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

Elaine S Fileman[1*], Penelope K Lindeque[1], Rachel A Harmer,[1] Claudia Halsband [2] and Angus Atkinson [1]

1-Plymouth Marine Laboratory, Prospect Place, West Hoe, Plymouth, Devon, PL1 3DH

2- Akvaplan-niva AS, Fram Centre, N-9296 Tromsø, Norway.

*corresponding author email : ese@pml.ac.uk

Running title :

Feeding of decapod larvae

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 picoplankton 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.

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 1956). 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, Burnett 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 12km 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 two months from one 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 1967), 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 on-going (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, 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 pre-ashed 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^{R} 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 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 methods section below). Sub-samples were taken from each incubated bottle; 100mL 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 protozoo-plankton 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 protozoo-plankton assemblages was carried out using a combination of analytical flow cytometry and inverted light microscopy.

Flow cytometry was used to characterise and enumerate *Synechococcus* (0.8-2µm)(SYN), pico-eukaryotes (0.2-2.0µm) (PEUK) and nanoeukaryotes (2-20µm) (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, 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 x200 magnification using

148 an Olympus IMT-2 inverted microscope (Utermöhl 1958). Depending on cell density either the
149 whole chamber, half of it or two transects across its maximum diameter were enumerated, with a
150 minimum number of cells counted being 300. Each cell >20µm was identified and enumerated,
151 however in the case of ciliates all were counted regardless of size. Cells were identified to genus
152 whenever possible and these were further combined according to taxonomic group: diatoms
153 (counts of chain-forming diatoms refer to cells not chains), armoured dinoflagellates, unarmoured
154 dinoflagellates and ciliates. Cell volumes were calculated by approximating to simple geometric
155 shapes according to Kovala and Larrance (1966) using average cell length, width and depth
156 measurements for each individual taxon (Widdicombe et al. 2010a). Carbon contents per cell were
157 then estimated using the empirically derived C:vol conversions of Menden Deuer and Lessard
158 (2000).

159 Decapod clearance and ingestion rates were calculated for each prey type or group of prey
160 types from differences in the rates of change of prey abundance in experimental bottles with and
161 without addition of decapods (Frost 1972). Clearance was only calculated when there were more
162 than 25 of each cell type enumerated in an aliquot/subsample and where there was a significant
163 difference in prey concentration between controls and experimental bottles, as determined by *t*-
164 tests (df=3, p=<0.1). The reduction in prey concentration in experimental bottles ranged from 4-
165 37%. Carbon specific ingestion rates were calculated by dividing the ingestion rates (µg C ind^{-1} d^{-1})
166 by the average larval mass in µg C, shown in Table 1. We used regression analysis to determine
167 whether there was a relationship between ingestion rates and available food. We used one-way
168 ANOVA to determine whether there were any significant differences in clearance rates of the
169 different food types by i) each decapod genus ii) between body C content of the decapod larval
170 stages and iii) between experiments for each decapod genus. Where ANOVA results were
171 significant (p=<0.05) we performed post-hoc comparisons using Tukey HSD (honestly significant
172 difference) to determine the cause of the variation.

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

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

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

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

where F_i is the clearance rate of the i th food type and $\sum F_i$ 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, the whole process was carried out as quickly as possible to minimize 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 mins. Total lysis was left for 4 h at 56°C and the DNA eluted in 2 x 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. (submitted).

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

211 prymesiales. One microliter of each PCR product was used as a template for a nested PCR using
212 the same PCR components described above but with 10 µM of the custom designed forward and
213 reverse primers Nest-Hapto-F 5'-TGA CAC AGG GAG GTA GTG ACA AG-3' and Nest-Hapto-R 5'-GGT
214 CGA AAC CAA CAA AAT AGC ACC-3' again following the cycling parameters described by Lindeque
215 et al. (submitted).
216 Individual amplicons were separated from the mix of amplicons within the PCR products by
217 clean-up of the PCR product using a QIAquick purification kit (Qiagen UK Ltd) and subsequent
218 cloning using the pGEM®-T Easy Vector System and JM109 competent cells (Promega UK Ltd)
219 following the standard protocol. Colony PCR with 10 µM of primers M13f and M13r (Heidecker et
220 al. 1980) preceded sequencing by LGC Genomics GmbH, Germany of each successful
221 amplification.
222 Sequences were opened in Mega 5 (Heidecker et al. 1980) and trimmed to the forward and
223 reverse primers. The Qiime pipeline (Caporaso et al. 2010) was used to assign the sequences to
224 Operational Taxonomic Units (OTUs) at the 97% similarity level and to generate representative
225 sequences. The representative sequences were then manually assigned taxonomy by searching
226 the EMBL DNA database for sequence similarities using a Basic Local Alignment Search Tool
227 (BLAST).
228
229 **Results**
230 *Seasonal and inter-annual changes in the natural food assemblage*
231 While the absolute amounts of food varied only about 3-4 fold throughout the 8
232 experiments, prey composition varied greatly, both seasonally and between the 2 years (Fig. 1a).
233 Chl-*a* concentrations in initial seawater samples (<200 µm) ranged between 0.5 and 1.6 µg chl *a* L^{-1}
234 with values often lower in 2010 than 2009 (Table 1). Diatoms were most abundant in May (mostly
235 *Thalassiosira* spp. and *Guinardia delicatula*) and July 2009 (*Chaetoceros* spp.) but comprised the
236 highest percentage of total biomass in May for both years. The diatoms present in spring 2010
237 were mostly *Chaetoceros densus*, *Rhizosolenia* spp, *Guinardia flaccida* and *G. delicatula* but the
238 latter were 10 times less abundant than in 2009 (Table 1). Dinoflagellates were more abundant in
239 2009 than 2010 and reached maximum abundance in August 2009 which corresponded with high
240 abundance of the unarmoured dinoflagellate *Karenia mikimotoi* and an unidentified Gymnodinoid
241 species of a similar size; during this time dinoflagellates comprised 90% of the total biomass. In
242 contrast, pico- and nanoeukaryotes were more abundant in April to June 2010 than 2009 (Table 1)
243 when they comprised 40 to 82 % of the total biomass. Ciliate abundance was greatest in August
244 2009 but their contribution to total biomass at this time was <1%. One ciliate species, *Mesodinium*
245 *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 $^{-1}$ d^{-1} for *Necora*; 1-27 µg C ind^{-1} d^{-1} for *Liocarcinus* and 3-11 µg C ind^{-1} d^{-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 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 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 $F=<1.2$, $p>0.2$, $df=4$) (Fig. 4), even the very small pico- and nanoeukaryotes were cleared and all three species could feed across 4 to 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 > 1000μm^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 Figure 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 armoured dinoflagellates and diatoms significantlyfaster than *Synechococcus* ($p=<0.05$). This general picture, of higher clearance rates of dinoflagellates or diatoms are 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 $df=5$, $p=>0.1$).

Molecular analysis of gut content

Using primers designed to target the haptophyte group, a total of 181 sequences were obtained from *Necora, Liocarcinus* and *Upogebia* feeding experiments and *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 at 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 meetings with prey and is independent of food type or quality (Factor & Dexter 1993; Welch and Epifanio 1995). Whilst 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 which 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). Whilst some species of pelagic copepods (e.g. *Oithona* spp.) have been shown to select motile prey over non-motile 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 a.l 2001). In this study we saw no evidence of increased clearance rates of motile cells relative to diatoms. The fact that decapod larvae in this study were ingesting a broad range of cell

384 sizes is more consistent with suspension feeding than the raptorial or ambush feeding that has
385 been found in later megalopae stages (Chen et al. 2013). While suspension feeding is likely a
386 requirement to capture the pico-and nanoplankton cells perhaps too small and numerous for
387 individual handling (Price, 1983), we cannot discount the possibility that other feeding modes are
388 employed on food items that were too large and rare for enumeration in our experiments, thereby
389 increasing their daily ration (Wirtz, 2012).

