the zooplankton assemblage here during their seasonal maximum in March–April (Highfield et al. 2010). The timing of the spring bloom at L4 can vary by up to 2 months from 1 year to the next (Irigoien et al. 2000a), and since meroplankton can also show substantial variability in phenology, there is the potential for these larvae to be mismatched with their food in some years (Edwards and Richardson 2004). The hypothesis driving this study was that a broad diet, including smaller nanoplankton, would reduce the effects of such mismatches.

In this study, we examine feeding of three major genera of decapods, the velvet swimming crab *Necora puber* (Holthuis 1987), the crab genus *Liocarcinus* and the mud shrimp, *Upogebia* spp., on their natural food assemblages at L4. All three species are commonly found in the English Channel (http://www.marlin.ac.uk/biotic/biotic.php). Our objective was to investigate the impact of decapod larvae on the plankton community in the Western English Channel and their ability to consume nano- and pico-size cells. This was achieved through traditional feeding experiments, supported by molecular gut content analysis, to determine feeding selectivity and natural diets of decapod larvae during spring and summer.

Methods

Site and sampling

This paper reports on the temporal distribution of decapod larvae and the ingestion rates of 3 genera: *Necora puber*, *Upogebia* spp. and *Liocarcinus* spp. (hereafter referred to as *Necora*, *Liocarcinus* and *Upogebia*) over a two-year period at L4. Zooplankton samples were collected throughout 2009 and 2010 (Atkinson et al. 2013) as part of the ongoing (1988–present) weekly monitoring of

the L4 time-series site (water depth ~ 54 m). These were obtained by duplicate vertical 4-min tows from 50 m to the surface using a 200-µm mesh WP-2 net. The contents of the cod end were preserved in 4 % buffered formaldehyde for later identification and quantification of decapod larvae by microscopy. Live decapods for feeding experiments were collected during mid-morning using the WP-2 net and towed slowly (10 m min^{-1}) vertically from 50 m to the surface. Once on deck, the samples were kept in a cool box until their return approx. 2–3 h later to a laboratory set to the ambient temperature of the upper mixed layer. Water (30 L) for the feeding experiments was collected from 10 m depth with the CTD Niskin bottles at the same time as collecting the decapods for each experiment. This water was gently siphoned into a polycarbonate carboy using silicon tubing with a 200-µm mesh bag attached to the end, to exclude mesozooplankton. The water was then left overnight in a temperature-controlled laboratory set at ambient sea surface temperature (Table 1).

Feeding experiments

We conducted a series of 8 decapod feeding experiments during the spring and summer of 2009 and 2010 to determine larval ingestion rates on a natural mixed phytoplankton and microzooplankton assemblage. Table 1 summarises these experiments, which consisted of bottle incubations of the natural plankton community with and without the addition of decapod larvae as grazers. Zoea larvae of *Necora*, *Liocarcinus* and *Upogebia* were identified using a dissecting microscope, and actively swimming individuals were carefully picked out, placed into filtered seawater and left overnight in the dark at ambient sea surface temperature to acclimate. The abundance of decapod species and their developmental stages varied throughout the study period (Table 1). For stage-specific carbon estimates, further specimens were picked from live net samples. Twelve individuals from each stage were picked out (*Liocarcinus* stages 1–5, *Necora* stages 1–5 and *Upogebia* stages 1–2), and three replicates of 4 individuals were filtered onto preashed glass fibre filters and oven dried (60 °C), for subsequent CHN analysis. After drying, individual decapod carbon and nitrogen content was measured using a Carlo Erba Carbon and Nitrogen analyser, model 'Flash EA 1112 series'.

Experimental seawater was gently mixed by rotating the carboy and carefully siphoned into 1.2-L glass Duran® bottles. When available, five individuals of each decapod genus within a similar range of developmental stage were added to four replicate incubation bottles. The bottles were carefully filled to the top with the incubation water to exclude air bubbles, placed onto a rotating plankton wheel with four replicate control bottles and rotated at 1 rpm. All

Table 1 Experimental details, decapod larval species incubated and biomass, initial chlorophyll-*a* concentration and prey abundance (cells l⁻¹) in initial seawater samples collected at L4 in the Western English Channel

Date	Incubation Temp. (°C)	Decapod species incubated	Stage	Mean C mass (µg) (± SD)	<200 µm screened chl-*a* conc (µg l⁻¹ ± SD)	Mean prey abundance (cells ml⁻¹) and growth rate (day⁻¹) in brackets						
						Ciliates	Diatoms	Armoured dinos	Unarmoured dinos	Syn (0.8–2 µm)	Peuk (0.2–2 µm)	Neuk (2–20 µm)
22 May 09	12.4	*Necora*	IV, V	71.5 (22.0)	1.51 (± 0.86)	1.5 (−0.07)	158 (−0.06)	3.3 (0.02)	19.2 (0.15)	268 (−0.12)	1413 (0.19)	48 (0.17)
		Liocarcinus	II–IV	8.7 (3.3)								
		Upogebia	I, II	15.4 (6.4)								
16 Jun 09	14.6	*Liocarcinus*	II–V	13.3 (9.7)	0.86 (± 0.03)	6.1 (−0.12)	2.9 (0.4)	6.9 (0.1)	41.4 (0.07)	1,451 (0.01)	14,330 (−0.14)	944 (0.49)
21 Jul 09	14.8	*Necora*	III, IV	38.5 (20.0)	1.32 (± 0.09)	4.3 (0.46)	1,045 (−0.01)	6.9 (0.67)	19.1 (0.35)	6,630 (−0.10)	18,311 (−0.46)	2,153 (0.24)
		Liocarcinus	1–IV	6.5 (3.8)								
		Upogebia	I, II	15.4 (0.4)								
25 Aug 09	15.3	*Necora*	I, III	15.3 (2.0)	2.5 (± 0.30)	9.4 (0.01)	6.9 (−0.7)	3.8 (0.79)	128.4 (0.81)	19,650 (−0.41)	8,585 (−0.42)	499 (0.12)
		Liocarcinus	I, II	4.3 (1.9)								
		Upogebia	I, II	15.4 (0.4)								
1 Mar 10	8.0	*Necora*	II	15.3 (2.0)	0.74 (± 0.02)	3.0 (−0.04)	18.3 (−0.06)	0.3 (0.32)	2.5 (0.21)	5,747 (−0.05)	10,795 (−0.16)	2,204 (−0.05)
		Liocarcinus	I–III	5.2 (1.9)								
12 Apr 10	9.1	*Liocarcinus*	V	27 (4.3)	1.63 (± 0.03)	3.4 (−0.08)	35.6 (−0.6)	0.5 (0.08)	19 (−0.10)	797 (−0.79)	7,279 (−1.19)	2,619 (−0.8)
17 May 10	12.2	*Necora*	V	87 (21.5)	1.2 (± 0.07)	1.6 (−0.07)	20.3 (0.06)	2.5 (0.02)	4.0 (0.15)	328 (0.21)	6,143 (−0.1)	5,041 (−0.21)
		Liocarcinus	IV, V	20 (8.6)								
15 Jun 10	13.3	*Liocarcinus*	I–III	5.2 (1.9)	0.46 (± 0.02)	4.7 (−0.01)	<0.1	1.1 (0.69)	5.4 (0.21)	9,131 (−0.02)	13,064 (−0.09)	3,256 (0.08)
		Liocarcinus	IV, V	20 (8.6)								
		Upogebia	I, II	15.4 (0.4)								

Dinos, dinoflagellates; Syn, *Synechococcus*; Peuk, picoeukaryotes; Neuk, nanoeukaryotes

experimental bottles were incubated for 24 h in the dark in the temperature-controlled laboratory.

