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Low predation rates on the larvae of three species of barnacles by the ctenophore *Pleurobrachia pileus*

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ABSTRACT: Predation is considered a major source of mortality during the planktonic larval phase of most species of benthic marine invertebrates; however, direct estimates of predation are scarce. We estimated predation rates (d^{-1}) on nauplii of 3 species of barnacles (*Balanus balanus*, *Balanus crenatus*, *Semibalanus balanoides*) by the ctenophore *Pleurobrachia pileus* in the Northwest Arm, Halifax, in winter and early spring 2014. Ingestion rates (prey d^{-1}) were predicted from the number and digestion time of barnacle nauplii in the pharynx of *P. pileus*. Predation rates were estimated by multiplying the ingestion rate by the ratio of the predator to prey concentration. The digestion time (mean ± SE) of barnacle nauplii was significantly longer at 2°C (7.1 ± 0.4 h, n = 5 to 8.6 ± 0.3 h, n = 9) than at 6°C (4.9 ± 0.4, n = 6 to 6.6 ± 0.3 h, n = 8), in 3 laboratory experiments. The digestion time of cyprid larvae could not be reliably estimated because they were egested alive as freely swimming individuals or trapped within a prey bolus. Nauplii of *B. crenatus* were positively 'selected for' by *P. pileus*, and estimates of predation rate were generally highest for this species. The predation rates of each species were frequently <0.005 d^{-1}, indicating that predation by *P. pileus* was negligible. Concentrations of *P. pileus* were within the normal range for this area (on the order of 0.1 ind. m^{-3}) and probably would need to be sustained at anomalously high levels (1 to 10 ind. m^{-3}) to have an ecologically significant impact on populations of larval barnacles.

KEY WORDS: Digestion time · Larval ecology · Larval invertebrates · Larval mortality · Predation impact · Larval predation

INTRODUCTION

Populations of most species of benthic marine invertebrates are seeded by the settlement of planktonic larvae. The spatial and temporal variability in the abundance of larvae and recruits is highly sensitive to variation in the magnitude of the mortality rate (Underwood & Fairweather 1989, Cowen et al. 2000). Reliable estimates of larval mortality are, therefore, critical to the accuracy of predictions of larval dispersal and population connectivity from biophysical models (Metaxas & Saunders 2009). The disparity between the number of eggs produced and the abundance of adults within benthic populations indicates that the magnitude of mortality that occurs during early life stages must be enormous (Thorson 1950); however, most estimates of larval mortality are uncertain, due to the difficulty of tracking larvae in nature (Rumrill 1990). Vertical life table methods (Aksnes & Ohman 1996) have recently been used to estimate stage-specific mortality rates of larval crustaceans (Tapia & Pineda 2007, White et al. 2014). This approach is promising, but is limited in its applicability because the model is dependent on the availability of data on durations of discrete larval stages, sensitive to cohort structure among larval stages, and dependent on several assumptions that require careful consideration (Aksnes & Ohman 1996, Gentleman et al. 2012).

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Larval mortality may be caused by predation, starvation, disease, developmental abnormalities, physiological stress, and transport away from suitable habitat (Thorson 1950, Rumrill 1990). Thorson's (1950) suggestion that predation is a major source of larval mortality has been supported by the high diversity of larval predators, presence of larval traits that defend against predation, and susceptibility of larvae to ingestion in the laboratory (see reviews by Young & Chia 1987, Rumrill 1990, Morgan 1995).

Few attempts have been made to measure larval predation directly. Olson & McPherson (1987) tracked ascidian larvae visually and found extremely high predation rates (0.41 s^{-1}) from ingestion by fish. Allen & McAlister (2007) tethered crab megalopae to moorings and also found high predation rates ranging from 0.62 d^{-1} in the water column during the day to 25.03 d^{-1} near the benthos at night. Lastly, Johnson & Shanks (2003) measured larval predation in mesocosms containing natural plankton assemblages and found low larval predation rates ranging from 0 to 0.07 d^{-1}. The variability in predation estimates among these studies highlights the need for further research on larval predation (Vaughn & Allen 2010).

The magnitude of predation is a function of the predator community composition and the functional response of each predator (Bailey & Houde 1989). Therefore, estimates of larval ingestion rates by predators that are abundant and readily feed on larvae are needed to assess the potential effect of predation on larval populations (Vaughn & Allen 2010). The quantification of predation rates from specific predators requires the use of an ingestion rate model coupled with estimates of the abundance of the predator and its prey. This method has been used to study plankton grazing (Båmstedt et al. 2000) and occasionally predation on larval fish (Purcell 1981, 1989, Jaspers et al. 2011, Purcell et al. 2014). Reports of predation rates by specific predators on larvae of benthic marine invertebrates are rare (Kuipers et al. 1990, Hansson et al. 2005, Hansson & Kiørboe 2006) and have not been presented in the context of their importance in larval ecology.

We evaluated predation rates of the nauplii of 3 species of barnacles from ingestion by the ctenophore *Pleurobrachia pileus* using a model that derives ingestion rate from the pharynx content of *P. pileus* and the digestion time of its prey (Bajkov 1935). This approach is restricted to predators that consume their prey whole (i.e. gelatinous zooplankton and fish), but does not require laboratory-derived estimation of ingestion rates, which are known to vary with turbulence (Saiz & Kiørboe 1995), temperature (Uye & Kayano 1994), the presence of other plankton (Johnson & Shanks 1997), and container size (Gibbons & Painting 1992).

In the Northwest Atlantic, the peak abundance of barnacle nauplii occurs in late winter and early spring (Paranjape & Conover 1973, Townsend 1984). Larval barnacles progress through 6 naupliar stages and a cyprid stage over a larval duration of several weeks (Walker et al. 1987). *P. pileus* is commonly encountered in the zooplankton communities of the Northwest Atlantic during the spring and summer (Bigelow 1924). At this time, *P. pileus* may reach maximum concentrations on the order of 1 to 10 ind. m^{-3} off the coast of Nova Scotia (Milne & Corey 1986).

The magnitude of predation by *P. pileus* on zooplankton depends on the encounter rate and susceptibility of prey to ingestion after capture (Greene et al. 1986). Frank (1986) and Båmstedt (1998) estimated that *P. pileus* reduced crustacean zooplankton biomass by 8 to 9% d^{-1} when present at concentrations of ≥1 ind. m^{-3}. On the other hand, Kuipers et al. (1990) found that *P. pileus* reduced copepod densities at a rate on the order of 0.1 to 1% d^{-1} when present at concentrations on the order of 1 to 10 ind. m^{-3}. Larval barnacles are a component of the natural diet of *Pleurobrachia* spp. (Fraser 1970, Rowe 1971, Hirota 1973, Anderson 1974, Frank 1986, Larson 1987, Kuipers et al. 1990, Båmstedt 1998). Kuipers et al. (1990) estimated that the maximum predation rate of larval barnacles by predation from *P. pileus* was ~2% d^{-1}. However, these estimates may not be reliable as Kuipers et al. (1990) used copepod digestion times to estimate the ingestion rate, and did not identify larval barnacles to species.

In this study, we improved on previous estimates of predation rates on larval barnacles by *P. pileus* (i.e. Kuipers et al. 1990) by quantifying species-specific predation rates of barnacle nauplii, evaluating the digestion time of larval barnacles in the pharynx of *P. pileus*, assessing the validity of the assumptions associated with the estimation of predation rates, and quantifying the temporal variation in abundance of other potential pelagic predators over the duration of the study.

MATERIALS AND METHODS

Sample collection

We obtained zooplankton samples in the Northwest Arm, Nova Scotia, Canada (Fig. 1). The North-

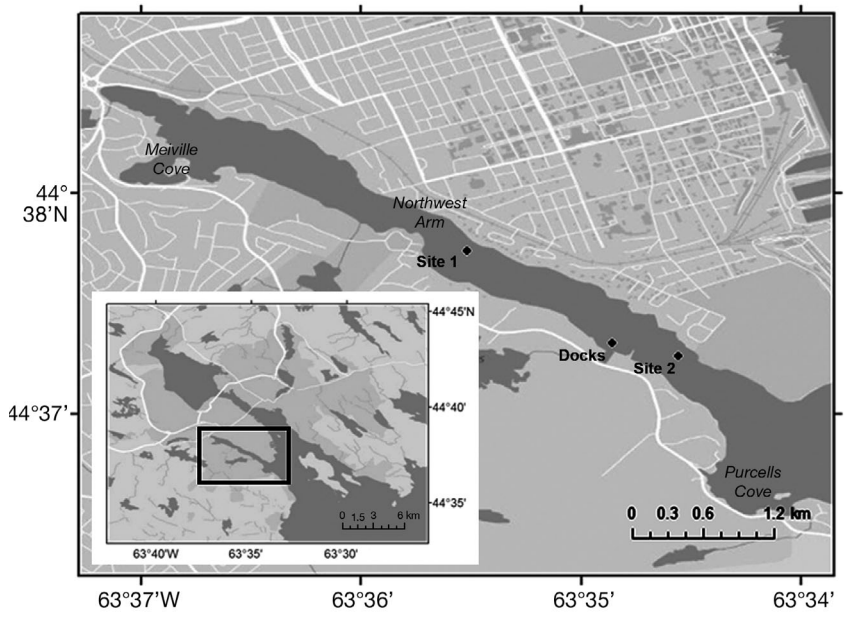

Fig. 1. Halifax Harbour (inset) and Northwest Arm. Plankton tows were done at Sites 1 (44°37′44″N, 63°35′31″W) and 2 (44°37′16″N, 63°34′31″W). Individuals of *Pleurobrachia pileus* were collected for gut content analysis at docks. The Basemap was obtained from Esri ArcMap v. 10.3 software

west Arm is a small inlet (5 km long, 0.5 km wide) in Halifax, on the eastern coast of Nova Scotia, with a maximum depth of 18 m and flushing time of 2.7 d (Gregory et al. 1993). At each of 2 sites, approximately 1.5 km apart (Site 1: 44° 37′ 44″ N, 63° 35′ 31″ W, Site 2: 44° 37′ 16″ N, 63° 34′ 31″ W, Fig. 1), we conducted 2 successive oblique plankton tows (15 m to surface, 0.5 to 1 m s^{-1} tow speed) using a 0.75-m diameter plankton net (125-µm mesh size) with a General Oceanics flowmeter. On deck, we measured the polar diameter (length along oral–aboral axis) of individuals of *Pleurobrachia pileus* prior to preserving the plankton sample in 95% ethanol.