390 The fact that decapod larvae fed on the small pico- and nano-size fraction was a noteworthy
391 finding of our study. Due to the functional morphology of the decapod larval mouthparts, it is
392 thought that as single cells, these food size classes are not easily accessible to most decapod
393 larvae (Anger 2001) but they could become more important as food items when they are
394 associated with detritus (Kiørboe 2000). We did not enumerate aggregates here but a study by
395 Factor and Dexter (1993) demonstrated that green crab larvae were capable of ingesting small
396 particles such as bacteria, small algal cells and organically enriched detrital particles (1-10 μm) in
397 their natural diet. Ingestion of pico and nano-sized cells has been shown to enhance survival time
398 compared to starved larvae (e.g. Lehto et al 1998) and therefore could provide an important food
399 source particularly when larger food items are scarce.

400 This is one of the very few studies of decapod larval feeding rates on natural plankton
401 communities. Other studies have been carried out with brachyuran crab larvae (Schwamborn et al.
402 2006), shrimp larvae (Harvey and Morrier 2003) and mud crab larvae (Epifanio et al. 1994) but
403 these studies only looked at feeding on food items in the >50 μm food size range. Thus direct
404 comparison with other studies is difficult, although feeding rates of other meroplanktonic larvae
405 have been investigated to some extent (e.g. Vargas et al. 2006; Almeda et al. 2010) and the
406 ingestion of natural phytoplankton pigments by decapod larvae has been shown (Harms and
407 Harms, 1993). Once thought to be exclusively carnivorous (Thorson 1946) laboratory studies have
408 shown that phytoplankton can also be eaten by decapod larvae (e.g. Harms and Seeger 1989).
409 However, the nutritional gain is variable and dependent on species and stage (Sulkin et al. 1998)
410 and thought to be more important for the younger stages (Anger, 2001). Further studies have
411 shown that heterotrophic protists provide a supplementary food source for larval crabs (Sulkin et
412 al. 1998; Hinz et al. 2001). Our results show that phytoplankton and protozooplankton, which are
413 abundant in coastal waters and are a potentially rich source of carbon (Azam et al. 1983), are
414 common components of the natural diet of decapod larvae. Diatoms, dinoflagellates,
415 nanoeukaryotes and to a lesser extent ciliates represented a significant food source for decapod
416 larvae in coastal waters, together contributing average daily carbon rations of 17-60% for the
417 three species. These findings are in line with those of Anger (1990) who reported that early crab
418 zoeae may ingest up to ca 40% body C and provide further evidence of a trophic link between the

419 microbial loop and decapod larvae (Lehto et al. 1998; Sulkin et al. 1998). Whilst there can be a
420 degree of uncertainty associated with applying carbon to volume conversion factors to estimates
421 of plankton abundance (Montagnes et al. 1994) our estimates of prey biomass and fall within the
422 ranges reported for coastal waters of the English Channel (Irigoien et al. 2000a, Fileman et al.
423 2010, Fileman et al. 2011) and when compared with chlorophyll concentrations provide realistic
424 C:chl ratios of between 30-70 (Irigoien et al. 2000b) .

425 These food sources, while important, likely only represent part of the total ingestion by
426 decapod larvae. Other food sources such as micro-metazoans and marine snow aggregates (not
427 counted here) could also contribute to the decapod diet (e.g. Incze and Paul 1983; Epifanio et al.
428 1994; Harvey and Epifanio 1997; Lehto et al. 1998) and a mix of both algal and animal food is
429 thought to be best for survival and development of *Necora* and *Liocarcinus* larvae (Choy, 1991).
430 The ability to feed on a wide spectrum of food sources, including nano- and microplankton, would
431 enhance the nutritional value of the larval food supply (Harms and Seeger, 1989).

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

448 *Unpredictability of food resources*

449 The ability of decapod larvae to ingest a wide variety of prey has important ecological
450 implications. Meroplankton can be seasonally abundant, and the timing of their appearance needs
451 to be matched with the seasonality of their food (Thorson 1946, Edwards and Richardson 2004).
452 The seasonal cycle and vertical distribution of decapod larvae around the UK have been described

453 by Lindley (1987), and Lindley et al. (1994) shows some evidence of decapod larvae being found in
454 areas of greater food concentrations. However, the timing of the seasonal occurrence of decapod
455 larvae in the plankton is also related to temperature, and this temperature-dependence of
456 decapod reproduction may be a stronger governing factor than seen in the holoplankton (Lindley
457 1998). Bloom timing can be highly variable from year to year (e.g. Bigelow et al. 1940) relative to
458 the timing of appearance of these larvae in the plankton (Fig. 6a). However, phenology studies
459 have shown that the average timing of diatom blooms over longer time scales has changed little
460 (Edwards and Richardson 2004; Wiltshire et al. 2008). In contrast, meroplanktonic species such as
461 decapods are more dependent on temperature to stimulate larval release and are suggested to
462 have shifted their development forward in response to earlier warming, such that a mismatch
463 could arise between grazers and their food (Edwards and Richardson 2004). Even though food
464 levels in terms of total C and Chl-*a* seemed to be fairly high in this study, the composition varied
465 enormously, and for example in March and May 2010 despite reasonable food concentrations,
466 most was of very small size (Fig. 1). Conversely the highest decapod larval abundances during both
467 2009 and 2010 occurred outside of bloom periods, with chl *a* concentrations < 0.5 mg m^{-3} (Fig. 6a)
468 Thus the ability of decapod larvae to consume small prey items if mismatched with blooms of
469 larger diatoms or dinoflagellates may increase their chances of survival.

470 *Trophic impact*

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

477 The zooplankton at L4 are very diverse (Highfield et al. 2010; Lindeque et al. 2013) so it is
478 unlikely that a single taxonomic group will ever be dominant in terms of grazing impact on its prey
479 (e.g. Fig. 7). However the advantage of the high resolution data derivable from bottle incubations
480 is that we can calculate grazing impact for each species on each prey item. In this study we found
481 the highest grazing impact by decapod larvae to be in the summer post-bloom period when the
482 population was dominated by dinoflagellates. It is therefore possible that the grazing impact of
483 decapod larvae could contribute to a high total zooplankton impact at certain times of the year
484 and on certain prey taxa (e.g. selected diatoms and dinoflagellates). When combined with other
485 predator and prey-specific grazing studies from this site, (e.g. Fileman et al. 2010, Lindeque et al.
486 submitted) 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).

Acknowledgements

This work was in part funded by the EU INTERREG IV CHARM 3 project (www.charm-project.org) (Management number 4037/1938) and the Natural Environment Research Council National Capability funding for the Western Channel Observatory (Agreement number R8-H12-84). We are grateful to Denise Cummings and the crew of PML RV Quest for water collection, James Highfield for experimental help, Katharine Pemberton for molecular analysis, Glen Tarran for help and advice on flow cytometry and Claire Widdicombe for L4 phytoplankton data Denise Cummings for L4 chlorophyll data and John Bruun for his expert statistical advice. We also thank three anonymous reviewers whose comments have greatly improved this manuscript.

References:

Almeda R, Messmer AM, Sampedro N, Gosselin LA (2010) Feeding rates and abundance of marine invertebrate planktonic larvae under harmful algal bloom conditions off Vancouver Island, Harmful Algae, 10, 194-206

Anger K (2001) The Biology of Decapod Crustacean Larvae. In: Vonk R (ed) Crustacean Issues 14 A.A. Balkema Publishers, Lisse, The Netherlands, 405 pp.