At the initial time point, T_{zero}, 100 mL of 200-µm mesh filtered seawater was filtered onto a glass fibre filter (GF/F) and frozen at -20 °C prior to chlorophyll-*a* (chl-*a*) analysis. Triplicate 250 mL sub-samples were taken from the 200-µm mesh filtered seawater and fixed in acid Lugol's iodine solution (2 % final concentration), and triplicate 2 mL sub-samples were fixed in paraformaldehyde (1 % final concentration) for approximately 1 h before being flash frozen with liquid nitrogen and then stored at -20 °C prior to flow cytometric analysis.

After 24 h, the experiments were stopped and the decapod larvae were removed using a pipette, immediately processed and stored in 95 % ethanol for molecular characterisation of gut content of experimental samples (see 'Molecular analysis of gut content' section below). Subsamples were taken from each incubated bottle: 100 mL for chlorophyll analysis, 500 mL were fixed in acid Lugol's solution (2 % final conc.) and 2 mL sub-samples fixed in paraformaldehyde as described for T_{zero}. In all experiments, decapod mortality was <5 %.

Sample processing and data analysis

To determine chl-*a* concentration, frozen filters were placed into 10 mL of 90 % acetone and left overnight at 4 °C in the dark. Samples were analysed using a Turner fluorometer and chl-*a* concentrations measured in µg L^{-1}. Changes within the phyto- and protozooplankton community due to predation by the 3 decapod larvae were estimated by comparing the abundance of phyto- and protozooplankton between the grazed and control bottles. Enumeration of the phyto- and protozooplankton assemblages was carried out using a combination of analytical flow cytometry and inverted light microscopy.

Flow cytometry was used to characterise and enumerate *Synechococcus* (0.8–2 µm) (SYN), pico-eukaryotes (0.2–2.0 µm) (PEUK) and nanoeukaryotes (2–20 µm) (NEUK). This analysis, based on the light scattering and fluorescence properties of the cells, was carried out using a Becton Dikinson FACSort™ flow cytometer equipped with an air-cooled laser providing blue light at 488 nm following the method of Tarran et al. (2006). Samples were analysed for a minimum of 4 min and maximum of 15 min at a mean flow rate of 87 ± 35 µL min^{-1} calibrated using Beckman Coulter™ Flowset™ fluorospheres at a known concentration. Flow rate calibrations were made prior to the analysis of each experiment. PEUK and NEUK abundances were converted to carbon using a conversion factor of 0.22 pg C µm^3 (Booth et al. 1988) and by applying this to cell volumes calculated from median cell diameter measurements (Tarran et al. 2006).

To enumerate microplankton, single aliquots of up to 50 mL of the Lugol's fixed water samples were concentrated by sedimentation for 24 h and examined at 200× magnification using an Olympus IMT-2 inverted microscope (Utermöhl 1958). Depending on cell density either the whole chamber, half of it or two transects across its maximum diameter were enumerated, with a minimum number of cells counted being 300. Each cell >20 µm was identified and enumerated; however, in the case of ciliates, all were counted regardless of size. Cells were identified to genus whenever possible, and these were further combined according to taxonomic group: diatoms (counts of chain-forming diatoms refer to cells not chains), armoured dinoflagellates, unarmoured dinoflagellates and ciliates. Cell volumes were calculated by approximating to simple geometric shapes according to Kovala and Larrance (1966) using average cell length, width and depth measurements for each individual taxon (Widdicombe et al. 2010a). Carbon contents per cell were then estimated using the empirically derived C:vol conversions of Menden-Deuer and Lessard (2000).

Decapod clearance and ingestion rates were calculated for each prey type or group of prey types from differences in the rates of change of prey abundance in experimental bottles with and without the addition of decapods (Frost 1972). Clearance was only calculated when there were more than 25 of each cell type enumerated in an aliquot/subsample and where there was a significant difference in prey concentration between controls and experimental bottles, as determined by *t* tests ($df = 3$, $p = <0.1$). The reduction in prey concentration in experimental bottles ranged from 4 to 37 %. Carbonspecific ingestion rates were calculated by dividing the ingestion rates (µg C ind^{-1} day^{-1}) by the average larval mass in µg C, as shown in Table 1. We used regression analysis to determine whether there was a relationship between ingestion rates and available food. We used one-way ANOVA to determine whether there were any significant differences in clearance rates of the different food types by (1) each decapod genus, (2) between body C content of the decapod larval stages and (3) between experiments for each decapod genus. Where ANOVA results were significant ($p = <0.05$), we performed post hoc comparisons using Tukey HSD (honestly significant difference) to determine the cause of the variation.

Selective feeding by decapod larvae of the various food types was evaluated from positive feeding rates using the electivity index (E_i) of (Vanderploeg and Scavia 1979):

$$E_i = \frac{K_i - (1/n)}{K_i + (1/n)}$$

where *n* is the total number of prey types in a given experiment, and the coefficient K_i is defined by

$$K_i = \frac{F_i}{\Sigma F_i}$$

where F_i is the clearance rate of the i th food type and ΣF_i is the sum of clearance rates of all food types. The index, E_i, ranges from -1 to $+1$ where 0 corresponds to no selectivity, negative values correspond to avoidance and positive values represent selection. We used a one-sample Student's t test to determine whether electivity significantly deviated from 0.

Molecular analysis of gut content

Molecular analysis was performed on two sample dates, August 2009 and June 2010. This was a small-scale analysis, supplementary to the main bottle incubations, and was aimed partly to better resolve some of the species within the NEUK group that were potential prey for the decapod larvae. Its second aim was to compare the gut content of larvae after the bottle incubation experiments and the gut content of decapod larvae taken directly from the field. Molecular analysis was carried out firstly on the decapods that had been feeding in the August 2009 and June 2010 experiments and secondly on decapod larvae (between 10 and 22 from each genus, where possible) sorted directly from the field samples.

The small size of the decapod larvae, 1–3 mm, hinders dissection of their stomachs, so instead DNA was extracted from pooled whole individuals. To eliminate contamination from material stuck to the outside of the larvae, they were thoroughly washed repeatedly in 0.2 μm filtered seawater and then in ethanol before finally being stored in 95 % ethanol, and the whole process was carried out as quickly as possible to minimise digestion of DNA in the gut. The samples were removed from the ethanol, pooled for each experiment for each species and rehydrated overnight at room temperature in 1 mL MillQ water prior to DNA extraction. DNA was extracted using the DNeasy Blood & Tissue Kit (Qiagen) following the standard protocol for animal tissue. An RNase step was included; 4 μL RNase A (100 mg mL^{-1}) was added to the lysis and incubated at room temperature for 2 min. Total lysis was left for 4 h at 56 °C, and the DNA eluted in 2 × 100 μL MilliQ water. Success of the DNA extraction was checked by running a 5-μL aliquot on a 0.8 % electrophoresis gel.

Primers designed to target the haptophyte group were used for gut content amplification and tested for negative amplification of the prey DNA. Partial 18S rDNA solely found in haptophytes was selectively amplified using the forward primer Prym-429f: 5′-GCG CGT AAA TTG CCC GAA-3′ (Coolen et al. 2004) and the reverse primer PRYM02: 5′-GGA ATA CGA GTG CCC CTG AC-3′ (Simon et al. 2000) following the methods described in Lindeque et al. (2014).

