Species-specific grazing and significant trophic impacts by two species of copepod nauplii, *Parvocalanus crassirostris* and *Bestiolina similis*

Michelle J. Jungbluth[1,3,*], Karen E. Selph[1], Petra H. Lenz[2], Erica Goetze[1]

[1]**Department of Oceanography, University of Hawaii at Manoa, Honolulu, Hawaii 96822, USA**
[2]**Pacific Biosciences Research Center, University of Hawaii at Manoa, Honolulu, Hawaii 96822, USA**

[3]***Present address:* San Francisco State University, Romberg Tiburon Center for Environmental Studies, 3150 Paradise Dr., Tiburon, CA 94920, USA**

ABSTRACT: The ingestion rates of *Parvocalanus crassirostris* and *Bestiolina similis* mid-stage (N3 and N4) nauplii feeding on a natural prey assemblage from a subtropical embayment were measured to evaluate differences in prey preferences and estimate the trophic impact of grazing by each species. During the 2 wk period of our experiments, the *in situ* 2–35 µm cell biomass increased over time and ranged from 45 to 187 µg C l^{-1}. The 2 studied copepod species overlapped in the range of prey removed; however *P. crassirostris* nauplii grazed less selectively than nauplii of *B. similis*, which selected against the 2–5 µm prey size group and increased selection over time for 20–35 µm prey. Total ingestion rates were comparable between the 2 species, and the majority of the grazing impact was due to *P. crassirostris*, which had a 2- to 11-fold higher *in situ* naupliar abundance. Grazing impacts by the 2 species combined ranged from 1.0 to 8.7 % of the total 2–35 µm prey biomass. Prey removal on individual prey size groups by *P. crassirostris* ranged from 0.9 to 23.7 %, with maximum impacts on prey >10 µm. Daily chlorophyll *a* removal was up to 12.9 % by *P. crassirostris*, and 5.7 % by *B. similis*. Our results suggest that naupliar ingestion rates and prey selectivity can vary on timescales of 2 to 3 d and differ between 2 closely related species, and also that nauplii can have a significant grazing impact on prey populations, particularly through positive selection for prey size classes that are less abundant.

KEY WORDS: Naupliar grazing · Copepod nauplii · Subtropical plankton · *Parvocalanus crassirostris* · *Bestiolina similis*

INTRODUCTION

Microzooplankton are the dominant phytoplankton consumers in the ocean and can remove up to 75 % of primary production in marine ecosystems (Calbet & Landry 2004). Because of year-round reproduction of subtropical copepods (Webber & Roff 1995), high growth rates (e.g. Kiørboe & Sabatini 1995, McKinnon & Duggan 2003), rapid development times (Hart 1990, Kiørboe & Sabatini 1995, Peterson 2001), and rapid response to increases in their prey communities (Hopcroft & Roff 1998, McKinnon & Duggan 2003, Hoover et al. 2006), copepod nauplii can be an epi-sodically significant component of the microzooplankton. For example, in response to the first rainfall event after a long dry summer in Hawaiian waters, Hoover et al. (2006) found that copepod nauplii increased to roughly 6-fold greater than their pre-storm abundance within 10 d of the storm. During peaks of abundance, nauplii can exert significant grazing pressure on their prey (White & Roman 1992, Lonsdale et al. 1996, Böttjer et al. 2010), in part because their specific ingestion rates can be greater than adult conspecifics (Paffenhöfer 1971, White & Roman 1992, Saiz & Calbet 2007, Böttjer et al. 2010, Almeda et al. 2011). Due to episodically high abun-

dance and high specific ingestion rates, nauplii may be an important pathway by which microbial production is transferred to higher trophic levels.

Diverse species of nauplii are capable of consuming prey of a wide range of types and sizes (Uye & Kasahara 1983, Saiz et al. 2014), including phytoplankton (Meyer et al. 2002, Vogt et al. 2013), microzooplankton (Merrell & Stoecker 1998), bacteria (Turner & Tester 1992, Roff et al. 1995), and detritus (Heinle et al. 1977), with diet breadth often overlapping adult conspecifics. For example, *Oithona davisae* nauplii and adults are capable of consuming a similar range of prey types, although the highest *O. davisae* nauplii clearance rates occurred on a small dinoflagellate (18 μm equivalent spherical diameter [ESD], 1.6 ml ind.$^{-1}$ d^{-1}) which is half the size of a ciliate that was cleared at the highest rate by adults (30 μm ESD, 11.7 ml ind.$^{-1}$ d^{-1}) (Saiz et al. 2014). Additional studies have shown that conspecific nauplii and adults ingest a similar range of different food types (Merrell & Stoecker 1998, Finlay & Roff 2004, Vogt et al. 2013). However, the preferred prey in a mixed assemblage of food has not been well studied for many species, and may differ between nauplii and adults.