Anger K and Dawirs RR (1981) Influence of starvation on the larval development of *Hyas araneus* (Decapoda, Majidae). Helgol Wiss Meeresunters, 34: 287-311

Anger K, Dawirs RR, Anger RV, Costlow, JD (1981) Effects of early starvation periods on zoeal development of brachyuran crabs . Biol Bull 161: 199-212

Atkinson A (1995) Omnivory and feeding selectivity in five copepod species during spring in the Bellingshausen Sea, Antarctica. ICES J Mar Sci 52: 385-396

Atkinson A, Fileman ES, Widdicombe C, Harmer R, McEvoy A, Harris RP, Smyth T (2013) Plymouth L4 (Site 48) In: O'Brien TD, Wiebe PH, Falkenhaug T (eds.) ICES Zooplankton Status Report 2010/2011. ICES Cooperative Research Report No. 318 pp 127-131

Azam F, T Fenchel , J G Field, J S Gray, L A Meter-Reil and F Thingstad (1983) The ecological role of water-column microbes in the sea. Mar Ecol Prog Ser 10: 257-263

Barnes MK, Tilstone G, Smyth T, Widdicombe CE, Glöel J, Robinson C, Suggett DJ. Drivers and effects of *Karenia mikimotoi* blooms in the western English Channel. Prog Oceanog (in review 2014).Bigelow HB, Lillick LC, Sears M (1940) Phytoplankton and planktonic protozoa of the

522 offshore waters of the Gulf of Maine. Trans Amer Phil Soc 31:149-237
523 Boidron-Métairon I F (1995) Larval nutrition. Ecology of Marine Invertebrate Larvae. Editor
524 McEdward L, New York: CRC Press. pp 223-248
525 Booth BC, Lewin J, Lorenzen CJ (1988) Spring and summer growth rates of subarctic Pacific
526 phytoplankton assemblages determined from carbon uptake and cell volumes estimated using
527 epifluorescence microscopy. Mar Biol 98: 287– 298
528 Burnet N, Sulkin S (2007) Characteristics of feeding on dinoflagellates by newly hatched larval
529 crabs. Mar Biol 151: 851–861
530 Caporaso JG, Kuczynski J, Stombaugh J, Bittinger K, Bushman FD, Costello EK, Fierer N, Pena AG,
531 Goodrich JK, Gordon JI, Huttley GA, Kelley ST, Knights D, Koenig JE, Ley RE, Lozupone CA,
532 McDonald D, Muegge BD, Pirrung M, Reeder J, Sevinsky JR, Tumbaugh PJ, Walters WA, Widmann J,
533 Yatsunenko T, Zaneveld J, Knight R (2010) QIIME allows analysis of high-throughput community
534 sequencing data. Nat Methods 7: 335-336
535 Chen X, Lin Q, Wang G, Li S (2013) Feeding in the megalopae of the mud crab (*Scylla*
536 *paramamosain*): mechanisms, plasticity, role of chelipeds and effect of prey density. Mar Fresh
537 Behav Phys , 46, 321-336.Choy SC (1991) Embryonic and larval biology of *Liocarcinus holsatus* and
538 *Necora puber* (Crustacea: Brachyura: Portunidae). J Exp Mar Biol Ecol 148: 77-92
539 Coolen MJL, Muyzer G, Rijpstra WIC, Schouten S, Volkman JK, Damste JSS, 2004. Combined DNA
540 and lipid analyses of sediments reveal changes in Holocene haptophyte and diatom populations in
541 an Antarctic lake. Earth Planet Sc Lett 223: 225-239
542 Durbin EG, Casas MG, Rynearson TA (2012) Copepod feeding and digestion rates using prey DNA
543 and qPCR. J Plankton Res 34: 72-82
544 Edwards M, Richardson AJ (2004) Impact of climate change on marine pelagic phenology and
545 trophic mismatch. Nature 430: 881-884
546 Eloire D, Somerfield PJ, Conway DVP, Halsband-Lenk C, Harris R, Bonnet D (2010) Temporal
547 variability and community composition of zooplankton at station L4 in the Western Channel: 20
548 years of sampling. J Plankton Res 32: 657-679
549 Epifanio CE, Lobanoff MA, Connaughton VP, Welch JM (1994) Growth and development of
550 Atlantic mud crab larvae fed natural zooplankton prey. J Exp Mar Biol Ecol 180: 165-174
551 Factor J R, Dexter BL (1993) Suspension feeding in larval crabs (*Carcinus maenas*). Journal of the
552 Marine Biological Association of the United Kingdom 73: pp. 207-211
553 Fileman, E, Petropavlovsky, A, Harris, R (2010) Grazing by the copepods *Calanus helgolandicus* and
554 *Acartia clausi* on the protozooplankton community station L4 in the Western English Channel.
555 Journal of Plankton Research, 32: 709-724

556 Fileman ES, Fitzgeorge-Balfour T, Tarran GA, Harris RP (2011) Plankton community diversity from
557 bacteria to copepods in bloom and non-bloom conditions in the Celtic Sea in spring. Estuar Coast
558 Shelf Sci, 93: 403–414

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

561 Gentleman W, Neuheimer AB (2012) Functional responses and ecosystem dynamics: how
562 clearance rates explain the influence of satiation, food-limitation and acclimation. J Plankton Res
563 30: 1215-1231

564 Harms J, Meyer-Harms B, Dawirs RR, Anger K (1994) Growth and physiology of *Carcinus maenas*
565 (Decapoda: Portunidae) larvae in the field and in laboratory experiments. Mar Ecol Prog Ser, 108:
566 107–118

567 Hartman MC Letterman GR (1978) An evaluation of three species of diatoms as food for *Cancer*
568 *magister* larvae. Proc. 9th Annu. Meet. World Maricult. Soc. pp. 271-276

569 Heidecker G, Messing J, Gronenborn B (1980) A Versatile Primer for DNA Sequencing in the
570 M13mp2 Cloning System. Gene 10: 69-73

571 Highfield JM, Eloire D, Conway D, Lindeque P, Attrill MJ, Somerfield PJ (2010) Seasonal dynamics of
572 meroplankton assemblages at station L4. J Plankton Res 32:681-691

573 Harms J, Seeger B (1989) Larval development and survival in seven decapod species (Crustacea) in
574 relation to laboratory diet. J Exp Mar Biol Ecol 133:129-139

575 Harms J, Meyer-Harms B, Dawirs RR, Anger K (1994) Growth and physiology of *Carcinus maenus*
576 (Decapoda: Portunidae) larvae in the field and in laboratory experiments. Mar Ecol Prog Ser 108:
577 107-118

578 Harvey EA, Epifanio CE (1997) Prey selection by larvae of the common mud crab *Panopeus*
579 *herbstii* Milne-Edwards. J Exp Mar Biol Ecol 217: 79-91

580 Harvey M, Morrier G (2003) Laboratory feeding experiments on zoea of northern shrimp *Pandalus*
581 *borealis* fed with natural zooplankton. Mar Ecol Prog Ser 265:165-174

582 Hinz S, Sulkin S, Strom S, Testermann J (2001) Discrimination in ingestion of protistan prey by
583 larval crabs. Mar Ecol Prog Ser 222: 155-162

584 Holthuis LB (1987) *Necora*, a new genus of European swimming crabs (Crustacea Decapoda,
585 Portunidae) and its type species, *Cancer puber* L., 1767. Zoologische Mededelingen 61: 1–14

586 Incze LS, Paul AJ (1983) Grazing and predation as related to energy needs of stage I zoeae of the
587 tanner crab *Chionoecefes bairdi* (Brachyura, Majidae). Biol Bull (Woods Hole) 165: 197-20

588 Irigoien X, Harris RP, Head RN, Harbour D (2000a) North Atlantic Oscillation and spring bloom
589 phytoplankton composition in the English Channel. J Plankton Res 22: 2367-2371