We did not use individuals of *P. pileus* obtained from plankton tows for pharynx content analysis because collection with plankton nets may cause unnatural feeding and egestion (Båmstedt et al. 2000). Instead, on each sampling date, we collected 29 to 49 individuals of *P. pileus* from 1 m depth to the sea surface from an array of floating docks near the 2 sites where we conducted plankton tows (Fig. 1). We collected *P. pileus* with a 10-cm diameter plastic container attached to a 1.5-m long stick. We measured the polar diameter of *P. pileus* before gently transferring each individual into a centrifuge tube containing 10% buffered formalin.

We collected samples in winter and spring 2014 and spring 2015. In all cases, we conducted plankton tows immediately before or after dipping. In 2014, we obtained samples weekly from 21 February to 30 April to determine the abundance of larval barnacles and *P. pileus* and the number of larval barnacles in the pharynx of *P. pileus* over most of the larval duration of the barnacles. On any one date, we collected plankton samples between 10:00 and 16:00 h and dipped ctenophores from docks near maximum ebb or flood tide between 11:30 and 15:30 h.

In 2015, we collected samples twice over a ~24 h period, on 6 to 7 April and 15 to 16 April to quantify short-term variability in the abundance of *P. pileus* and larval barnacles and the pharynx content of *P. pileus*. On each pair of dates, we dipped ctenophores in the late afternoon or evening of Day 1 and in the early morning of Day 2 (Table S1 in the Supplement at www.int-res.com/articles/suppl/m541p105_supp.pdf). We collected consecutive samples only twice because of poor weather in winter and apparent senescence of ctenophores in spring. We observed several individuals of *P. pileus* that were irregularly shaped and apparently in a state of disintegration during dipping on 15 to 16 April 2015. Collection of these individuals was avoided, and those that were collected were excluded from analysis.

In the laboratory, we split the plankton samples obtained from plankton tows with a Folsom splitter and enumerated larval barnacles and their potential predators. For n = 7, we counted all subsamples and determined that enumerating a minimum of 80 individuals of each species resulted in <10% error. We identified larval barnacles to species and stage following Crisp (1962), Lang (1980), and Branscomb & Vedder (1982). Four species of balanid barnacles release larvae in the winter and spring months in the Northwest Atlantic including *Amphibalanus improvisus* (formerly *Balanus improvisus*), *Balanus balanus*, *Balanus crenatus*, and *Semibalanus balanoides* (Bousfield 1954). Nauplii of *B. crenatus* and *A. improvisus* are morphologically similar (Lang 1980), and no attempt was made to distinguish these species, which are collectively referred to hereafter as *B. crenatus*. We did not enumerate the first larval barnacle stage because this stage was not abundant in our samples and is difficult to identify to species. We identified barnacle cyprids as either *S. balanoides* or *Balanus* sp. We identified potential predators of larval bar-

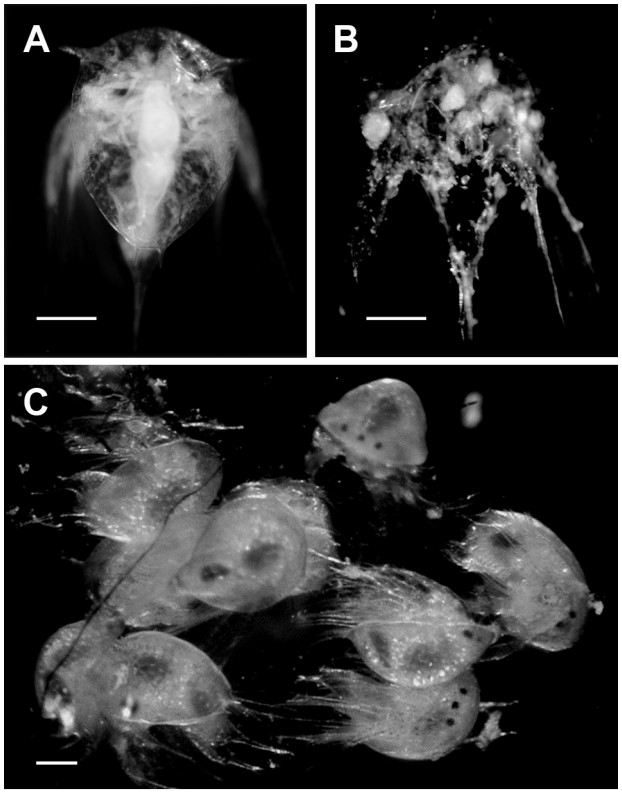

Fig. 2. (A) Undigested nauplius of *Semibalanus balanoides*, (B) digested barnacle nauplius, and (C) bolus of several Stage 6 nauplii of *S. balanoides*. Scale bars = 200 µm

nacles following Fahay (1983) and Johnson & Allen (2012).

We excised the pharynx of each ctenophore collected from floating docks and enumerated the number of larval barnacles of each species. We grouped consecutive naupliar stages (Stages 2–3 and 4–5) because larvae partially digested or wrapped in mucus were difficult to identify to stage. All other prey items were identified as copepods, which we enumerated but did not identify further taxonomically. We only enumerated larval barnacles and copepods if they were intact and their exoskeleton had not been completely cleared of tissue (Fig. 2A,B).

Digestion experiments

We measured digestion time of barnacle nauplii in the pharynx of *P. pileus* at 2°C and 6°C in 3 experiments (Table 1). The temperature treatments reflected the range of temperatures measured from HOBO® pendant data loggers (accuracy ± 0.47°C; Onset Computer), deployed at depths of 1 and 10 m in the Northwest Arm over the field sampling period in 2014 (Fig. S1 in the Supplement.

For each experiment, we collected ~50 individuals of *P. pileus* from floating docks in the Northwest Arm, immediately transported them to the Dalhousie University Aquatron facility, and evenly allocated them in 2 cylindrical tanks (0.65 m diameter, 0.41 m height) filled with ~100 l of continuously flowing sand-filtered seawater. We then held the ctenophores for 24 to 72 h without food prior to feeding them larval barnacles (Table 1). In all cases, we acclimated the ctenophores at 2 or 6°C (±0.5°C) for 24 h before feeding. In Expt 1, we acclimated the ctenophores immediately after collection, whereas in Expts 2 and 3, we acclimated the ctenophores after holding them at ambient temperature for 24 h or 48 h, respectively (Table 1).

After the acclimation period, we offered larval barnacles to *P. pileus* for 15 to 30 min. We transferred each ctenophore that had consumed at least 1 barnacle nauplius into a 200 ml beaker filled with 1 µm filtered seawater at 2 or 6°C (±0.5°C). We placed the beaker in a temperature-controlled room maintained at the experimental temperatures. We monitored the

Table 1. Details of methods used in digestion experiments. Temp: mean temperature measured during the experiment ± range of 0.2°C; N: number of ctenophores; Starvation period: sum of holding and acclimation periods; Meal size: number of larval barnacles fed to each ctenophore; Bb: *Balanus balanus*; Bc: *Balanus crenatus*; Sb: *Semibalanus balanoides*; lab: laboratory-reared

Experiment, treatment (°C)	Date	Temp (°C)	N	Starvation period (h)	Mean ctenophore diameter ± SD (cm)	Prey type (species; stage)	Meal size (min., max.)
1, 2	30 Mar 2014	2.52	9	24	1.79 ± 0.37	Sb (lab); 3–4	1, 1
1, 6	30 Mar 2014	5.55	8	24	1.85 ± 0.20	Sb (lab); 3–4	1, 1
2, 2	12 Apr 2014	1.76	8	48	2.08 ± 0.22	Bb, Bc, Sb; 4–6	1, 9
2, 6	12 Apr 2014	6.17	8	48	1.99 ± 0.12	Bb, Bc, Sb; 4–6	1, 10
3, 2	13 Apr 2014	2.52	5	72	1.58 ± 0.31	Bb, Bc, Sb; 4–6	1, 2
3, 6	13 Apr 2014	5.76	6	72	1.86 ± 0.46	Bb, Bc, Sb; 4–6	2, 12
4, 6	12 Mar 2015	6	13	72	–	Sb (lab); 6	2, 29
5, 6	4 Apr 2015	6	34	36	–	Sb (lab); cyprid	1, 22

temperature in each room throughout each experiment using a HOBO® pendant data logger (Table 1). We visually monitored larvae in the pharynx of each ctenophore at 30 min intervals, and recorded the time when each nauplius was completely cleared of tissue (i.e. digested; Fig. 2A,B). We defined the digestion time of each nauplius as the time elapsed between the time that *P. pileus* was transferred to the 200 ml beaker and the midpoint of the last 2 times the nauplius was checked. At the end of the experiment, we measured the polar diameter of each ctenophore.