To increase amplification of any haptophyte DNA from the guts, internal nested PCR primers were designed specifically from an alignment of 12 haptophyte sequences. These consisted of sequences from within the orders coccolithales, isochrysidales, phaeocystales and prymesiales. One microliter of each PCR product was used as a template for a nested PCR using the same PCR components described above but with 10 μM of the custom-designed forward and reverse primers Nest-Hapto-F 5′-TGA CAC AGG GAG GTA GTG ACA AG-3′ and Nest-Hapto-R 5′-GGT CGA AAC CAA CAA AAT AGC ACC-3′ again following the cycling parameters described by Lindeque et al. (2014).

Individual amplicons were separated from the mix of amplicons within the PCR products by clean-up of the PCR product using a QIAquick purification kit (Qiagen UK Ltd) and subsequent cloning using the pGEM®-T Easy Vector System and JM109 competent cells (Promega UK Ltd) following the standard protocol. Colony PCR with 10 μM of primers M13f and M13r (Heidecker et al. 1980) preceded sequencing by LGC Genomics GmbH, Germany, of each successful amplification.

Sequences were opened in Mega 5 (Heidecker et al. 1980) and trimmed to the forward and reverse primers. The Qiime pipeline (Caporaso et al. 2010) was used to assign the sequences to Operational Taxonomic Units (OTUs) at the 97 % similarity level and to generate representative sequences. The representative sequences were then manually assigned taxonomy by searching the EMBL DNA database for sequence similarities using a Basic Local Alignment Search Tool (BLAST).

Results

Seasonal and inter-annual changes in the natural food assemblage

While the absolute amounts of food varied only about 3–4 fold throughout the 8 experiments, prey composition varied greatly, both seasonally and between the 2 years (Fig. 1a). Chl-a concentrations in initial seawater samples (<200 μm) ranged between 0.5 and 1.6 μg chl-a L^{-1} with values often lower in 2010 than 2009 (Table 1). Diatoms were most abundant in May (mostly *Thalassiosira* spp. and *Guinardia delicatula*) and July 2009 (*Chaetoceros* spp.) but comprised the highest percentage of total biomass in May for both years. The diatoms present in spring 2010 were mostly *Chaetoceros densus*, *Rhizosolenia* spp, *Guinardia flaccida* and *G. delicatula*, but the latter were 10 times less abundant than in 2009 (Table 1). Dinoflagellates were more abundant in 2009 than 2010 and reached maximum abundance in August 2009 which corresponded with high abundance of the unarmoured dinoflagellate *Karenia mikimotoi* and an unidentified Gymnodinoid species of a similar size; during this time, dinoflagellates comprised 90 % of the

Fig. 1 **a** Initial total biomass (µg C L^{-1}) and the relative contributions of the different prey to total biomass (µg C L^{-1}) and **b**–**d** total biomass ingested (µg C ind^{-1} day^{-1}) and % daily C ration (*numbers above bars*) by *Necora*, *Liocarcinus* and *Upogebia*
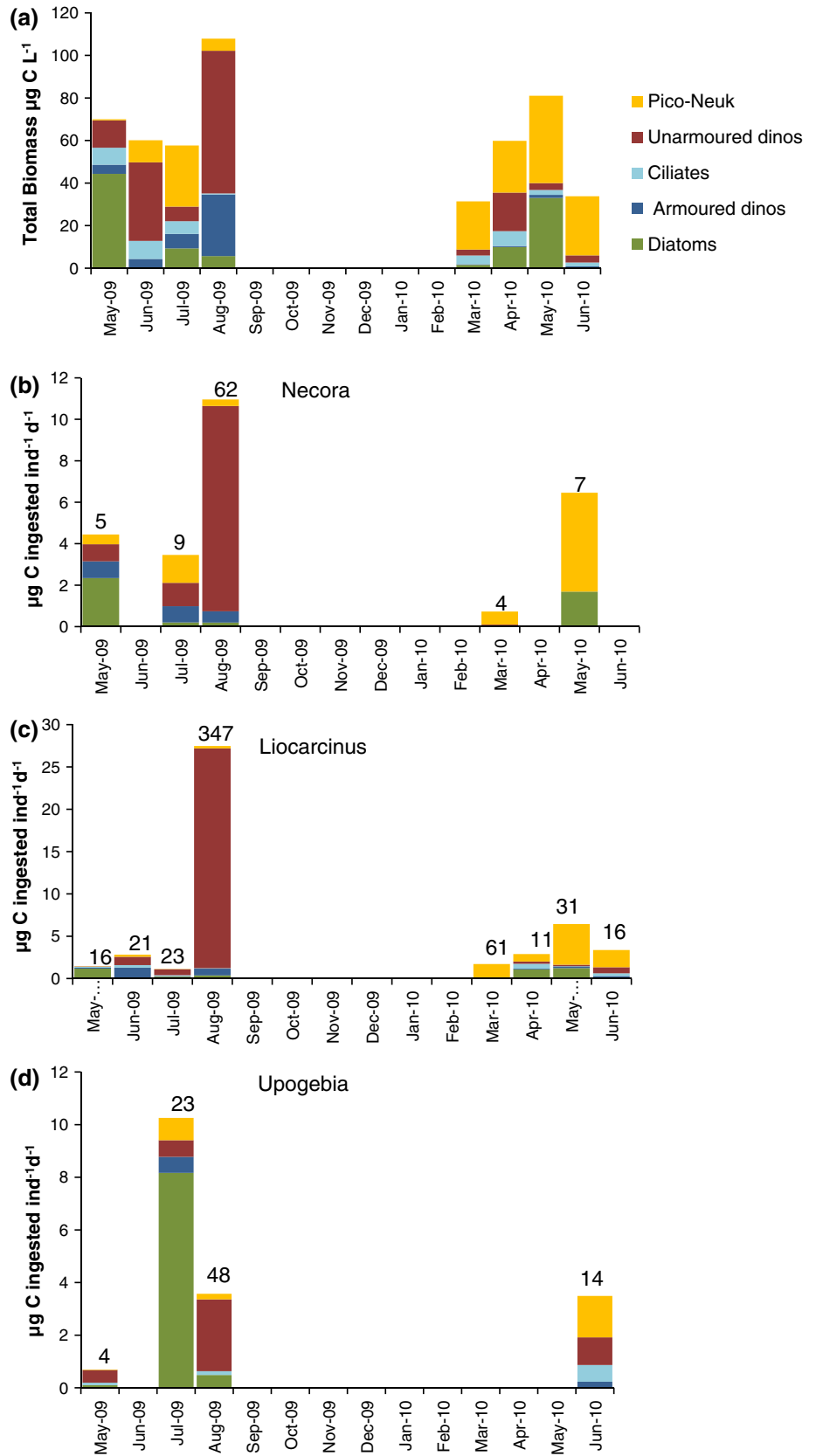
total biomass. In contrast, pico- and nanoeukaryotes were more abundant in April to June 2010 than 2009 (Table 1) when they comprised 40–82 % of the total biomass. Ciliate abundance was greatest in August 2009, but their contribution to total biomass at this time was <1 %. One ciliate species, *Mesodinium rubrum,* was also abundant during May 2009. Despite large differences in absolute cell abundance and prey biomass contributions during May of the two years, Fig. 1a shows that the total biomass was relatively similar (70 µg C L^{-1} in 2009 and 81 µg C L^{-1} in 2010).