590 Irigoien X, Head RN, Harris RP, Cummings D, Harbour D, Meyer-Harms B (2000b) Feeding
591 selectivity and egg production of *Calanus helgolandicus* in the English Channel. Limnol Oceanogr
592 45 : 44-54
593 Irigoien X, Flynn KJ, Harris RP (2005) Phytoplankton blooms: a "loophole" in microzooplankton
594 grazing impact? J Plankton Res 27: 313-321
595 Johnson PR, Tiselius P (1990) Feeding behaviour, prey detection and capture efficiency of the
596 copepods *Acartia tonsa* feeding on planktonic ciliates. Mar Ecol Prog Ser 60: 35-44
597 Kovala PE Larrance JD, (1966)Computation of phytoplankton cell numbers, cell volume, cell surface
598 area and plasma volume per litre, from microscopical counts. Special Report, vol. 38, University of
599 Washington, Seattle, 1-91
600 Kiørboe T (2000) Colonization of marine snow aggregates by invertebrate zooplankton:
601 Abundance, scaling, and possible role. Limnol Oceanogr 45: 479-484
602 Kiørboe T (2011) How zooplankton feed: mechanisms, traits and trade-offs. Biol Rev 86: 311-339.
603
604 Lebour M, (1922) The food of plankton organisms. J Mar Biol Ass UK, 12: 644-677
605 Lehto J, Sulkin SD, Strom S, Johnson D (1998) Protists and detrital particles as prey for the first
606 larval stage of the brachyuran crab, *Hemigrapsus oregonensis*. J Exp Mar Biol Ecol 230: 213-224
607 Lindeque PK, Parry HE, Harmer RA, Somerfield PJ, Atkinson A (2013) Next Generation Sequencing
608 Reveals the Hidden Diversity of Zooplankton Assemblages. PloS one 8 (11): e81327
609 Lindley, JA (1987) Continuous plankton records: the geographical distribution and seasonal cycles
610 of decapod crustacean larvae and pelagic post-larvae in the north-eastern Atlantic Ocean and the
611 North Sea. J Mar Biol Ass UK, 122: 195-211
612 Lindley JA, Williams, R, Conway, DVP (1994) Variability in dry weight and vertical distributions of
613 decapod larvae in the Irish Sea and North Sea during the spring. Mar Biol 120: 395-395
614 Lindley, JA (1998) Diversity, biomass and production of decapod crustacean larvae in a changing
615 environment. Invertebrate Reproduction & Development. Volume 33: 209-219
616 McConaugha JR, (2002) Alternative feeding mechanisms and prey selection in post-larval
617 megalopae of the blue crab, *Callinectes sapidus*. Mar Biol 140: 1227-1233
618 Menden-Deuer S, Lessard EJ (2000) Carbon to volume relationships for dinoflagellates, diatoms,
619 and other protist plankton. Limnol Oceanogr 45: 569-579
620 Montagnes DJS, Berges, JA, Harrison, PA Taylor FJR (1994) Estimating carbon, nitrogen, protein
621 and chlorophyll a from volume in marine phytoplankton. Limnol Oceanogr 39: 1044–1060
622 Nejstgaard JC, Frischer ME, Raule CL, Gruebel T, Kohlberg KE, Verity PG (2003) A new approach to
623 an old problem: molecular detection of algal prey in copepod guts and faecal pellets. Limnol
624 Oceanogr Meth 1: 29-38

625 Nejstgaard JC, Frischer ME, Simonelli P, Troedsson C, Brakel M, Adiyaman F, Sazhin AF, Artigas AF
626 (2008) Quantitative PCR to estimate copepod feeding. Mar Biol 153: 565-577
627 Olson RR, Olson MH (1989) Food limitation of planktotrophic marine invertebrate larvae: does it
628 control recruitment success? Ann Rev Ecol Syst 20: 225-247
629 Pan M, Pierce GJ, Cunningham CO, Hay SJ (2011) Seasonal and interannual variation of decapod
630 larval abundance from two coastal locations in Scotland, UK. J Mar Biol Ass UK, 91: 1443-1451
631 Perez M F, Sulkin SD (2005) Palatability of autotrophic dinoflagellates to newly hatched larval
632 crabs. Mar Biol 146: 771-780
633 Pingree RD, Griffiths DK (1978) Tidal fronts on the shelf seas around the British Isles, Journal of
634 Geophysical Research 83: 4615–4622.
635 Price HJ, Paffenhöfer G-A (1983) Capture of small cells by the copepod *Eucalanus elongatus*.
636 Limnol Oceanogr 31: 189-194
637 Roman MR, Rublee PA (1980) Containment Effects in Copepod Grazing Experiments: A Plea to End
638 the Black Box Approach. Limnol Oceanogr 25:982-990
639 Schwamborn R, Ekau W, Silva AP, Neumann-Leitào S, Saint-Paul U (2006) Ingestion of large centric
640 diatoms, mangrove detritus, and zooplankton by zoeae of *Aratus pisonii* (Crustacea: Brachyura:
641 Grapsidaae). Hydrobiologia: 560, 1–13
642 Shaber K, Sulkin S (2007) Feeding on dinoflagellates by intermediate and late stage crab zoeae
643 raised in the laboratory and collected from the field J Exp Mar Biol Ecol 340:149-159
644 Simon N, Campbell L, Ornolfsdottir E, Groben R, Guillou L, Lange M, Medlin LK (2000)
645 Oligonucleotide probes for the identification of three algal groups by dot blot and fluorescent
646 whole-cell hybridization. J Eukaryot Microbiol 47:76-84
647 Smyth T, Fishwick J, Al-Moosawi L, Cummings DG, Harris C, Kitidis V, Rees A, Martinez-Vicente V,
648 Woodward EMS (2010) A broad spatio-temporal view of the Western English Channel
649 observatory. J Plankton Res 32: 585-601
650 Strathmann RR (1995) Are planktonic larvae of marine benthic invertebrates too scarce to
651 compete within species ? Oceanologica acta 19: 399-407
652 Sulkin SD, Lehto J, Strom S, Hutchinson D (1998) Nutritional role of protists in the diet of first stage
653 larvae of the Dungeness crab *Cancer magister*. Mar Ecol Prog Ser 169:237-242.
654 Tarran GA, Heywood JL, Zubkov, MV (2006) Latitudinal changes in the standing stocks of nano- and
655 picoeukaryotic phytoplankton in the Atlantic Ocean. Deep-Sea Res II: 53: 1516-1529
656 Thorson G (1946) Reproduction and larval development of Danish Marine bottom invertebrates.
657 Medd Komm Dan Fisk-Havunders Ser Plankton: 4 pp 1–523
658 Utermöhl H, (1958) Zur vervollkommnung der quantitativen phytoplankton methodik. Mitteilungen
659 der Internationalen Vereinigung für Theoretische und Angewandte Limnologie 9: 1-38

660 Vargas CA, Manriquez P, Navarrette SA (2006) Feeding by larvae of intertidal invertebrates:
661 Assessing their position in pelagic trophic webs. Ecology 87: 444–457
662 Welch, J M Epifanio, C E (1995) Effect of variations in prey abundance on growth and
663 development of crab larvae reared in the laboratory and in large, field-deployed enclosures.
664 Marine Ecology Progress Series 116: 55–64
665 Widdicombe, CE Eloire D, Harbour D, Harris R, Somerfield PJ (2010a) Time series of phyto- and
666 microzooplankton abundance and composition at station L4 in the English Channel from 1988 to
667 2009. doi:10.1594/PANGAEA.758061
668 Widdicombe C, Eloire D, Harbour D, Harris R, Somerfield PJ (2010b) Long-term phytoplankton
669 community dynamics in the Western English Channel. J Plankton Res 32: 643-655
670 Wirtz K W. 2012. Who is eating whom? Morphology and feeding type determine the size relation
671 between planktonic predators and their ideal prey. Mar Ecol Prog Ser 445: 1-12
672
673