In Expt 1, we offered the ctenophores Stage 3 to 4 nauplii of *S. balanoides* reared in the laboratory. We induced larval release by feeding adults high concentrations of diatoms (*Thalassiosira weissflogii*), and cultured the larvae at a concentration of 1 larva ml^{-1} in 4 l glass jars containing 1 µm filtered seawater at 6°C, and 10^5 phytoplankton cells ml^{-1} (3 to 1 mixture of *T. weissflogii* to *Isochrysis* sp.). In Expts 2 and 3, we offered the ctenophores a mixture of 3 species of Stage 4 to 6 nauplii (Table 1) collected from horizontal plankton tows (0.75 m diameter plankton net with closed cod end) near the sea-surface in the Northwest Arm. Prior to the experiment, we kept these larvae under the same culture conditions as *S. balanoides* in Expt 1.

We attempted to conduct similar experiments with field-collected barnacle cyprids as prey, but the majority of ctenophores egested all cyprids prey within hours of feeding. This was unexpected, as only a single barnacle nauplius was egested in the nauplii-digestion experiments described above. To further investigate larval digestion and egestion, we conducted 2 additional experiments using laboratory-reared sixth stage nauplii and cyprids of *S. balanoides* as prey (Expts 4 and 5, Table 1). For these experiments, we held each ctenophore in a 5.7 l PVC cylinder (radius = 10 cm, height = 18 cm) with a 120 µm Nitex mesh bottom immersed in continuously flowing sand-filtered seawater at 6°C while digesting their prey. We used this holding container to prevent stress-induced larval egestion that may have occurred in previous experiments, when the ctenophores were held in much smaller (200 ml) containers, and accommodate ctenophores digesting larvae over long time periods. We monitored temperatures throughout these experiments using a hand-held thermometer. We checked the pharynx content of the ctenophores, and removed egested prey from the holding containers at ~15 min intervals to prevent re-ingestion of egested prey. We removed ctenophores that had egested all of their prey from their container and replaced them with newly fed ctenophores. Egested prey were either expelled alive individually or as part of a bolus composed of many prey items held together by mucus (Fig. 2C).

Data analysis

Prey composition

For each sampling date, we compared the observed frequency of each species of barnacle nauplii in the pharynx of *P. pileus* (larvae pooled across ctenophores) with the expected frequency calculated from the proportion in the plankton (larvae pooled across sites) using χ^2-tests. To determine whether each species was over- or under-represented in the pharynx, we calculated a selectivity index, α, following Chesson (1978):

$$\alpha_a = \frac{\frac{r_a}{p_a}}{\sum_{i=1}^{n} \frac{r_i}{p_i}} \quad (1)$$

where r is the proportion of larvae in the pharynx and p is the proportion of larvae in the plankton. The subscript a represents the species of interest, in n species of prey. For $\alpha_a < 0.5$, a lower proportion of prey type a was found in the pharynx than the plankton. For $\alpha_a > 0.5$, a higher proportion of prey type a was found in the pharynx than the plankton. Lastly, for $\alpha_a = 0.5$, prey type a was found in equal proportions in the pharynx and plankton. We did not include cyprid larvae in this analysis, as they were not identified to species.

Size and pharynx contents of *P. pileus*

A mismatch between the polar diameters of individuals collected from plankton tows and those dipped from floating docks could bias projected feeding rates (see following subsection) if there is a relationship between ctenophore size and the number of larval barnacles in the pharynx. We explored the relationship between polar diameter and the total number of barnacle nauplii consumed for each survey using a generalized linear model with a negative binomial error structure and log-link function. We used a negative binomial error structure (rather than Poisson) because the ratio of variance to mean (σ^2/μ) indicated the data were aggregated ($\sigma^2/\mu > 1$, in all cases). We tested the hypothesis that the mean polar diameter of individuals of *P. pileus* collected by dipping was greater than that collected by plankton tow

using a 1-tailed Students *t*-test. We did not include individuals of *P. pileus* with a polar diameter <0.3 cm in the estimates of ctenophore abundance from the plankton samples because the polar diameter of ctenophores collected for pharynx content analysis was always >0.5 cm.

Prey digestion time and barnacle predation rate

For Expts 1 to 3, we examined the effects of temperature (fixed factor, 2 levels) and experiment (random factor, 3 levels) on digestion time with a 2-way ANOVA using Type III SS due to unbalanced sample size (Table 1). We did not test for normality because the sample sizes were low ($5 \leq n \leq 9$); this was not a concern because ANOVA is robust to deviation in normality (Underwood 1997). The Levene's test indicated that variances were not significantly heterogeneous ($p = 0.97$). We used the mean digestion time from Expts 1, 2, and 3 at 2°C (8.1 h) to estimate the average ingestion rate I (prey 0.34 d^{-1}) of *P. pileus* on each species of larval barnacle on each sampling date. This digestion time represents an overall average value from experiments that used different prey types, meal sizes (number of prey ingested), and acclimation/starvation periods (Table 1). We estimated the mean ingestion rate on each sampling date following Bajkov (1935):

$$I = \left(\sum_{i=1}^{n} \frac{G_i}{D} \right) / n \quad (2)$$

where G_i is number of prey in the pharynx in the i^{th} ctenophore, D is the digestion time (d), and n is the number of ctenophores examined for gut contents.

Bajkov's (1935) model requires that the following assumptions are met: (1) the rate of change of G_i is in steady state (i.e. ingestion rate equals digestion rate; Bromley 1994) and (2) prey are digested linearly over the time after the first prey item is digested (Bochdansky & Deibel 2001). We investigated the validity of these assumptions using data from our digestion experiments. Assumption 1 is supported if there is no relationship between digestion time and meal size, in which case the digestion rate increases with meal size, and therefore with ingestion rate. We evaluated the relationship between the average digestion time and initial meal size for each ctenophore using simple linear regression. Assumption 2 is supported if there is a negative linear relationship between the number of undigested prey in the pharynx of *P. pileus* and the time after the first prey item is digested. We fitted a power model ($y = ax^{-c}$) to this relationship and tested for non-linearity by examining the confidence interval of the exponent, c. Only relationships between undigested pharynx content and time with a minimum of 5 data points were evaluated (n = 8 ctenophores).

Ingestion rates can be potentially affected by prey egestion. Egestion of individual live prey will result in an overestimate of ingestion rates because it is assumed that all prey in the pharynx are killed and digested. Undigested prey were frequently egested as a bolus, and probably would die if unable to detach from one another. This would result in an underestimate of effective ingestion rates (ingestion that results in prey mortality) because the egestion time is inherently shorter than the digestion time. To explore the extent to which ctenophore egestion may affect estimates of ingestion rate, we computed the median egestion time (pooled among ctenophores) from Expts 4 and 5 (Table 1), as well as the frequency of ctenophores that egested prey as a bolus.

For each sampling date, we estimated the mean predation rate, μ (d^{-1}), for nauplii of each barnacle species from predation by *P. pileus* as:

$$\mu = \frac{IP}{C} \quad (3)$$

where P and C are the mean concentrations of *P. pileus* and barnacle nauplii (no. m^{-3}), respectively, averaged across the 2 sampling sites (Fig. 1). The standard deviation of μ was calculated by propagating the error of I (from D), P, and C (Hughes & Hase 2010). The standard deviation associated with D was calculated from the pooled variance among experiments (Zar 1984). By not propagating error in G_i we assume that the mean number of barnacle nauplii eaten per ctenophore is an accurate representation of predation within the ctenophore population. Cyprid larvae were not included in this analysis because their digestion time is unclear. All analyses were carried out using R v.3.1.1.

RESULTS

Zooplankton concentrations and pharynx contents of *Pleurobrachia pileus*

Balanus balanus was the most abundant species of larval barnacle throughout the sampling period in 2014, peaking in concentration on 7 March (Fig. 3A). Concentrations of larval *Semibalanus balanoides* and *B. crenatus* peaked on 17 March and 2 April 2014,

respectively (Fig. 3A). Progression through the developmental stages was exhibited by larvae of all barnacle species from 21 February to 2 April 2014, and was characterized by a decline in abundance of the second naupliar stage and an increase in abundance of successive stages (Fig. 3). The proportion of Stage 6 nauplii of S. balanoides decreased and earlier naupliar stages of this species became relatively more abundant over the last 4 sampling dates (Fig. 3B). Cyprid larvae first appeared on 2 April 2014; cyprids of *S. balanoides* and *Balanus* sp. peaked in abundance on 9 and 17 April 2014, respectively (Fig. S2A in the Supplement at www.int-res.com/articles/suppl/m541p105_supp.pdf).