Ingestion rates and daily rations

The total amount of carbon ingested by the three decapod species ranged between 1 and 11 µg C ind^{-1} day^{-1} for *Necora*; 1–27 µg C ind^{-1} day^{-1} for *Liocarcinus* and 3–11 µg C ind^{-1} day^{-1} for *Upogebia* (Fig. 1b–d). Carbon ingestion by *Necora* and *Liocarcinus* was highest in August, corresponding to highest biomass of unarmoured dinoflagellates and total prey, but for *Upogebia* highest ingestion was in July. Ingestion rates corresponded to average daily rations of 17 % (range 4–62 %) for *Necora*, 60 % (range 11–347 %) for *Liocarcinus* and 22 % (range 4–48 %) for *Upogebia*. These rations comprised varying contributions of the available food, such that usually (but not always) when a food category dominated the available food (Fig. 1a), it also dominated the diet (Fig. 1b–d). However, overall, we found no significant differences between ingestion rates of the different food types by each group of decapod larvae (ANOVA F = ≤1, p ≥ 0.4). Ingestion rates of the different food groups increased with increase in food availability (Fig. 2) as did the total amount of C ingested by each decapod (Fig. 3) although this correlation was only significant for *Necora* ($R^2 = 0.94$, $p = 0.005$). A correlation analysis between carbon-specific ingestion rates and prey availability (µg C L^{-1}) was significant for *Necora* ($R^2 = 0.69$, $p = 0.05$), but not for *Liocarcinus* ($R^2 = 0.44$, $p ≥ 0.05$) or *Upogebia* ($R^2 = 0.55$, $p ≥ 0.05$).
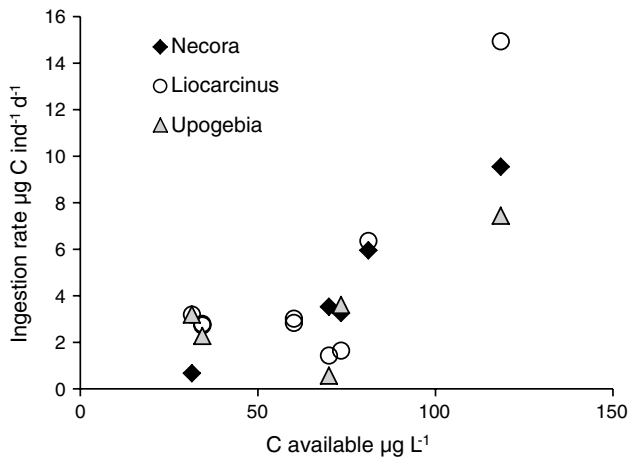
Clearance rates

A comparison of clearance rates of each decapod genus on the different food types between experiments revealed that there were no significant differences with food type between experiments for *Necora* or *Upogebia*, but *Liocarcinus* showed a significant difference in clearance rates of unarmoured dinoflagellates (ANOVA F = 7.9, p = 0.0002). Results of a post hoc Tukey analysis showed that *Liocarcinus* clearance rates of unarmoured dinoflagellates were significantly higher in August 2009 than in other months (p = 0.05) and these higher clearance rates coincided with the highest biomass of unarmoured dinoflagellates (Fig. 1a). We also examined for differences in clearance
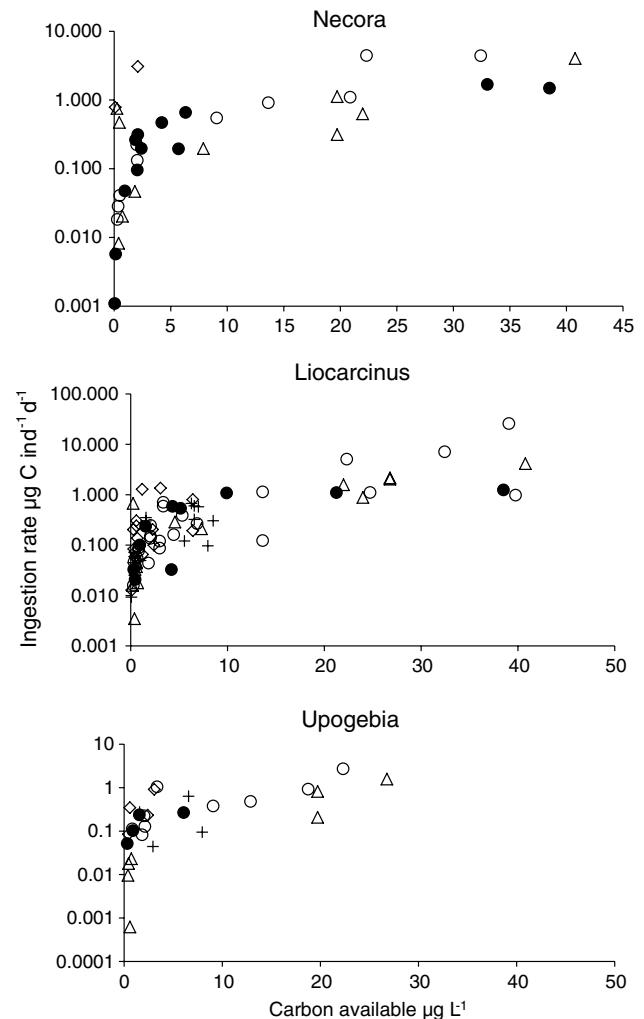
Fig. 2 Ingestion rates (µg C ind^{-1} day^{-1}) of different prey types by *Necora* sp., *Liocarcinus* spp. and *Upogebia* spp. as a function of their concentration (µg C L^{-1}). *Open diamond* armoured Dinoflagellates, *open circle* unarmoured Dinoflagellates, *closed circle* diatoms, *plus symbol* ciliates, open triangle pico- and nanoplankton

rates across the range of decapod larval body size used in the 8 experiments and found no differences for stages I–IV, but Stage 5 *Necora* (ANOVA F = 3.4, p ≤ 0.03, df = 3) and *Liocarcinus* (ANOVA F = 15.0, p = 0.001, df = 6) showed significantly higher clearances rates.

In order to combine the experiments to derive broad inferences on selective feeding, we have attempted to control potential effects of the varying ambient temperature. This is because ambient sea surface temperatures on the experimental days ranged substantially, from 8.0 °C in March to 15.3 °C in August (Table 1). Clearance rates of microplankton prey by *Necora* and *Liocarcinus* (the genera which were incubated across the full temperature range) increased with temperature (Y = 0.13x + 0.11, $R^2 = 0.68$, p ≤ 0.01), although no such relationship was observed for

Fig. 3 Total C ingestion (μg C ind^{-1} d^{-1}) by Necora, Liocarcinus and Upogebia as a function of totalfood concentration (μg C L^{-1})

nanoplankton prey. Using the relationship between Log_{10} median clearance rates and temperature ($Y = 0.13x + 0.11$, $R^2 = 0.68$, $p < 0.01$), we adjusted clearance rates of microplankton to a nominal mid-range temperature of 12 °C.