Figure 1

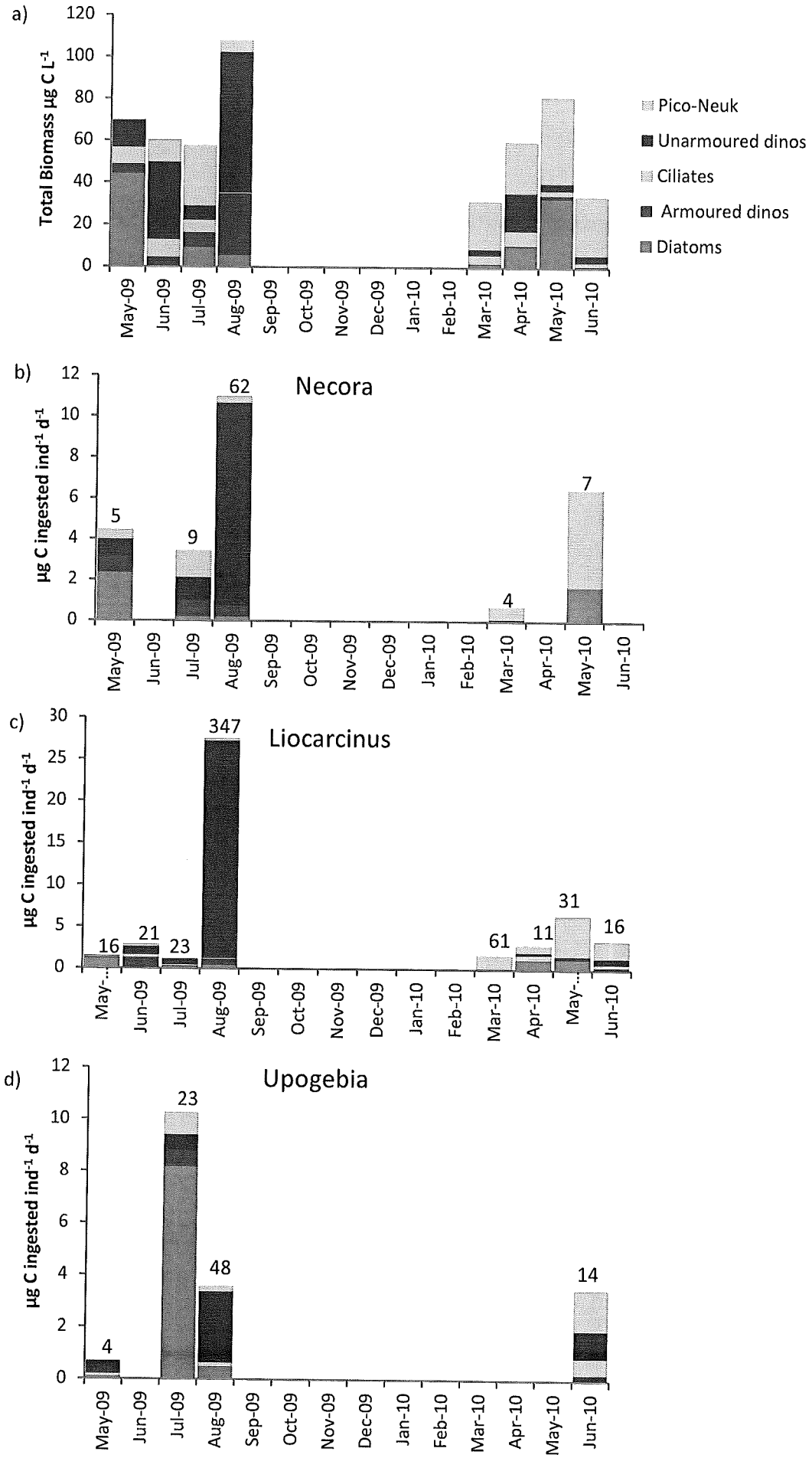
a)
Total Biomass µg C L-1
120
100
80
60
40
20
0
May-09
Jun-09
Jul-09
Aug-09
Sep-09
Oct-09
Nov-09
Dec-09
Jan-10
Feb-10
Mar-10
Apr-10
May-10
Jun-10
Pico-Neuk
Unarmoured dinos
Ciliates
Armoured dinos
Diatoms
b)
Necora
µg C ingested ind-1 d-1
12
10
8
6
4
2
0
5
9
62
4
7
c)
Liocarcinus
µg C ingested ind-1 d-1
30
25
20
15
10
5
0
16
21
23
347
61
11
31
16
May-...
d)
Upogebia
µg C ingested ind-1 d-1
12
10
8
6
4
2
0
4
23
48
14

Figure 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} d^{-1}) and % daily C ration (numbers above bars) by *Necora*, *Liocarcinus* and *Upogebia*.