The abundance of *P. pileus* fluctuated over the sampling period in 2014, but gradually decreased between 21 February and 30 April (Fig. 4A); the polar diameter of ctenophores gradually increased

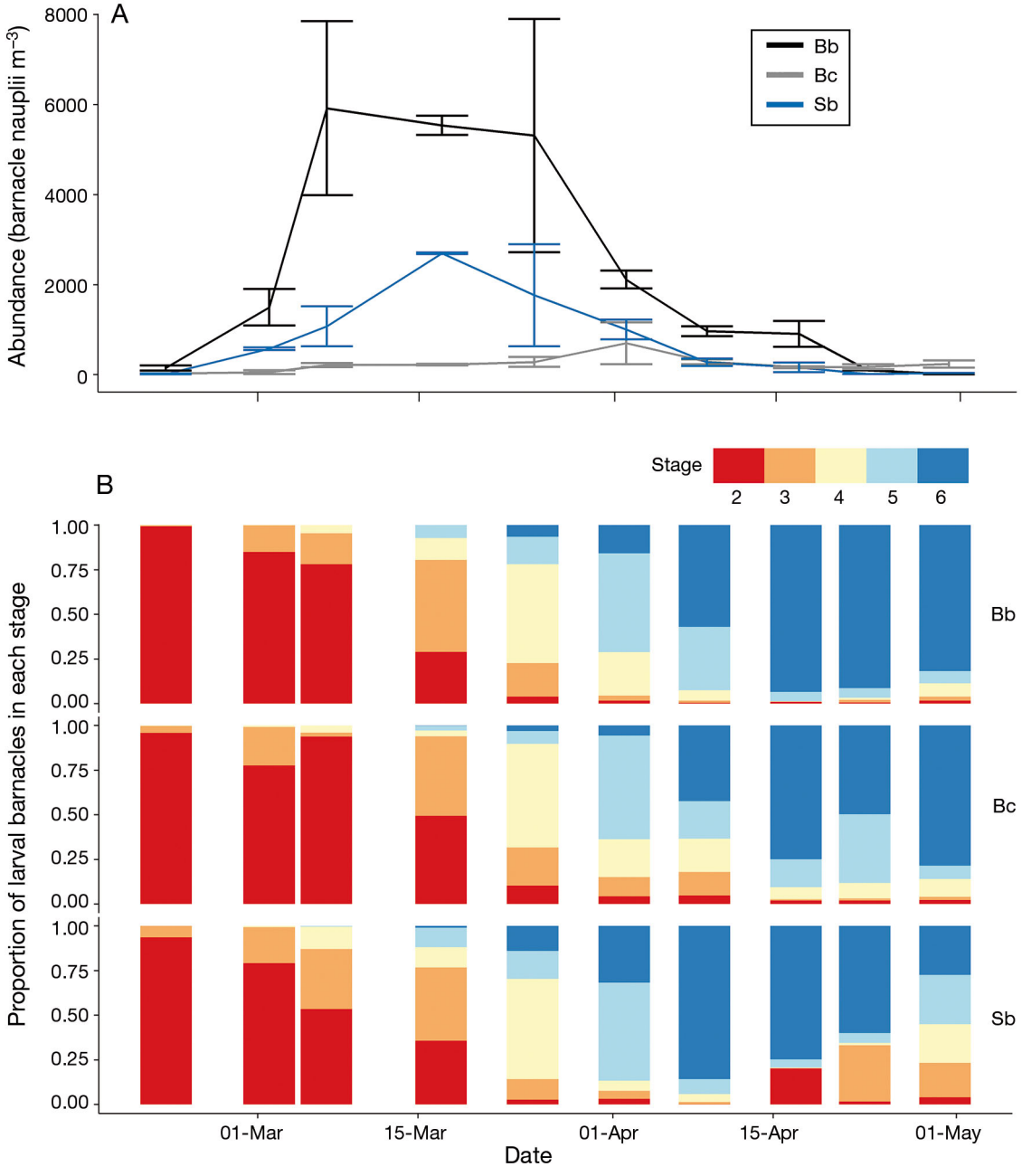

Fig. 3. Time series of (A) mean (±1 SE; n = 2 sites) concentrations of nauplii of *Balanus balanus* (Bb), *Balanus crenatus* (Bc), and *Semibalanoides balanoides* (Sb) and (B) proportions of each naupliar stage pooled across sites

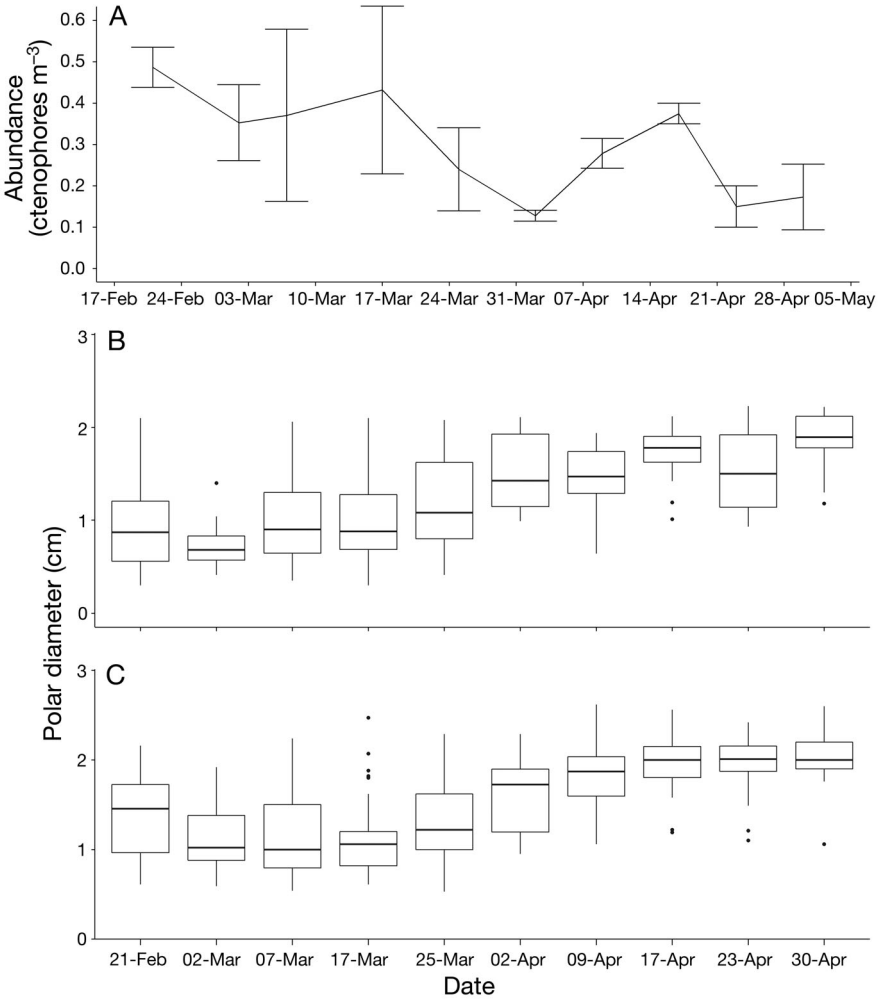

Fig. 4. (A) Time series of mean (±1 SE; n = 2 sites) concentrations of ctenophores. (B,C) Box plots (median ± interquartile range [IQR]; whiskers 1.5 IQR) of polar diameters of *Pleurobrachia pileus* collected by (B) plankton tow and (C) dip net

over the same period (Fig. 4B,C). A wide range of other (potential) predators were collected from the plankton tows (Table S2 in the), but only a few were consistently abundant throughout the sampling period (Fig. 5).

In 2014, the number of prey items in the pharynx of *P. pileus* was composed of at least 70% larval barnacles, except on 21 February and 23 April (Fig. 6). Barnacle cyprids appeared in the diet of ctenophores from 2 to 30 April 2014, but were not as abundant in the diet as were nauplii (except on 23 April 2014). The pharynx of any ctenophore was never completely full in our study. The mean and maximum number of nauplii retrieved from the pharynx of *P. pileus* varied from 0.66 to 10.0 nauplii ctenophore^{-1} and 3 to 70 nauplii ctenophore^{-1}, respectively (Fig. 7A). The mean and maximum number of cyprids retrieved from the pharynx of *P. pileus* varied from 0.1 to 2 cyprids ctenophore^{-1} and 2 to 14 cyprids ctenophore^{-1}, respectively, from 2 to 30 April 2014 (Fig. S2B in the Supplement). Nauplii of *B. balanus* dominated the pharynx contents from 2 to 25 March 2014, but their frequency slowly declined thereafter (Fig. 7B). In contrast, nauplii of *B. crenatus* were rarely found in the pharynx from 2 to 25 March 2014, but became the dominant prey item by 30 April 2014 (Fig. 7B). The majority of cyprid larvae in the diet of *P. pileus* were *Balanus* sp. from 17 to 30 April (Fig. S2C in the Supplement).

Prey selectivity (α index; Chesson 1978) was consistent across sampling dates, and χ^2 tests indicated that the null hypothesis of no preference was rejected ($p < 0.05$) in all cases except for *B. balanus* on 2 April (Table S3 in the Supplement). We therefore calculated these statistics for data pooled across sampling dates. *P. pileus* selected against *B. balanus* ($\alpha_{pooled} = 0.11$; $\chi^2_{pooled} = 179$) and *S. balanoides* ($\alpha_{pooled} = 0.06$; $\chi^2_{pooled} = 150$), but selected for *B. crenatus* ($\alpha_{pooled} = 0.83$; $\chi^2_{pooled} = 2260$).

On 4 of the 10 sampling dates in 2014, the number of prey in the pharynx was significantly positively related to the polar diameter of *P. pileus* (Fig. S3 in the Supplement; Table 2). On 6 of the 10 sampling dates, the polar diameter of *P. pileus*, collected by dipping from floating docks, was significantly greater than individuals collected from plankton tows (Table 2).