Decapod larvae fed on a range of prey items in comparable experiments from small nanoeukaryotes through to

large tintinnid ciliates (Table 2). *Liocarcinus* cleared some species of diatom, armoured dinoflagellates and ciliates at higher rates than *Necora* or *Upogebia* (Table 2, Fig. 4). *Necora* larvae did not feed on ciliates, whereas *Upogebia* larvae did not feed on the potentially toxic dinoflagellate *Karenia mikimotoi* despite it being available in relatively high concentrations during August 2009. Comparing temperature-adjusted data, we found no significant differences in clearance rates with food size for any of the decapod genera (ANOVA $F \leq 1.2$, $p > 0.2$, $df = 4$) (Fig. 4), even the very small pico- and nanoeukaryotes were cleared and all three species could feed across 4–5 orders of magnitude of size range. However, there was some evidence for larger cells being selected if data were not temperature-adjusted, with *Necora* larvae having significantly higher clearance rates on the largest cells >1,000 μm^3 ($p = 0.01$ ANOVA, Tukey test, $df = 4$). There was no obvious difference between clearance rate of motile versus non-motile cells (diatoms), shown as filled symbols in Fig. 4.

A one-way ANOVA showed that for most of the food items, the 3 decapods had statistically similar clearance rates. However, when comparing clearance rates on the various food items for each species in turn, clearance rates differed according to prey type (Table 3). All three decapods cleared

Table 2 Minimum and maximum decapod larval clearance rates (temperature-adjusted) on commonly encountered food types

Prey category	Taxon	Max average abund ± SD cells/ml	*Necora* sp. (ml ind^{-1} day^{-1})	*Liocarcinus* sp. (ml ind^{-1} day^{-1})	*Upogebia* sp. (ml ind^{-1} day^{-1})
Diatoms	*Guinardia delicatula*	125 ± 26	20–76	13–50	55–63
	Thalassiosira rotula	3.6 ± 0.3	40–145		
	Thalassiosira spp.	10.6 ± 0.8	133		
	Chaetoceros densus	15.8 ± 11		715	
	Chaetoceros spp				18
	Rhizosolenia spp	19.3 ± 14		49–369	84
	Pennates	70 ± 44	11–24		
Armoured dinoflagellates	Protoperidini-ales < 30 μm	5.4 ± 0.6	20–68	30–167	118
	Prorocentrum micans	1.7 ± 0.1		19–128	52
Unarmoured dinoflagellates	Gymnodiniales < 30 μm	21.7 ± 1.8	20–72	16–120	41–129
	Gymnodiniales 30–50 μm	17.2 ± 2	18–92	11–116	20–93
	Gymnodiniales > 50 μm	2.5 ± 0.7	17–69	24–65	25
	Karenia mikimotoi	64 ± 3.6	39–50	75–120	
Ciliates	Tintinnids	0.9 ± 0.09		42	34
	Mesodinium rubrum	8.7 ± 0.4		139–398	
	Aloricate oligotrichs 30–50 μm	3.9 ± 0.6		35–60	40
Pico- and nanoplankton	*Synechococcus* sp.	19,650 ± 2,567	24	10–18	27
	Picoeukaryotes	18,311 ± 2,180	38–57	12–49	44
	Nanoeukaryotes	4,451 ± 763	20–65	24–97	77–106

Empty cells represent no data for that prey category
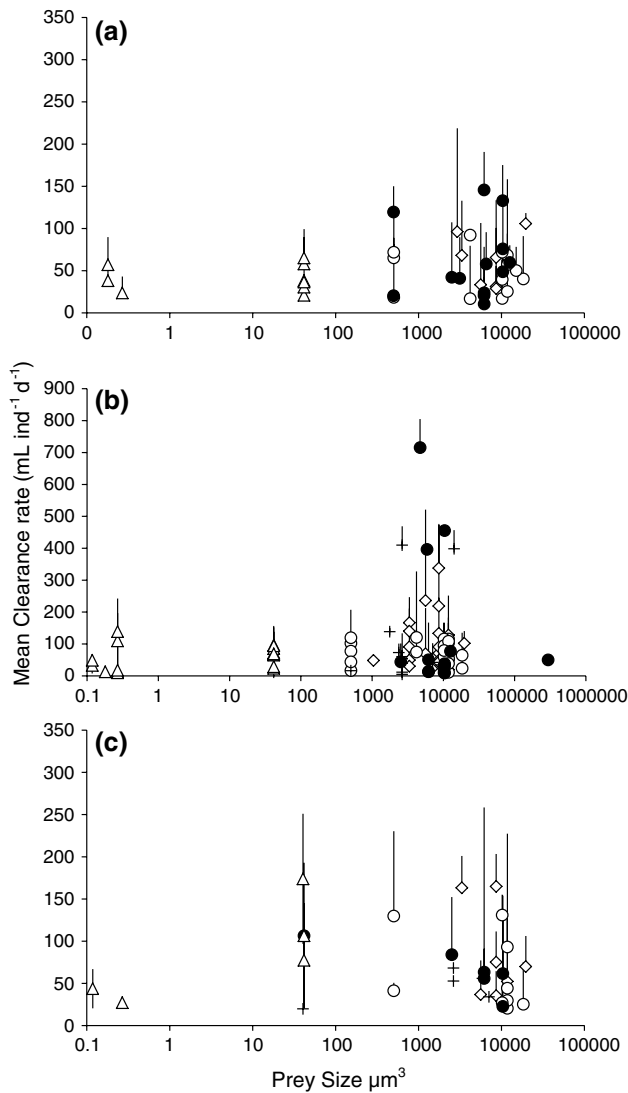
Fig. 4 Mean clearance rates (mL ind^{-1} day^{-1}) with standard *error bars* of each prey category by **a** *Necora*, **b** *Liocarcinus* and **c** *Upogebia* in relation to prey cell size (μm^3). Microplankton clearance rates have been temperature-adjusted to 12 °C. *Open diamond* armoured Dinoflagellates, *open circle* unarmoured Dinoflagellates, *filled circle* diatoms, *plus symbol* ciliates, *open triangle* pico- and nanoplankton

Table 3 Summary of ANOVA test and post hoc Tukey HSD test to compare clearance rates on different food groups

Decapod	One-way ANOVA		Tukey HSD
	F	*p*	
Necora	2.56	0.025	Adinos = Neuk = Diat = Udino > Syn
Liocarcinus	8.2	<0.0001	ADino > Neuk = Cils = Peuk = Syn
			Diat > Peuk = Syn
			Udinos > Syn
Upogebia	4.1	0.001	ADino = Diat > Syn