Figure 2

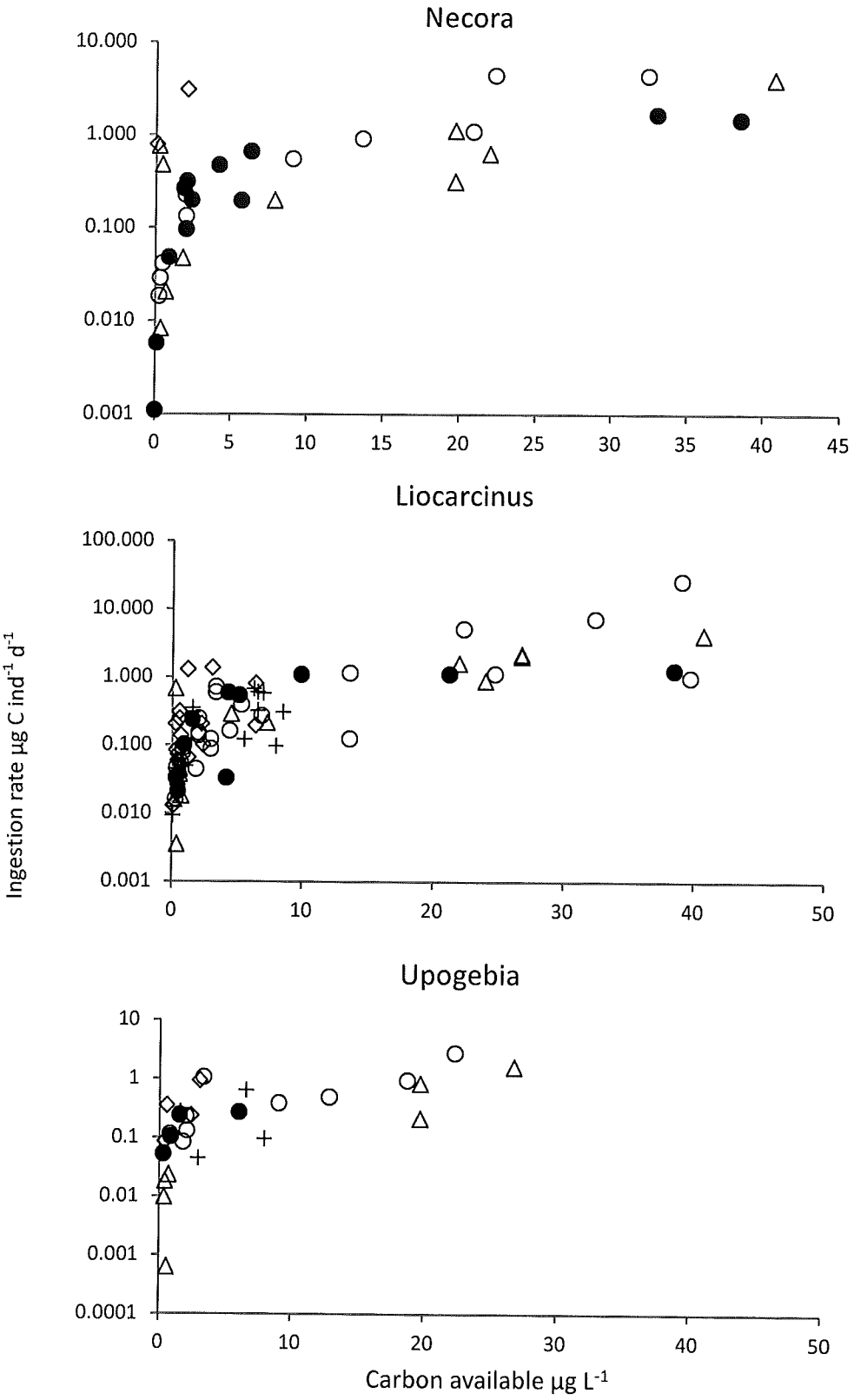

Figure 2: Ingestion rates (µg C ind^{-1} d^{-1}) of different prey types by *Necora* sp., *Liocarcinus* spp. and *Upogebia* spp. as a function of their concentration (µg C L^{-1}) ◊ = armoured Dinoflagellates; ○ = unarmoured Dinoflagellates; ● = diatoms; + = ciliates; Δ= pico- and nanoplankton.

Figure 3

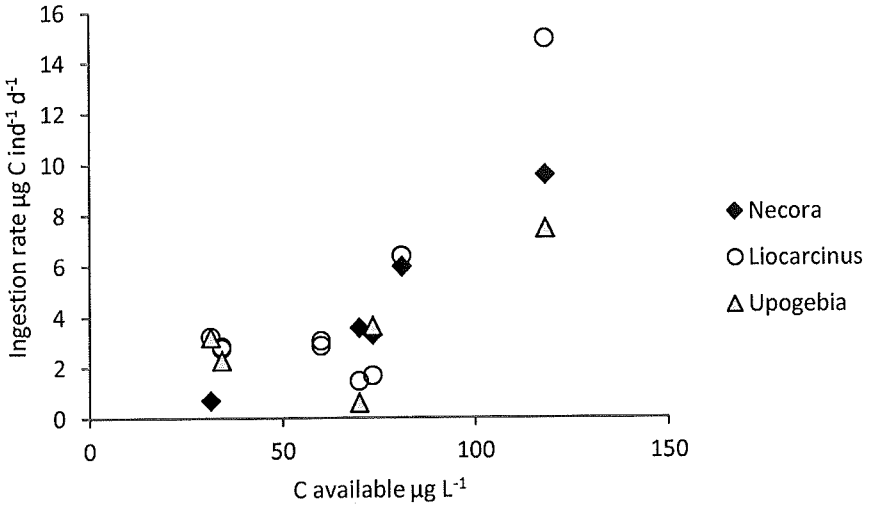

Figure 3: Total C ingestion (µg C ind^{-1} d^{-1}) by *Necora*, *Liocarcinus* and *Upogebia* as a function of total food concentration (µg C L^{-1}).

Figure 4

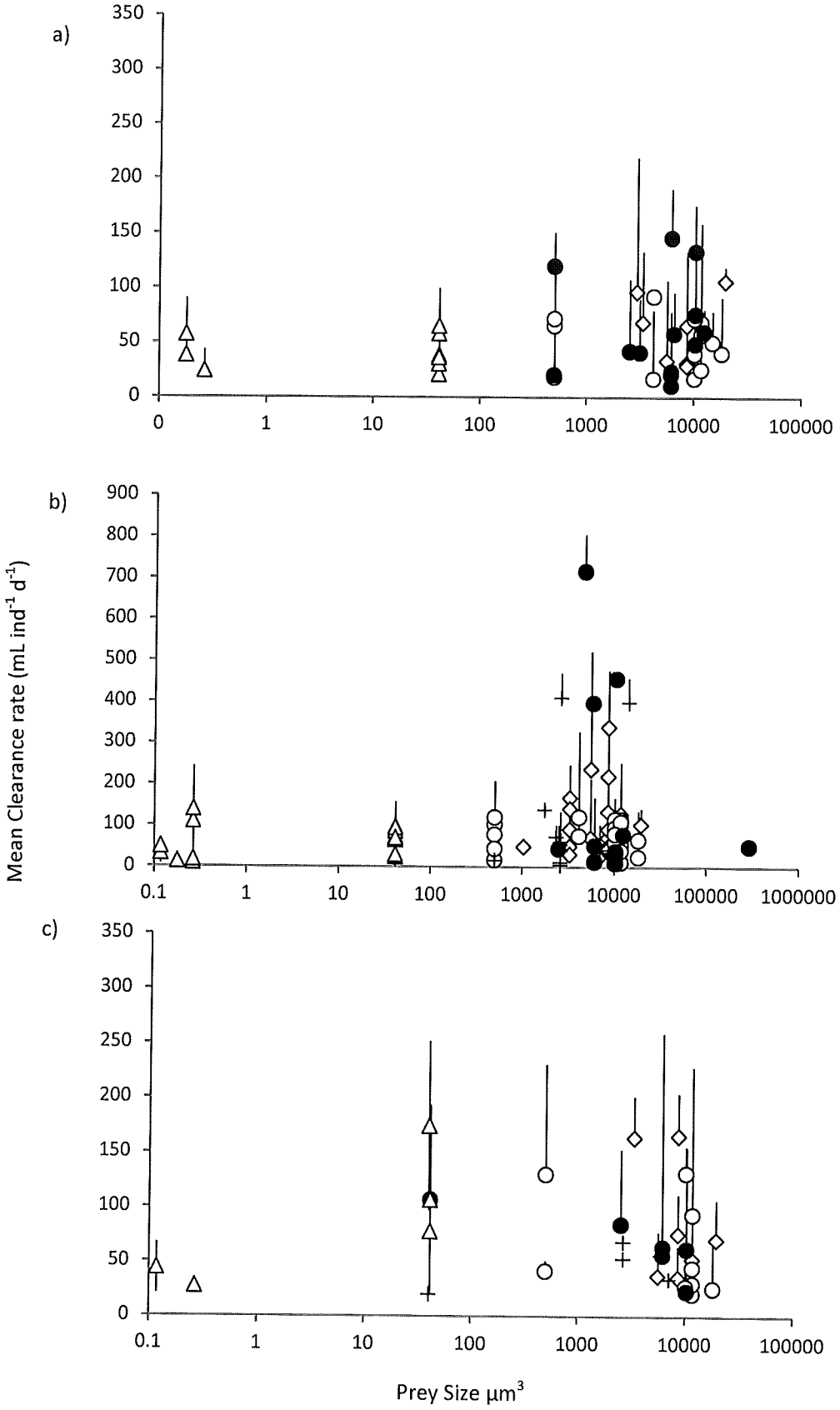

Figure 4: Mean clearance rates (mL ind^{-1} d^{-1}) with standard error bars of each prey category by a) *Necora, b*) *Liocarcinus and* c) *Upogebia* in relation to prey cell size (μm^3). Microplankton clearance rates have been temperature- adjusted to 12°C. ◊ = armoured Dinoflagellates; o = unarmoured Dinoflagellates; • = diatoms; + = ciliates; Δ= pico- and nanoplankton.