In 2015, *S. balanoides* was the most abundant species of larval barnacle in April (Fig. S4A in the Supplement). The variability between samples collected on consecutive sampling dates was low for each species, except for *S. balanoides* on 6 to 7 April (Fig. S4A in the Supplement). The mean abundance of *P. pileus* was <0.1 ind. m^{-3} and was consistent between sampling dates (Fig. S4B in the Supplement). The mean number of nauplii per ctenophore ranged from 0.05 nauplii on 16 April to 0.7 nauplii on 7 April (Table S1 in the Supplement).

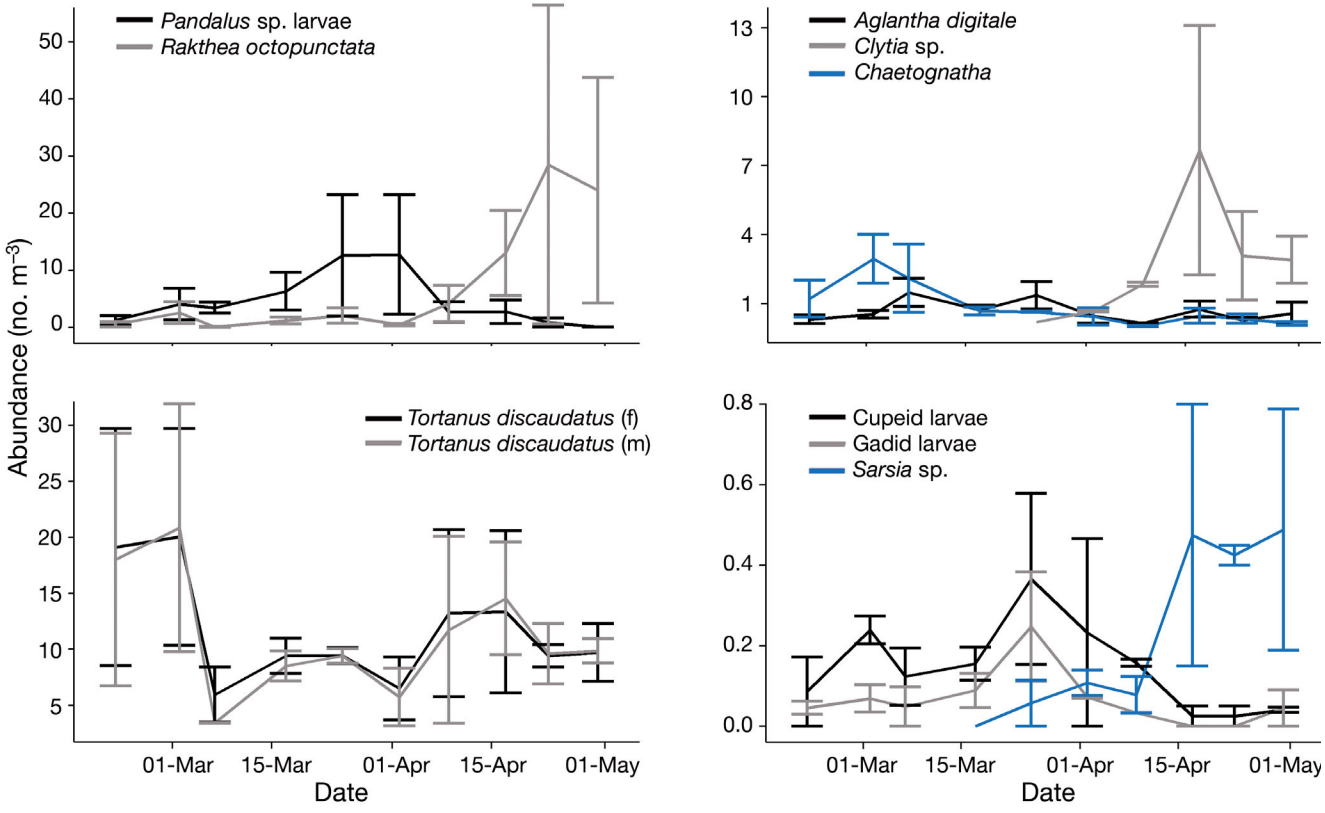

Fig. 5. Time series of mean (± 1 SE; n = 2 sites) concentrations of some potential predators of barnacle nauplii. Note the different scales on *y*-axes. f: female; m: male

Digestion time and predation rate of larval barnacles

Mean digestion times from Expts 1 to 3 were significantly longer at 2°C than at 6°C and varied significantly among experiments (Fig. 8, Table 3). At 2°C, mean digestion times (±SE) ranged between 7.1 ± 0.4 h (n = 5) and 8.6 ± 0.3 h (n = 9). At 6°C, mean digestion times ranged between 4.9 ± 0.4 h (n = 6) and 6.6 ± 0.3 h (n = 8). The mean digestion time (±SE) from Expt 4 (at 6°C) was 4.0 ± 0.2 h.

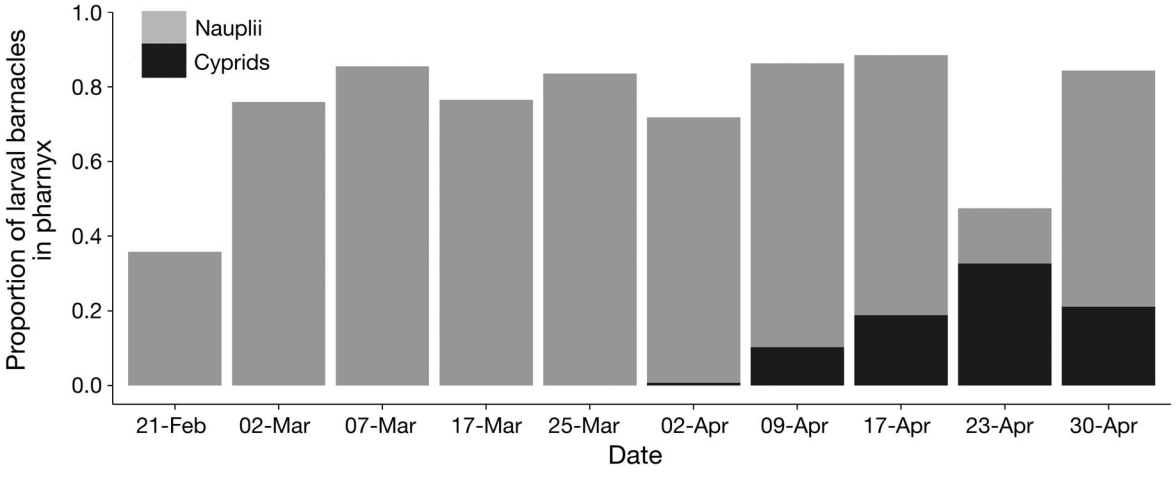

Fig. 6. The proportion of larval barnacles in the pharynx of *Pleurobrachia pileus* (pooled across ctenophores). Prey items other than larval barnacles consisted of unidentified copepods