$\alpha = 0.05$

ADino armoured dinoflagellates, *Diat* diatoms, *UDino* unarmoured dinoflagellates, *Neuk* nanoeukaryotes, *Cils* ciliates, *Peuk* picoeukaryotes, *Syn* synechococcus
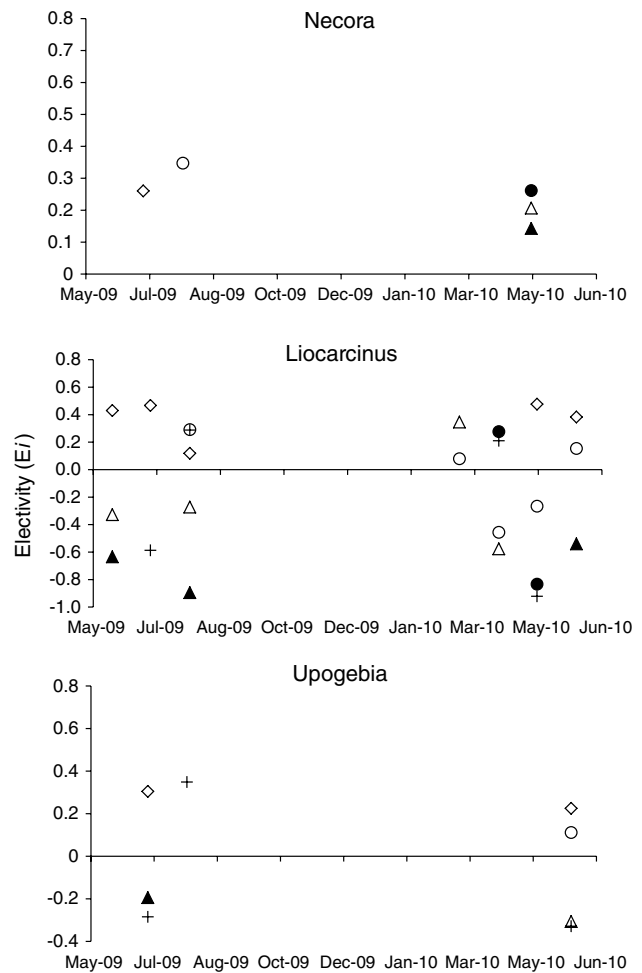
Fig. 5 Electivity index (E_i) of the different prey groups for all experiments where clearance rates were significant. Only the data points where the electivity index was shown to deviate significantly from 0 (Student's *t* test) are shown. *Open diamond* armoured dinoflagellates, *open circle* unarmoured dinoflagellates, *filled circle* diatoms, *plus symbol* ciliates, *open triangle* nanoeukaryotes, *filled triangle* picoeukaryotes/*Synechococcus*

armoured dinoflagellates and diatoms significantly faster than *Synechococcus* ($p \leq 0.05$). This general picture, of higher clearance rates of dinoflagellates or diatoms, is supported by their generally higher electivity indices (Fig. 5).

Clearance rates did not change significantly with total concentration of food (~0.2–40 µg C L^{-1}) in the incubations (one-way ANOVA $df = 5$, $p \geq 0.1$).

Molecular analysis of gut content

Using primers designed to target the haptophyte group, a total of 181 sequences were obtained from *Necora,*

Table 4 Proportion of diet made up by different prey as determined by molecular amplification of gut content using haptophyte primers

	Decapod species	Total no. of sequences	No. of sequences	
			OTU 1 (*P. globosa*)	OTU 2 (*I. galbana*)
August 2009				
Experimental	*Upogebia* sp.	20	0	20
	Necora puber	19	0	19
	Liocarcinus spp.	20	0	20
Field	*Upogebia* sp.	21	0	21
	Liocarcinus spp.	21	0	21
June 2010				
Experimental	*Liocarcinus* spp.	20	0	20
	Upogebia sp.	18	0	18
Field	*Liocarcinus* spp.	20	14	6
	Upogebia sp.	20	19	1
Sum		179	33	146

Liocarcinus and *Upogebia* feeding experiments, *Liocarcinus* and *Upogebia* field samples in August 2009 and *Liocarcinus* and *Upogebia* feeding experiments and field samples in June 2010. Analysis using Qiime assigned these sequences to four different Operational Taxonomic Units (OTU). Two OTUs each contained a single sequence neither of which showed >96 % homology to any sequences in the database and as a result were not used in the analysis. The representative sequence for OTU 1 (Accession number KF925469) showed 100 % homology to *Phaeocystis globosa*, and the representative sequence for OTU 2 (Accession number KF925470) showed 100 % homology to *Isochrysis galbana* (Table 4).

The proportion of the haptophyte diet, i.e., the number of haptophyte sequences assigned to each OTU, is shown in Table 3. The sequence analysis showed that 100 % of the haptophytes identified in August 2009, both in experiments and taken directly from the field, were *Isochrysis galbana*. In June 2010, haptophytes found in the gut content from experiments were *I. galbana* (100 % of sequences), while the decapods from the field had eaten a mixture of *I. galbana* and *Phaeocystis globosa*, dominated by the latter.

Seasonal abundance and grazing impact

At L4 decapod larvae generally occur from spring through to autumn and are scarce during the winter months (Fig. 6a). The seasonal cycle is usually characterised by a peak in March–April, a decrease in May–June and then a further increase in July–August. During the study period, total decapod abundance ranged from <1 to 96 individuals m^{-3} with a maximum in April 2009 (Fig. 6a, b). Brachyurans dominated during the spring months, whereas *Upogebia* was more abundant in June, July and August.

We estimated the potential trophic impact of decapod larval feeding on each of the different prey groups by multiplying the decapod C ingestion rate for each prey group by the monthly average abundance of each decapod genus (Fig. 7a) and relating this to the monthly average standing stocks of each prey group present at L4 at 10 m estimated from Widdicombe et al. 2010b. We estimated that potential trophic impact by *Necora* was lower than the other two species, and this showed a decreasing trend from May to August. Grazing impact by *Liocarcinus* was highest in August when 5 % of the standing stocks were removed daily. For *Upogebia*, their impact was generally lower in 2009 than in 2010 (Fig. 7).

Discussion

The annual cycle of decapod larval abundance at L4 follows the typical annual cycle of zooplankton observed in temperate waters around the UK (e.g. Eloire et al. 2010; Pan et al. 2011) which is usually characterised by an abundance peak in late spring followed by a secondary peak in autumn (Highfield et al. 2010). This coincides often (but not always) with peaks in phytoplankton abundance at L4 (Widdicombe et al. 2010b). Below, we discuss first the main features of decapod larval diet and feeding selectivity and second how this broad diet spectrum relates to unpredictability in the food resource for these potentially sensitive larval stages. Thirdly, we discuss the trophic impact of these larvae on the food sources.

Diet and Feeding selectivity

Decapod larvae are suspension feeders (Anger 2001), and it has been shown that suspension feeding is based on chance