Figure 5

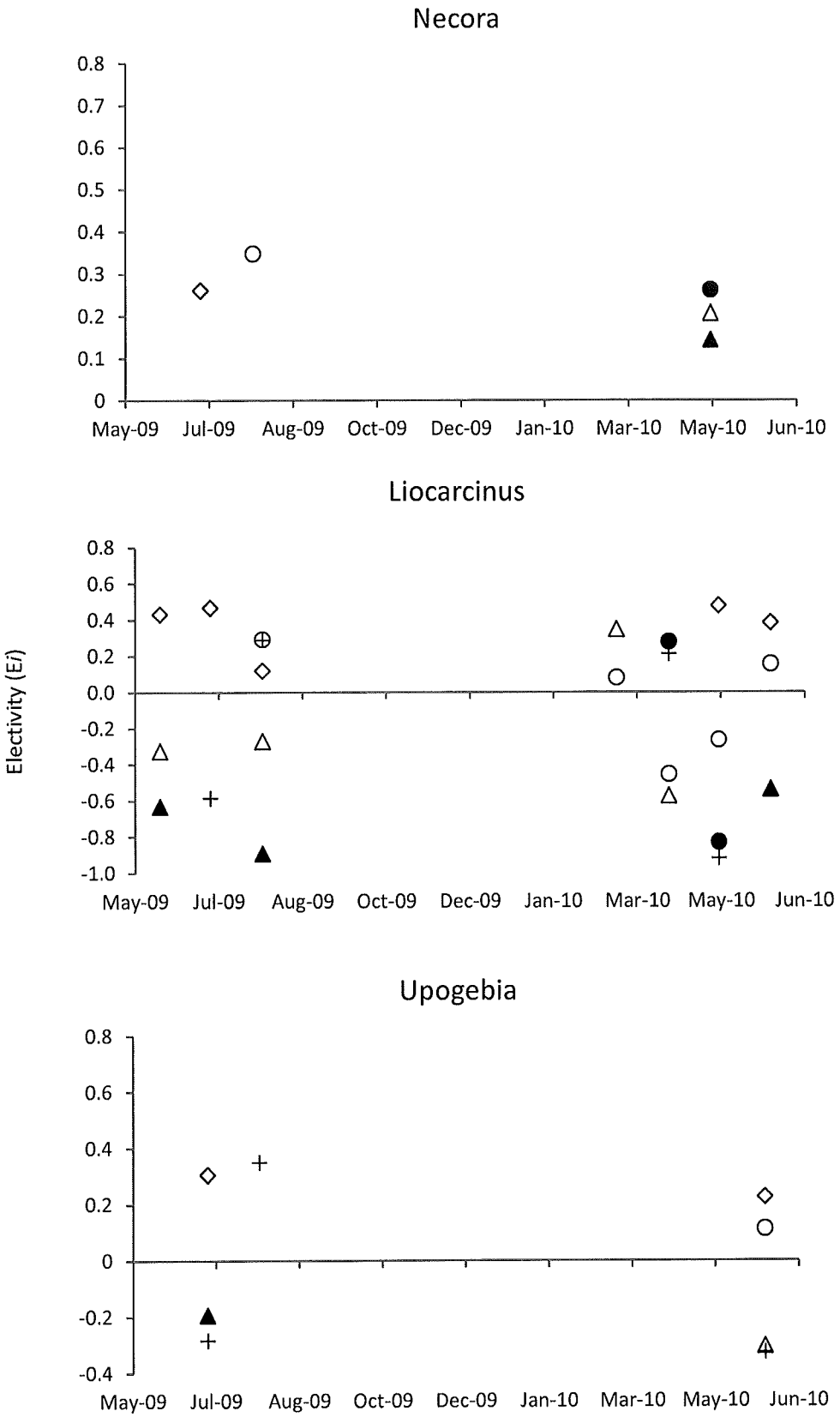

Figure 5: Electivity index (E_i) of the different prey groups for all experiments where clearance rates were significant. Only the data points where the electivity index was shown to deviate significantly from 0 (Student's t-test) are shown. ◊ = armoured dinoflagellates; ○ = unarmoured dinoflagellates; ● = diatoms; + = ciliates; Δ= nanoeukaryotes ▲ = picoeukaryotes/*Synechococcus*

Figure 6

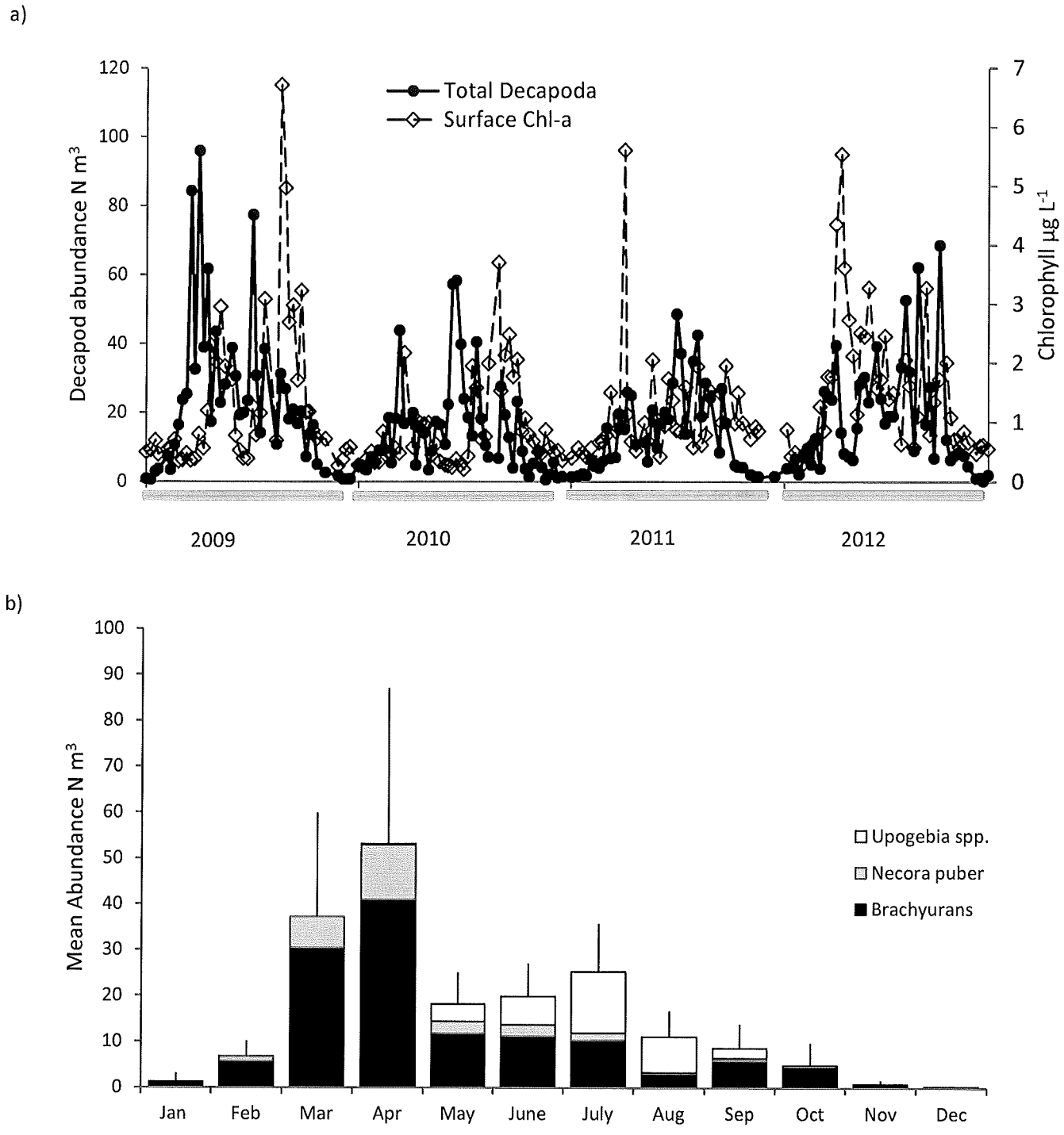

Figure 6: Seasonal variation in decapod abundance (Nm^3) at L4 a) total decapod abundance for 2009 and 2012 with total surface chlorophyll a concentrations ($\mu g\ L^{-1}$) b) monthly average abundance of *Necora puber*, Brachyurans (which includes *Liocarcinus* spp.) and *Upogebia* spp. during 2009-2010. Error bars indicate standard deviation of the mean total abundance (n= 8).