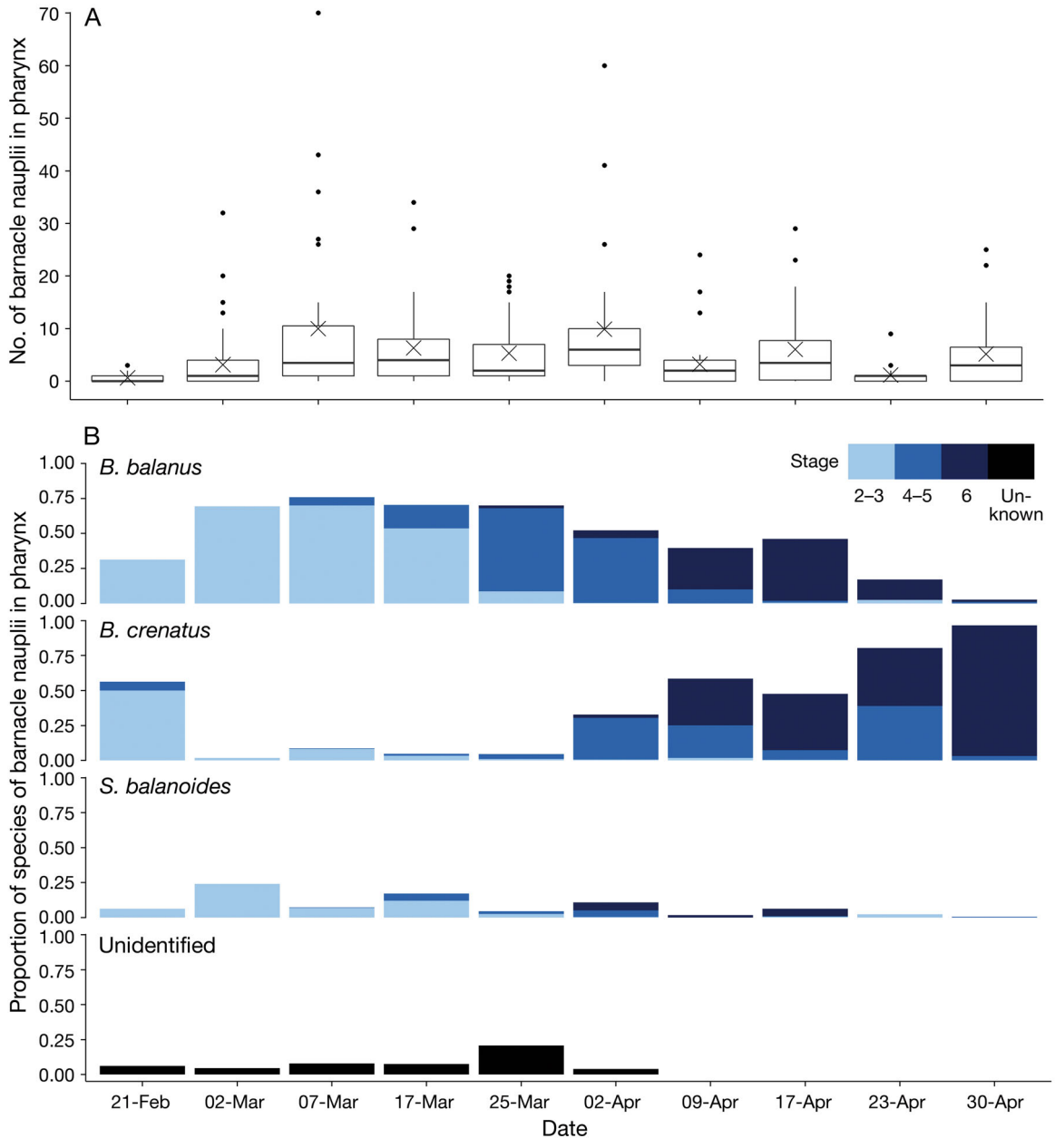

Fig. 7. Description of content of barnacle nauplii recovered from the pharynx of *Pleurobrachia pileus*. (A) Box plots (median ± interquartile range [IQR]; whiskers 1.5 IQR; X denotes mean) of the number of barnacle nauplii (species combined) per ctenophore. (B) The proportion of barnacle nauplii (pooled across ctenophores) catagorized as *Balanus balanus*, *Balanus crenatus*, *Semibalanoides balanoides,* and unidentified. Stacked bars represent naupliar stages according to the colour key. Stages not specified for 'unidentified category

No significant relationship was detected between meal size and the average digestion time of *P. pileus* (Table S4 in the Supplement). Also, 5 of the 8 relationships between the number of undigested nauplii in the phanyx of *P. pileus* and time were determined to be non-linear (Fig. 9).

Based on 4 observations in experiments carried out in 2014, the observed minimum cyprid digestion times were 23 and 17 h at 3 and 7°C, respectively. In Expts 4 and 5, 77% of ctenophores egested at least 1 nauplius and all ctenophores egested cyprids. Also, a prey bolus of at least 2 larvae was formed by 60% of ctenophores that egested nauplii, and 42% of ctenophores that egested cyprids. Median times to egestion of nauplii and cyprids were 0.5 and 0.3 h, respectively (Fig. 10).

Table 2. Results from analyses used to determine if a bias in ctenophore size affected the calculation of predation rates; *t*-tests were used to compare the polar diameter among ctenophores collected by dip and plankton tow methods. Generalized linear models (GLM; negative binomial error structure, log link) were used to determine the relationship between ctenophore size and the number of barnacle nauplii recovered from the pharynx. Significant differences (α = 0.05) are in bold

Date	*t*-test			GLM			
	t-statistic	df	p	*z*-statistic	Residiual df	Residual deviance	p
21 Feb 2014	3.57	60	**<0.001**	−2.55	26	27.36	**0.017**
2 Mar 2014	5.10	68	**<0.001**	1.03	48	49.68	0.304
7 Mar 2014	1.45	59	0.077	−0.42	28	33.95	0.677
17 Mar 2014	1.33	70	0.093	2.93	31	37.01	**0.003**
25 Mar 2014	1.07	59	0.145	2.42	35	40.90	**0.015**
2 Apr 2014	0.39	34	0.351	2.57	28	33.33	**0.010**
9 Apr 2014	3.83	47	**<0.001**	−0.26	30	34.77	0.799
17 Apr 2014	2.54	47	**0.007**	−0.01	32	38.55	0.989
23 Apr 2014	2.98	38	**0.002**	−1.46	32	34.16	0.144
30 Apr 2014	1.81	41	**0.040**	−0.61	31	35.79	0.545

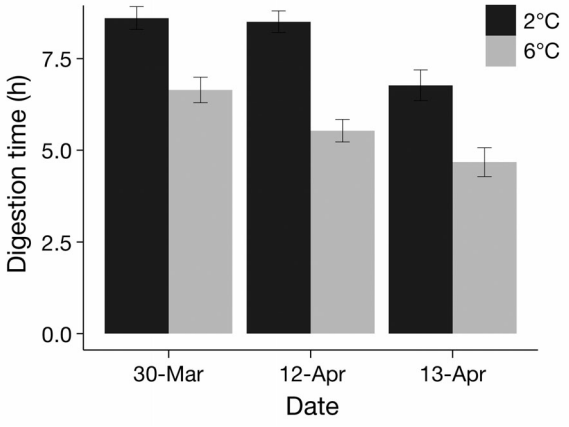

Fig. 8. Mean (±1 SE) digestion time of barnacle nauplii in the pharynx of *Pleurobrachia pileus* from Expts 1 to 3. See Table 1 for sample size and other methodological details on each experiment

The predation rates that we estimated were generally greater for *B. crenatus* than for *B. balanus* and *S. balanoides* throughout the sampling period (Fig. 11). In general, predation rates of *B. balanus* and *B. crenatus* declined from 21 February to 25 March 2014, then increased from 2 to 30 April 2014.

DISCUSSION

Phenology of prey and predators

The abundance and stage progression of *Balanus balanus* and *Semibalanus balanoides* indicated that hatching occurred primarily in early and mid-March, respectively. This is consistent with previous observations of hatching of *B. balanus* and *S. balanoides* occurring primarily in late winter or spring in the Northwest Atlantic (Bousfield 1954, Lang & Ackenhusen-Johns 1981). In our study, larval *Balanus crenatus* were present in winter and early spring, as previously reported by Lang & Ackenhusen-Johns (1981), although the primary hatching period of this species was reported to occur later in the spring and summer by Bousfield (1954, 1955). Variability in the abundance and size of *Pleurobrachia pileus* over the study period is consistent with the dynamics of this species in our region (Milne & Corey 1986, Matsakis & Conover 1991).

Other carnivorous zooplankton that were present and are known to feed on larval barnacles *in situ* include larval pandalid shrimp (Stickney & Perkins 1981), larval gadid and clupeid fish (Marshall et al. 1937, Bainbridge & McKay 1963), chaetognaths (Alvarez-Cadena 1993), and hydrozoan jellyfish (e.g. *Aglantha digitale*, *Clytia* sp., *Rakthea octopunktata*, *Sarsia* sp.; Purcell & Mills 1988). The copepod *Tortanus discaudatus* likely feeds on barnacle nauplii as this species ingests large copepods (Ambler & Frost 1974, Mullin 1979). A different species of *Tortanus* has been observed feeding on barnacle nauplii in the laboratory (Uye & Kayano 1994).

Because the majority of larvae were released in early March, the temporal overlap of larval barnacles with cnidarian predators was minimal, as several hydrozoan medusae (*Clytia* sp., *R. octopunktata*, *Sarsia* sp.) only co-occurred with late larval stages. Cnidarian medusae typically become abundant in late spring and summer in our study region (Bigelow 1924, Matsakis & Conover 1991). However, multiple

Table 3. Results of 2-way ANOVA (Type III SS) examining the effects of experiment (random factor) and temperature (fixed factor, 2 levels: 2°C, 6°C) on digestion time of barnacle nauplii in the pharynx of *Pleurobrachia pileus*. Significant differences (α = 0.05) are in bold

Source	SS	df	*F*	p
Experiment	24.3	2	14.3	**<0.001**
Temperature	57.9	1	48.8	**0.020**
Experiment × Temperature	2.37	2	1.40	0.244
Residual	32.1	40	–	–

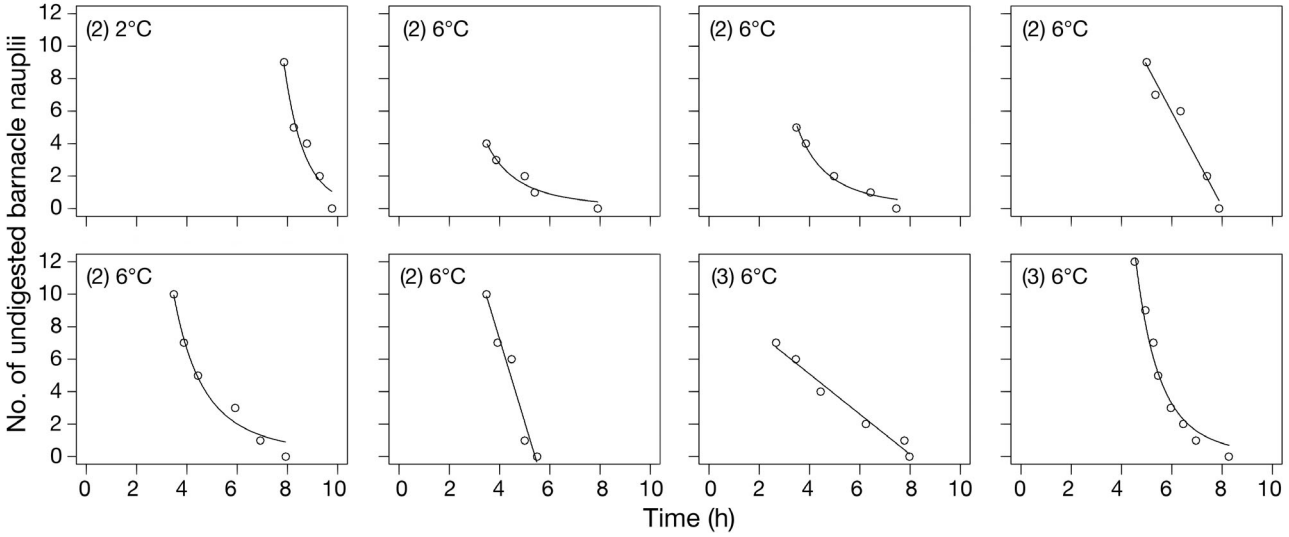

Fig. 9. Relationships between the number of undigested nauplii in the pharynx of a ctenophore and time, fitted with linear or power ($y = ax^c$) models. Non-linear relationships indicate a lower confidence limit of parameter $c > 1$. The experiment number (in parentheses) and temperature treatment (see Table 1) are presented in each panel