Fig. 6 Seasonal variation in decapod abundance (Nm³) at L4, **a** total decapod abundance for 2009 and 2012 with total surface chlorophyll-*a* concentrations (µg L⁻¹), **b** monthly average abundance of *Necora puber*, Brachyurans (which includes *Liocarcinus* spp.) and *Upogebia* spp. during 2009–2010. *Error bars* indicate standard deviation of the mean total abundance (n = 8)
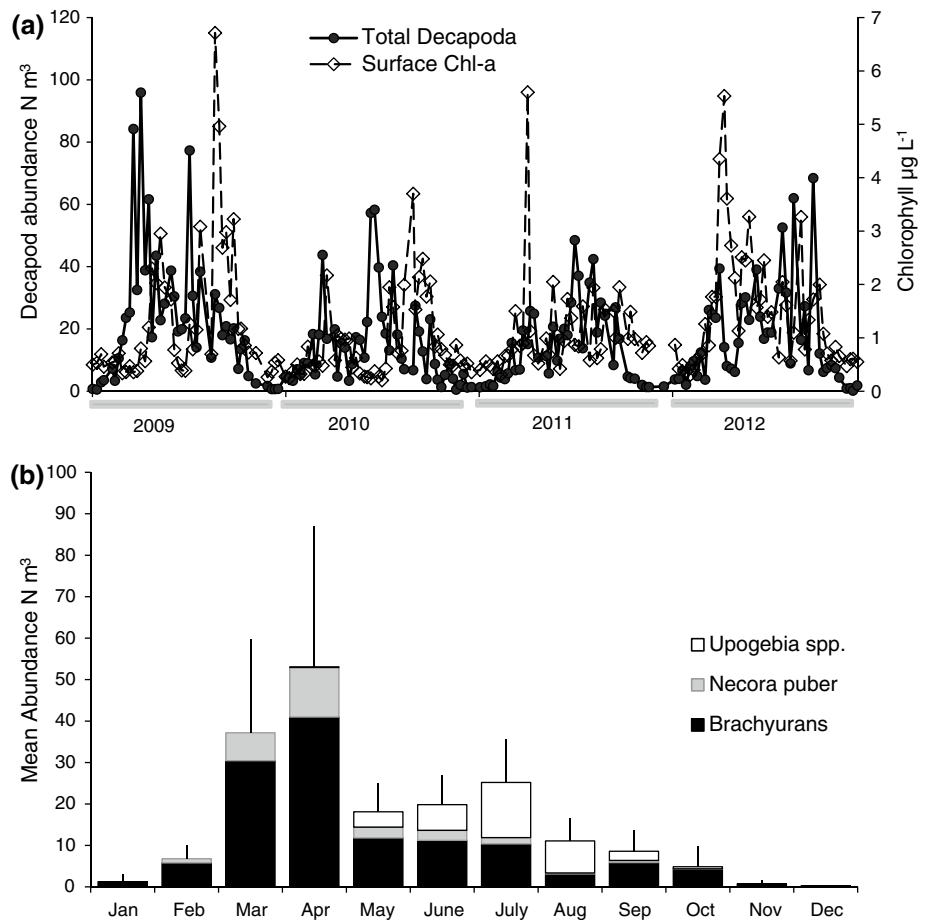

meetings with prey and is independent of food type or quality (Factor and Dexter 1993; Welch and Epifanio 1995). While zoea of the higher Decapoda (e.g. crab larvae) have been described as being primarily raptorial feeders with limited capability for filter feeding of small particles (Strathmann 1987), a number of studies on decapod larvae, including crab larvae, have demonstrated feeding on microplankton (e.g. Incze and Paul 1983; Harms and Seeger 1989). However, it was Lebour (1922) who first noted the presence of phytoplankton in decapod larval guts.

In this study, food availability and composition varied among experiments, reflecting the wide range of prey types to which decapod larvae are exposed in the water column at L4. The decapod larvae used in the experiments, representing 3 genera and a range of stages, were found to be omnivorous feeders which is in keeping with findings from other studies (e.g. Lehto et al. 1998; Perez and Sulkin 2005). They consumed a wide range of prey types of differing size including small pico-eukaryotes and *Synechococcus* spp., and differing motility, e.g. centric diatoms and ciliates. However, dinoflagellates (armoured and unarmoured), diatoms and occasionally ciliates were selected for over smaller-sized picoeukaryotes and *Synechococcus*.

Liocarcinus demonstrated high clearance rates of unarmoured dinoflagellates during August 2009; one species that we found within this group was *Karenia mikimotoi*, a potentially toxic dinoflagellate species that was particularly abundant during this particular season (Barnes et al. in review). At the same time, we found that *Upogebia* did not feed on *K. mikimotoi* and could, therefore, have been discriminating against this prey (Hinz et al. 2001; Perez and Sulkin 2005). Other studies have shown that a variety of dinoflagellates are readily ingested by crab larvae, even potentially toxic strains (e.g. Shaber and Sulkin 2007), and ingestion of dinoflagellate prey may enhance larval survival (Sulkin et al. 1998).

Studies of feeding mechanisms in zooplankton tend to focus on the dominant copepods and other holoplankton to a greater extent than on meroplankton (Kiørboe 2011). While some species of pelagic copepods (e.g. *Oithona* spp.) have been shown to select motile prey over nonmotile forms (Johnson and Tiselius 1990; Atkinson 1995), some decapod larvae are known to feed preferentially on suitably sized non-motile prey that provide biochemical cues on their cell surface (Hinz et al. 2001). In this study, we saw no evidence of increased clearance rates of motile
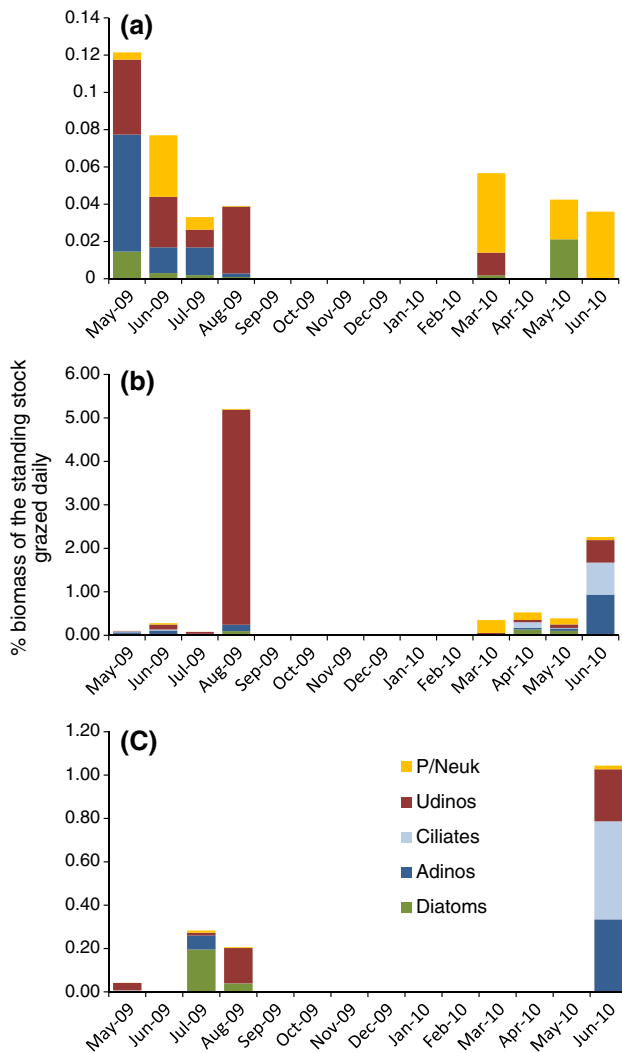
Fig. 7 Potential trophic impact (% biomass of the standing stock grazed day^{-1}) by **a** *Necora puber*, **b** *Liocarcinus* spp. **c** *Upogebia* spp. upon different prey

cells relative to diatoms. The fact that decapod larvae in this study were ingesting a broad range of cell sizes is more consistent with suspension feeding than the raptorial or ambush feeding that has been found in later megalopae stages (Chen et al. 2013). While suspension feeding is likely a requirement to capture the pico- and nanoplankton cells perhaps too small and numerous for individual handling (Price and Paffenhöfer 1983), we cannot discount the possibility that other feeding modes are employed on food items that were too large and rare for enumeration in our experiments, thereby increasing their daily ration (Wirtz 2012).

The fact that decapod larvae fed on the small pico- and nano-size fraction was a noteworthy finding of our study. Due to the functional morphology of the decapod larval mouthparts, it is thought that as single cells, these food size

classes are not easily accessible to most decapod larvae (Anger 2001), but they could become more important as food items when they are associated with detritus (Kiørboe 2000). We did not enumerate aggregates here but a study by Factor and Dexter (1993) demonstrated that green crab larvae were capable of ingesting small particles such as bacteria, small algal cells and organically enriched detrital particles (1–10 μm) in their natural diet. Ingestion of pico- and nano-sized cells has been shown to enhance survival time compared to starved larvae (e.g. Lehto et al. 1998) and therefore could provide an important food source particularly when larger food items are scarce.