Figure 7

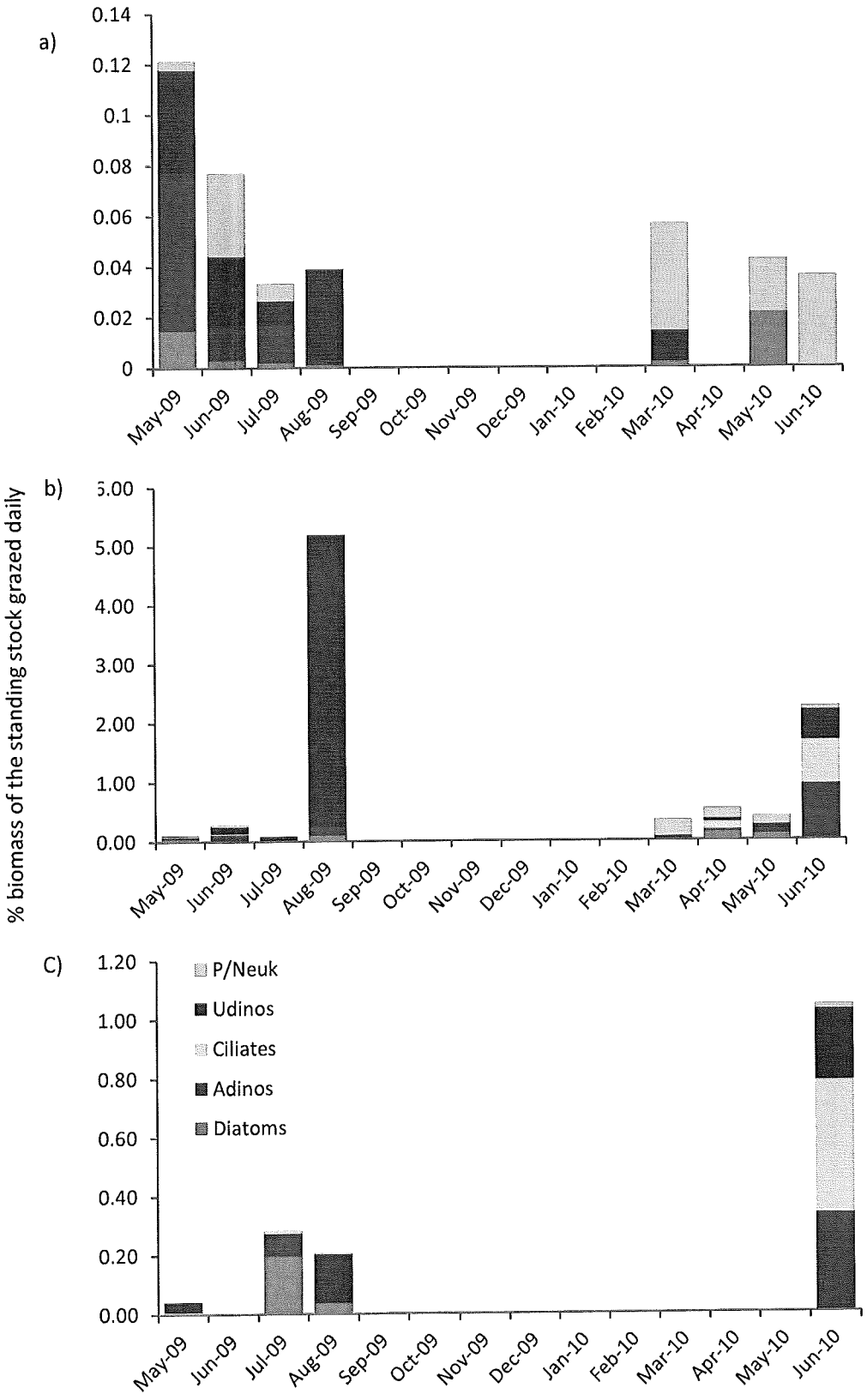

Figure 7: Potential trophic impact (% biomass of the standing stock grazed d^{-1}) by a) *Necora puber*, b) *Liocarcinus* spp. c) *Upogebia* spp. upon different prey.

Table 1

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. Syn = *Synechococcus,* Peuk=picoeukaryotes, Neuk=nanoeukaryotes.

Date	Incubation Temp. °C	Decapod Species Incubated	Stage	Mean C mass (µg) (±Stdev)	<200µm screened chl-*a* conc (µg l^{-1} ±stdev)	Mean prey abundance (cells ml^{-1}) and growth rate (d^{-1}) in brackets						
						Ciliates	Diatoms	Armoured dinos	Unarmoured dinos	Syn (0.8-2µm)	Peuk (0.2-2µm)	Neuk (2-20µm)
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)	1451 (0.01)	14330 (-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)	1045 (-0.01)	6.9 (0.67)	19.1 (0.35)	6630 (-0.10)	18311 (-0.46)	2153 (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)	19650 (-0.41)	8585 (-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)	5747 (-0.05)	10795 (-0.16)	2204 (-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)	7279 (-1.19)	2619 (-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)	6143 (-0.1)	5041 (-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)	9131 (-0.02)	13064 (-0.09)	3256 (0.08)
		Liocarcinus	IV, V	20 (8.6)								
		Upogebia	I, II	15.4 (0.4)								

Table 2: Minimum and maximum decapod larval clearance rates (temperature-adjusted) on commonly encountered food types. Empty cells represent no data for that prey category.

Prey Category	Taxon	Max average abund ± stdev cells/ml	*Necora* sp. (ml ind^{-1} d^{-1})	*Liocarcinus* sp. (ml ind^{-1} d^{-1})	*Upogebia* sp. (ml ind^{-1} d^{-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					
	Protoperidiniales <30µm	5.4 ±0.6	20-68	30-167	118
	Prorocentrum micans	1.7 ± 0.1		19-128	52
Unarmoured dinoflagellates					
	Gymnodiniales <30µm	21.7 ± 1.8	20-72	16-120	41-129
	Gymnodiniales 30-50µm	17.2 ± 2	18-92	11-116	20-93
	Gymnodiniales >50µm	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
	Myrionecta rubra	8.7 ± 0.4		139-398	
	Aloricate oligotrichs 30-50µm	3.9 ± 0.6		35-60	40
Pico- and Nano-plankton					
	Synechococcus sp.	19650 ± 2567	24	10-18	27
	Picoeukaryotes	18311 ± 2180	38-57	12-49	44
	Nanoeukaryotes	4451 ± 763	20-65	24-97	77-106

TABLE 3: Summary of ANOVA test and post-hoc Tukey HSD test to compare clearance rates on different food groups. ADino = armoured dinoflagellates; Diat = diatoms; UDino = unarmoured dinoflagellates; Neuk= nanoeukaryotes; Cils = ciliates; Peuk = picoeukaryotes; Syn = synechococcus; $\alpha = 0.05$.

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

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. sequences	No. 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
August 2009 Field	*Upogebia sp.*	21	0	21
	Liocarcinus spp.	21	0	21
June 2010 Experimental	*Liocarcinus spp.*	20	0	20
	Upogebia sp.	18	0	18
June 2010 Field	*Liocarcinus spp.*	20	14	6
	Upogebia sp.	20	19	1
SUM		**179**	**33**	**146**