In the subtropics, copepod nauplii may have an equal or greater daily carbon ration than observed in other ocean regions due to the effect of warmer temperatures on ingestion (White & Roman 1992) and growth rates (Ota & Landry 1984), as well as the inverse relationship between carbon ration and body size (Mauchline 1998). In combination with higher year-round abundance in the subtropics (Hopcroft et al. 1998, McKinnon & Duggan 2003, Hannides 2007), high daily carbon rations could result in grazing impacts in these ecosystems that are greater than those observed in temperate ecosystems. However, in subtropical ecosystems, the higher diversity, co-existence of closely related species, and year-round production of nauplii leave no distinct copepod cohorts to be followed and studied throughout development. In addition, morphological identification of nauplii to species is typically difficult or impossible due to their similar appearance, and therefore knowledge of the ecology of subtropical copepod nauplii at the species level is very scarce.

Despite the potential importance of nauplii as grazers in subtropical ecosystems, there are no prior studies of the impacts of subtropical nauplii grazing on natural prey assemblages. The current study focused on grazing by *Parvocalanus crassirostris* and *Bestiolina similis* nauplii in Kane'ohe Bay, a subtropical embayment on the eastern shore of Oahu, Hawaii.

Naupliar grazing on the natural prey assemblage was measured in 5 experiments over a 2 wk period in May and June 2013. Concurrent measurements of *in situ* naupliar abundance and their potential prey in field populations were made, as well as parallel seawater dilution experiments to estimate microzooplankton community grazing rates (Landry & Hassett 1982). The objectives of the study were to (1) quantify ingestion by 2 species of common subtropical copepod nauplii, (2) evaluate species' differences in prey selectivity and how these preferences change with variable prey availability in the field, and (3) estimate the species-specific impact of naupliar grazing on the standing stock of prey to determine whether nauplii are important grazers in subtropical ecosystems. Our results suggest that selectivity on prey differs between species and this leads to potentially different impacts on their prey depending on species-specific naupliar abundance and prey availability.

MATERIALS AND METHODS

Grazing experiments

A series of 5 bottle incubation experiments (hereafter referred to as E1 to E5; see Table 1) were conducted over a 10 d period (27 May to 5 June 2013) to measure grazing by copepod nauplii on the natural prey assemblage collected from Stn S3, located in the southern semi-enclosed basin of Kane'ohe Bay, Oahu, Hawaii (21° 25′ 56″ N, 157° 46′ 47″ W; Jungbluth & Lenz 2013). The copepods were N3 and N4 stage nauplii of *Parvocalanus crassirostris* and *Bestiolina similis*. Concurrent experiments were run to measure microzooplankton community grazing, and to quantify *in situ* predator and prey abundances. The results from these experiments were used to correct for multiple trophic interactions within the bottle incubations, as these interactions can mask the effect of metazoan grazing (Nejstgaard et al. 2001). Salinity and temperature in the field were measured using a YSI 6600V2 sonde prior to collecting water for bottle incubations. Daily rainfall estimates were obtained from a rain gauge located at Luluku (www.prh.noaa. gov), and weather station data from the Hawaii Institute of Marine Biology (HIMB) (www.himb.hawaii. edu/weatherstation/) were used for estimates of the wind magnitude, wind direction, and solar irradiance.

Copepod nauplii used in the incubations were obtained from laboratory culture populations of *P. crassirostris* and *B. similis* established from animals previously collected in Kane'ohe Bay (P. Lenz lab). Both

species are capable of completing naupliar development in less than 3 d and reaching the adult stage (C6) in approximately 7 to 8 d (McKinnon et al. 2003, VanderLugt et al. 2009). Use of these monospecific cultures enabled us to produce high abundance naupliar cohorts of a specific age for grazing incubations. To produce these cohorts, adults of each species were isolated and fed 1×10^6 cells ml^{-1} *Tisochrysis lutea* (formerly *Isochrysis galbana* Tahitian strain; Bendif et al. 2013) 18 h prior to the start of each experiment to increase naupliar production. The adults were removed 6 h later, resulting in a cohort of nauplii (N3 and N4; note that N3 are the first feeding stage of these nauplii) raised at the experimental temperature of 21°C by the beginning of each experiment. Sets of ~50 nauplii were isolated into small volumes (<10 ml) of 0.2 µm filtered seawater and held for 1 to 3 h prior to the start of each grazing experiment. This procedure resulted in minimal exposure of the N3 and N4 nauplii to prey prior to the start of the grazing experiments. Seawater for the prey assemblage was collected from 2 m depth using a 5 l General Oceanics Niskin bottle deployed by hand line, with the contents gently added (silicone tubing) to two 20 l polycarbonate carboys.