This is one of the very few studies of decapod larval feeding rates on natural plankton communities. Other studies have been carried out with brachyuran crab larvae (Schwamborn et al. 2006), shrimp larvae (Harvey and Morrier 2003) and mud crab larvae (Epifanio et al. 1994), but these studies only looked at feeding on food items in the >50 μm food size range. Thus, direct comparison with other studies is difficult, although feeding rates of other meroplanktonic larvae have been investigated to some extent (e.g. Vargas et al. 2006; Almeda et al. 2010), and the ingestion of natural phytoplankton pigments by decapod larvae has been shown (Meyer-Harms and Harms 1993). Once thought to be exclusively carnivorous (Thorson 1946), laboratory studies have shown that phytoplankton can also be eaten by decapod larvae (e.g. Harms and Seeger 1989). However, the nutritional gain is variable and dependent on species and stage (Sulkin et al. 1998) and thought to be more important for the younger stages (Anger 2001). Further studies have shown that heterotrophic protists provide a supplementary food source for larval crabs (Sulkin et al. 1998; Hinz et al. 2001). Our results show that phytoplankton and protozooplankton, which are abundant in coastal waters and are a potentially rich source of carbon (Azam et al. 1983), are common components of the natural diet of decapod larvae. Diatoms, dinoflagellates, nanoeukaryotes and, to a lesser extent, ciliates represented a significant food source for decapod larvae in coastal waters, together contributing average daily carbon rations of 17–60 % for the three species. These findings are in line with those of Anger (1990) who reported that early crab zoeae may ingest up to ca 40 % body C and provide further evidence of a trophic link between the microbial loop and decapod larvae (Lehto et al. 1998; Sulkin et al. 1998). While there can be a degree of uncertainty associated with applying carbon to volume conversion factors to estimates of plankton abundance (Montagnes et al. 1994), our estimates of prey biomass and fall within the ranges reported for coastal waters of the English Channel (Irigoien et al. 2000a; Fileman et al. 2010, 2011) and when compared with chlorophyll concentrations provide realistic C:chl ratios of between 30 and 70 (Irigoien et al. 2000b).

These food sources, while important, likely only represent part of the total ingestion by decapod larvae. Other food sources such as micro-metazoans and marine snow aggregates (not counted here) could also contribute to the decapod diet (e.g. Incze and Paul 1983; Epifanio et al. 1994; Harvey and Epifanio 1997; Lehto et al. 1998), and a mix of both algal and animal food is thought to be best for survival and development of *Necora* and *Liocarcinus* larvae (Choy 1991). The ability to feed on a wide spectrum of food sources, including nano- and microplankton, would enhance the nutritional value of the larval food supply (Harms and Seeger 1989).

In laboratory studies using bottle incubations, predator prey assemblages can experience a number of differences in a bottle that they do not experience in the field. These are known collectively as 'bottle effects' (Roman and Rublee 1980) and can be especially important at the lower end of the food chain such as the nano- and pico-size fraction due to food chain effects. We tested this independently on a specific group of nanoplankton (haptophytes) using a molecular approach. This provided a valuable in situ diet cross-check by confirming ingestion of small flagellated cells, *Isochrysis galbana* (8 μm) and *Phaeocystis globosa* (solitary cells 3–9 μm). Molecular techniques such as polymerase chain reaction (PCR) are increasingly used for the detection of prey within zooplankton trophic interactions (e.g. Nejstgaard et al. 2003, 2008; Durbin et al. 2012; Lindeque et al. 2013). In this study, the use of molecular techniques not only allowed better resolution of one targeted group of prey, the nanoeukaryotes, which are difficult to identify to genus using light microscopy or flow cytometry but also enabled us to identify prey in animals taken directly from the field. Further 'bottle effects' could occur as a result of excretion by the decapod predators in the experimental bottles, and this could stimulate phytoplankton growth relative to the controls. As we did not spike our incubation bottles with nutrients during this study, our grazing estimates on phytoplankton could be underestimated.

Unpredictability of food resources

The ability of decapod larvae to ingest a wide variety of prey has important ecological implications. Meroplankton can be seasonally abundant, and the timing of their appearance needs to be matched with the seasonality of their food (Thorson 1946; Edwards and Richardson 2004). The seasonal cycle and vertical distribution of decapod larvae around the UK have been described by Lindley (1987), and Lindley et al. (1994) show some evidence of decapod larvae being found in areas of greater food concentrations. However, the timing of the seasonal occurrence of decapod larvae in the plankton is also related to temperature, and this temperature dependence of decapod reproduction may be a stronger governing factor than seen in the holoplankton (Lindley 1998). Bloom timing can be highly variable from year to year (e.g. Bigelow et al. 1940) relative to the timing of appearance of these larvae in the plankton (Fig. 6a). However, phenology studies have shown that the average timing of diatom blooms over longer time scales has changed little (Edwards and Richardson 2004; Wiltshire et al. 2008). In contrast, meroplanktonic species such as decapods are more dependent on temperature to stimulate larval release and are suggested to have shifted their development forward in response to earlier warming, such that a mismatch could arise between grazers and their food (Edwards and Richardson 2004). Even though food levels in terms of total C and chl-*a* seemed to be fairly high in this study, the composition varied enormously, and for example in March and May 2010 despite reasonable food concentrations, most was of very small size (Fig. 1). Conversely, the highest decapod larval abundances during both 2009 and 2010 occurred outside of bloom periods, with chl-*a* concentrations <0.5 mg m^{-3} (Fig. 6a). Thus, the ability of decapod larvae to consume small prey items if mismatched with blooms of larger diatoms or dinoflagellates may increase their chances of survival.

Trophic impact

Overall, decapod larval grazing impact on their food supply was generally very low (<1 % of prey biomass removed per day for *Necora* and *Upogebia*; up to 5 % of prey biomass removed by *Liocarcinus*), due to low abundance. Almeda et al. (2010) also report low grazing impact of invertebrate larvae during a harmful algal bloom. These findings are in line with those of Strathmann (1995) who concluded that the larvae of most meroplankton species are at such low concentrations that their effect on their own food supply is expected to be negligible.

The zooplankton at L4 are very diverse (Highfield et al. 2010; Lindeque et al. 2013) so it is unlikely that a single taxonomic group will ever be dominant in terms of grazing impact on its prey (e.g. Fig. 7). However, the advantage of the high-resolution data derivable from bottle incubations is that we can calculate grazing impact for each species on each prey item. In this study, we found the highest grazing impact by decapod larvae to be in the summer post-bloom period when the population was dominated by dinoflagellates. It is therefore possible that the grazing impact of decapod larvae could contribute to a high total zooplankton impact at certain times of the year and on certain prey taxa (e.g. selected diatoms and dinoflagellates). When combined with other predator and prey-specific grazing studies from this site (e.g. Fileman et al. 2010; Lindeque et al. 2014) and with time-series observations of their abundances (Irigoien et al. 2005; Eloire et al. 2010), it will be possible to build up a picture of how grazing can structure populations.

Further, the information on prey selectivity and functional responses provide insights into feeding mechanisms, of use to developing modelling approaches such as trait-based models (Kiørboe 2011).

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