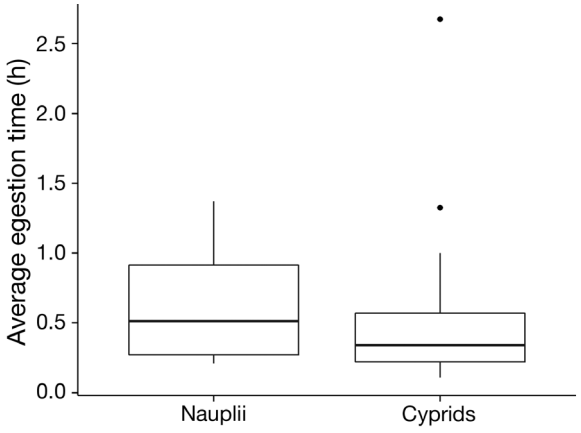

Fig. 10. Box plots (median ± interquartile range [IQR]; whiskers 1.5 IQR) of average egestion times for each ctenophore

predators were present during most of the larval duration of each barnacle species, including *P. pileus*, *T. discaudatus*, chaetognaths, larval fish, and larval shrimp. Future studies on the impact of these predators on larval barnacle populations would be useful in assessing the overall importance of carnivorous zooplankton on larval barnacle mortality in the region.

Magnitude of predation

We estimated low predation rates on larvae of each species of barnacle from predation by *P. pileus* over the sampling period, frequently on the order of 0.001 d^{-1}. Over a 6 wk naupliar duration (Bousfield 1954), this predation rate would reduce the larval population by only ~5%. Our estimates of the predation rate were low mainly because the abundance of larvae of each barnacle species was frequently at least 3 orders of magnitude more abundant than that of *P. pileus*. The total concentration of early stage barnacle nauplii was frequently on the order of 1 to 10 larvae l^{-1}, which is within the range of concentrations reported in the Northwest Atlantic (Bousfield 1955, Townsend 1984). The concentration of *P. pileus* in our study was also similar to those observed in previous studies in this region (Frank 1986, Milne & Corey 1986, Matsakis & Conover 1991). Therefore, our results appear to be representative of normal conditions, and we conclude that it would require an anomalously high abundance of *P. pileus* (1 to 10 ind. m^{-3}) sustained over long periods to substantially reduce populations of barnacle nauplii in the Northwest Arm.

Predation rates on *B. crenatus* were generally the highest among barnacle species. This is consistent with *B. crenatus* being positively 'selected' over the other barnacle species. This may have occurred because larval *B. crenatus* exhibited a higher degree of spatial overlap with *P. pileus*, were easier to capture and ingest, and/or were digested at a slower rate. Each species of barnacle was consistently selected either for or against over the study duration, suggesting that changes in the abundance or stage composition of barnacle nauplii did not substantially

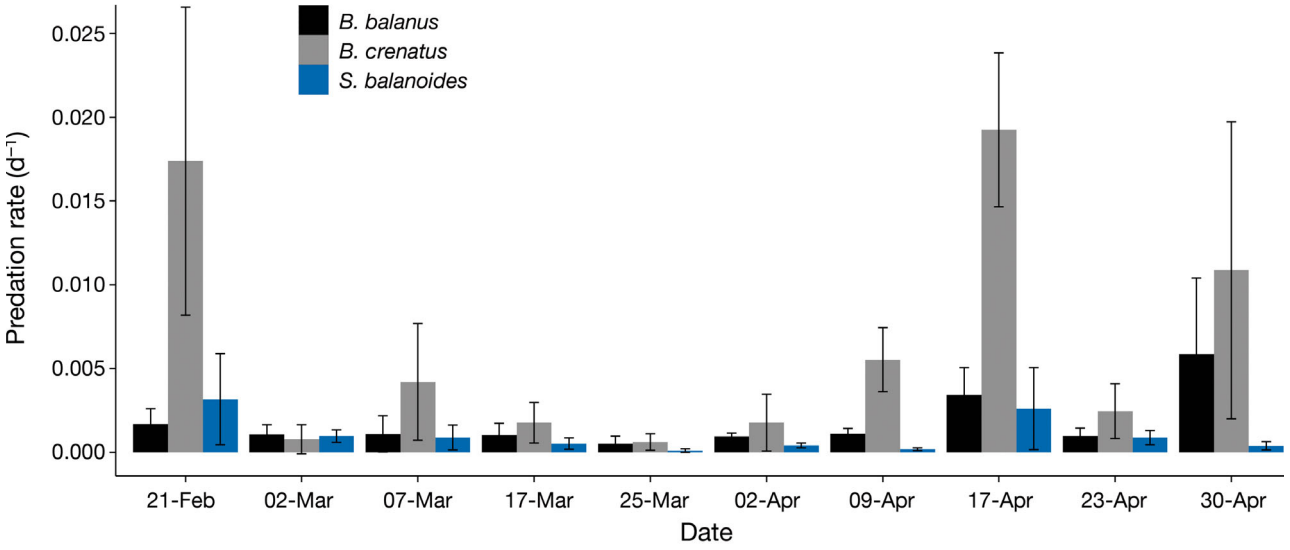

Fig. 11. Mean instantaneous mortality rate (±1 SD) of larval *Balanus balanus*, *Balanus crenatus*, and *Semibalanoides balanoides* from ingestion by *Pleurobrachia pileus* over the sampling duration. SD propagated from prey concentration (n = 2) and the pooled SD among digestion time experiments (each with different n, see Table 1)

change their selectivity to *P. pileus* relative to the other barnacle species on any one sampling date.

Although larval barnacles are frequently reported in the digestive tract of predators (Marshall et al. 1937, Bainbridge & McKay 1963, Fraser 1970, Stickney & Perkins 1981, Purcell & Mills 1988, Alvarez-Cadena 1993), estimates of predation rates are scarce. Available estimates are based on concentrations of larval barnacles not identified to species and on ingestion rates that were either extrapolated from laboratory incubations (Hansson & Kiørboe 2006) or from digestion times estimated indirectly (Kuipers et al. 1990, Hansson et al. 2005). Kuipers et al. (1990) reported predation rates of larval barnacles (nauplii and cyprids) by *P. pileus*, but used the digestion time of copepods to evaluate predation rates. Using data provided in Kuipers et al. (1990; their Table 1 and Fig. 9) and the barnacle nauplii digestion time provided by Larson (1987; his Table 4) for *Pleurobrachia bachei* at 12 to 14°C, we estimated that predation rates of barnacle nauplii from Kuipers et al. (1990) were similar to those in our study — on the order of 0.0001 to 0.001 d^{-1} and reached ~0.01 d^{-1} on only one occasion. In contrast to our study, Kuipers et al. (1990) reported lower average numbers of larval barnacles in the pharynx (~0.01 to 1 larva ctenophore^{-1}) and higher concentrations of ctenophores (1 to 10 ctenophores m^{-3}). Hansson & Kiørboe (2006) also estimated low predation rates of barnacle nauplii from ingestion by *Sarsia* sp. (often <0.001 d^{-1}). Hansson et al. (2005) reported average predation rates of larval barnacles from ingestion by *Aurelia aurita* in Limfjorden, Denmark, ranging from 0.01 d^{-1} in May to 0.10 d^{-1} in July. The authors suggested that *A. aurita* was capable of controlling larval barnacle populations.

The high level of temporal variability in predation rates of *B. crenatus* in our study and of barnacle nauplii in Hansson et al. (2005) underscores the importance of sampling at different times during the larval period. Variability in predation rate is caused by factors that influence (1) the ingestion rate and (2) the abundance of predator and prey populations. As larvae develop, their morphology, behaviour, and swimming and sensory abilities change (Chia et al. 1984, Kingsford et al. 2002). These factors likely influence their vulnerability to ingestion (Pennington et al. 1986). For example, Greene et al. (1986) attributed stage-specific variation in the clearance rate of copepods by *P. bachei* to differences in swimming speed and post-encounter escape abilities of the prey. Ontogenetic variation in vertical distribution, which has been reported for larval barnacles (Tapia et al. 2010), can also influence stage-specific vulnerability of larvae by affecting the realized concentration of larval prey to predators. Temporal variability in the depth integrated abundance of predators and prey is influenced by phenology on large time scales and patchiness on short time scales. The low daily variability of the abundance of barnacle nauplii and *P. pileus* and the number of barnacle nauplii in the pharynx that we observed in 2015 suggest that our weekly sampling in 2014 was adequate for resolving temporal variability in predation rates.

A limitation to our study was that predation by only a single predator was evaluated. We therefore obtained rough approximations of the predation rate of 2 other predators (*Sarsia* sp. and *T. discuadatus*) by multiplying predictions of the ingestion rate of barnacle nauplii from published laboratory-derived functional response models (Uye & Kayano 1994, Hansson & Kiørboe 2006) by the ratio of the concentrations of each predator and barnacle nauplii from our study. Using this method we estimate that predation rates of barnacle nauplii from *Sarsia* sp. were on the order of 0 to 0.001 d^{-1} and those from *T. discaudatus* were on the order of 0.001 d^{-1}. The ingestion rate models reported by Hansson & Kiørboe (2006) and Uye & Kayano (1994) are based on different species of predator and prey and higher prey concentrations and temperature ranges compared to our study. They can therefore only be used as first-order approximations at best, but suggest the impact of predation by these species is also low.

The low predation rates that we estimated in our study are consistent with the previous suggestion that predation may not be a major source of larval mortality (Johnson & Shanks 2003). However, it is not possible to determine the importance of predation to mortality in our study because the overall mortality rate and the predation rate integrated over the entire predator community were not measured. For example, ingestion from adult planktivorous fish and benthic predators, which were not enumerated, may be important sources of predation (Gaines & Roughgarden 1987, Navarrete & Wieters 2000). The few measurements of predation rates of planktonic larvae integrated over predator communities are highly variable, ranging from ≤0.07 d^{-1} (Johnson & Shanks 2003) to well over 1 d^{-1} (Olson & McPherson 1987, Allen & McAlister 2007). Vaughn & Allen (2010) point out that it is difficult to determine whether this variability is due to methodological differences between studies or spatial and temporal variation in predation.

Our results are limited to the Northwest Arm. Spatial variation in the abundance of prey and composition of predator species can lead to large variability in estimates of predation rate. For example, Hansson et al. (2005) found that estimates of predation rates of barnacle nauplii by *A. aurita* ranged from 0.006 to 0.99 d^{-1} and 0.02 to 2.31 d^{-1} from 2 separate surveys (n = 12 sites $survey^{-1}$) in Limfjorden, Denmark. Pepin et al. (2002) found that variation in larval fish mortality among sites was positively related to differences in the abundance of pelagic fish predators. Also, Acosta & Butler (1999) demonstrated that predation on lobster larvae was significantly higher in reef habitats rather than in lagoon or bay habitats. In our region, the highest biomass of chaetognaths is restricted to waters overlying deep basins (Sameoto 1973). Therefore, the potential effect of chaetognath predation on larval barnacle populations is probably stronger in Bedford Basin and St. Margarets Bay, which are adjacent to and much deeper than the Northwest Arm.

Sources of variation in ingestion rates

On each sampling date, the distribution of the number of larval barnacles retrieved from the pharynx of individuals of *P. pileus* was highly skewed. We expected that the size distribution of *P. pileus* would be a potential source of this variation as a positive relationship between ctenophore size and clearance rate has been demonstrated in the laboratory (Gibbons & Painting 1992). However, in our study a significant positive relationship between ctenophore size and pharynx content was only detected on 4 of the 10 sampling dates. On only one of these sampling dates, the size of ctenophores collected by dipping from floating docks was significantly higher than plankton tows. Therefore, the size bias that occasionally occurred in our ctenophore collection probably had little influence on our estimates of ingestion rates.

Other potential sources of variability in the pharynx content of *P. pileus* include feeding activity, prey concentration, and digestion time. When actively feeding, we expect that ctenophores exposed to higher prey concentrations over their digestion time (~8 h) should have more prey in their pharynx. Individual ctenophores collected on the same date may experience different prey densities over the time scale of the digestion time due to the combined effect of vertical migration and small-scale patchiness of prey. The digestion time of *P. pileus* may be influenced by ctenophore size, prey composition and starvation. Harris et al. (1982) reported no relationship between digestion time of copepods and size of *P. pileus*, but did not provide the size range tested. The presence of copepods and cyprids in the pharynx of *P. pileus* may have affected the digestion time of barnacle nauplii. We did not test for the effect of alternative prey on the digestion time; however, we demonstrated that digestion time did not vary with meal size of barnacle nauplii. This suggests that the presence of other prey items (copepods and barnacle cyprids) did not substantially affect the digestion

time of barnacle nauplii within the range of meal sizes in our digestion experiments.

The presence of zooplankton other than barnacle nauplii could have reduced the ingestion rate on barnacle nauplii by satiating *P. pileus*. In the laboratory, the ingestion rate of *Pleurobrachia* spp. increases linearly with the concentration of copepod prey up to ~60 prey l^{-1} (Reeve & Walter 1978, Chandy & Green 1995). In our study, the maximum concentration of barnacle nauplii was ~10 larvae l^{-1}. We did not quantify copepod concentrations in our study; however, in Bedford Basin, Matsakis & Conover (1991) found that copepod (>200 µm) concentrations ranged between 1 and 10 ind. l^{-1} during March and April. It is therefore unlikely that prey concentrations were high enough to satiate *P. pileus*. This is further supported by our observation that the pharynx of individuals of *P. pileus* was never full in our study. However, we acknowledge that the presence of other zooplankton may reduce the feeding rate of *P. pileus* without filling the pharynx. For example, it has been suggested that ctenophores reduce their feeding rate to maintain a certain number of prey items within the pharynx (Rowe 1971). It has also been suggested that ambient plankton reduce feeding rates on other zooplankton by interfering with prey detection (Johnson & Shanks 1997).

Our estimates of ingestion rate require the determination of digestion time and involve several assumptions. Firstly, the ingestion and digestion rates (prey d^{-1}) are assumed equal (Bromley 1994). Ingestion rates are therefore only accurate if ctenophores have been feeding for a sufficiently long period to reach steady state prior to collection. This assumption inherently requires that the digestion rate is not constant, but varies positively with ingestion rate. Our observations indicate that digestion rate increases with meal size, and therefore support this assumption. This relationship may not hold when the number of nauplii in the pharynx of *P. pileus* exceeds the maximum meal size tested in our experiments (12 nauplii), which occurred on many occasions in our study. For example, Rowe (1971) found a non-linear relationship between digestion rate of *P. pileus* and prey concentration (nauplii of *Artemia* sp.) that was consistent with the Michaelis-Menten saturation curve. If the presence of many prey items (including copepods and cyprids) does indeed reduce digestion rate, this would result in an overestimate of ingestion rate. This further emphasizes that predation rates on barnacle nauplii by *P. pileus* were low in our study.

Secondly, Bajkov's (1935) model assumes that digestion rate remains constant within each ctenophore as prey items in the pharynx are eliminated (Bromley 1994). However, the digestion rate of many marine organisms has been shown to decrease as food content is eliminated from the gut (see reviews by Bromley 1994, Båmstedt et al. 2000). We found that the digestion rate of *P. pileus* remained constant in some cases, but decreased over time in others. It is therefore possible that we did not predict ingestion rates accurately for some individuals. When the digestion rate is non-linear, our predictions of ingestion rate would be over- or underestimated at low or high pharynx content levels, respectively.

In our study, the average digestion time of barnacle nauplii by *P. pileus* ranged from 7 to 8 h at 2°C and 4 to 6.5 h at 6°C. Using a different method, Larson (1987) estimated that *P. bachei* digested barnacle nauplii in 4.2 h at 12 to 14°C. Although we expect digestion time to be negatively related to temperature, Kuipers et al. (1990) found that there was little difference in digestion time of copepods by *P. pileus* between 7 and 13°C, and that the digestion time was substantially reduced at 5°C. The digestion time of larval barnacles by *Pleurobrachia* spp. appears to be longer than that of copepods, as estimates of copepod digestion time vary between 1.2 and 3.5 h over a temperature range of 7 to 14°C (Sullivan & Reeve 1982, Harris et al. 1982, Larson 1987, Kuipers et al. 1990, Båmstedt 1998).

The time required for *P. pileus* to digest cyprid larvae appears to be much longer than that for nauplii. Unfortunately, the digestion time could not be determined with certainty because almost all cyprids were egested prior to complete digestion. Egestion was not induced by prey handling following feeding, as we observed the egestion of both Stage 6 nauplii and cyprids by undisturbed individuals of *P. pileus*. Observations *in situ* can determine whether this phenomenon occurs under natural conditions. We suspect that the high frequency of egestion and long digestion time that we observed were in response to the inability of digestive enzymes of *P. pileus* to penetrate the exoskeleton of the cyprid. The exoskeleton encloses the cyprid, except for a narrow opening on the ventral side, which can be tightly shut by an adductor muscle (Walley 1969).

Ctenophores and cnidarian medusae have been observed egesting undigested fish eggs (Jaspers et al. 2011, Purcell et al. 2014). Cnidarian medusae have also been observed egesting live bivalve larvae (Purcell et al. 1991). Barnacle cyprids and nauplii were frequently observed swimming freely after being egested; however, in many instances undigested larvae were egested as a bolus, in which case, if

unable to free themselves, these larvae would eventually die. Although our results suggest that some undigested Stage 6 nauplii of *S. balanoides* may be egested after feeding, we assumed all nauplii found in the pharynx would be eventually fully digested.

Conclusions

In our study, larval barnacle populations were not strongly affected by predation by *P. pileus*; however, larval predation could be significant when integrated across numerous pelagic and benthic predators. Studies of predation of benthic marine invertebrate larvae are scarce, and further investigations are required to improve our ability to predict predation rates based on the community composition of predators. Future studies using ingestion models should recognize and evaluate the assumptions by making direct measurements (i.e. digestion time). Also, predation rates should be quantified at several times during the larval duration and at the highest possible taxonomic resolution. We have made a first attempt at addressing these issues, and our results suggest that further studies such as this are warranted to accurately assess the importance of predation on mortality of larval benthic marine invertebrates.

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