Separate experiments indicated that longer incubation times decreased ingestion estimates within the grazing treatments, likely due to the fast development rates of our nauplii (<24 h inter-molt period) and the diverse and rapidly changing prey community in this relatively warm (>20°C) system (Jungbluth et al. 2017). Thus, 6 h incubations were chosen to give the most representative view of naupliar grazing rates on natural prey, with conditions closest to those *in situ,* and in order to minimize nutrient remineralization and other food web interaction effects that can be significant during longer incubations (Roman & Rublee 1980).

Grazing incubations were performed in pre-washed (10% HCl rinse, followed by 3 rinses with ambient 0.2 µm seawater) polycarbonate bottles (total volume: 1120 ml) with 35 µm gently pre-screened bulk seawater offered as prey. It is possible that our nauplii would consume prey >35 µm given the opportunity, however the small size of the copepod species in our study (~40 µm wide, ~70 µm long; *P. crassirostris* N1 dimensions) necessitated the removal of prey >35 µm to ensure removal of other nauplii from the field. Our initial expectations were that the optimum prey size for our species would be 2 to 7 µm (Berggreen et al. 1988, Hansen et al. 1994), therefore the prey included here (<35 µm) should represent a majority consumed naturally by our species.

The experimental nauplii were transferred into the 1120 ml grazing bottles at 2 densities (42–51 [moderate] and 81–95 [high] nauplii; see Table 1) and placed on a bottle roller (Wheaton) at 5 rpm in the dark for 6 h. The 2 nauplius densities were tested to ensure that we could detect removal of prey cells relative to controls over our incubation period, since a predator density that is too low may result in insignificant prey removal relative to control bottles. Removal of cells in treatments relative to control bottles was detected in the moderate density treatments and ingestion rate estimates were comparable to those from higher density bottles. Since results were comparable between moderate and high density treatments, results reported here focus on bottles with ~50 nauplii l^{-1}, also because treatment replication was better with moderate density bottles (n = 3 per experiment) than high density bottles (n = 2). This density of nauplii is well within the range of total nauplius concentrations reported in previous studies in Kane'ohe Bay (7 to 68 total nauplii l^{-1}; Hoover et al. 2006) and within the range of each species abundance we have previously measured following storm run-off events in the bay (M.J.J. pers. obs.).

Treatment bottles were run in triplicate, with 2 or 3 no-nauplii control bottles for each experiment (2 for E1 to E2, 3 for E3 to E5). Experiments were incubated at 21°C, which is within the range of the annual temperature fluctuation in Kane'ohe Bay (20 to 29°C during the previous 5 yr; HIMB weather data). No nutrients were added to the bottles, because controls and experimental bottles were considered approximately equally influenced by nitrogen remineralization from grazing processes, due to the presence of other <35 µm microzooplankton grazers in all bottles and the short incubation times (6 h). Nauplii are known to have low expected nitrogen remineralization (~10-fold lower than adults) due to their small biomass compared to adults (Vidal & Whitledge 1982, Mauchline 1998); at 50 nauplii l^{-1}, excretion rates were estimated to be 2 to 3 orders of magnitude below the *in situ* average nitrogen concentrations in Kane'ohe Bay (0.2 to 1.0 µM; Drupp et al. 2011).

Initial and final time-point measurements included samples to quantify particle size and abundance in the 2–35 µm size range from the Coulter counter (CC), as well as samples for specific prey types, including chlorophyll *a* (chl *a*) and the abundance and biomass of types of nano- and microplankton. Prey types and CC-quantified potential prey were not expected to be equal; some prey types include cells <2 µm, while the lower limit of the CC was 2 µm. Nauplii were recovered at the end of the experiments to check

their condition (alive/dead; no dead nauplii were found), then preserved in 10% paraformaldehyde, stained with 1% Rose Bengal, and enumerated using microscopy for use in clearance and ingestion rate estimates.

Prey size spectra and abundance (Coulter counter)

Due to the ambient prey community being largely spherical cells (few diatoms present, verified microscopically), initial and final time-point CC samples were taken for prey particle spectra by gently pouring 20 ml from each incubation bottle through a 35 µm cap filter into a clean beaker, then gently back-washing the filter into the experimental bottle to return any nauplii using a small volume of 0.2 µm filtered seawater. From this subsample, triplicate 2 ml volumes were measured with a Beckman Coulter Multisizer III CC with a 100 µm orifice tube, yielding a spectrum of particle sizes from 2–35 µm ESD, as well as quantitative abundance data. These raw data were further processed in R (R Core Team 2016) to streamline binning of prey size groups, for calculations of clearance and ingestion rates, and for statistical analyses.

Prey ESD was converted to biovolume (BV; µm^3), then to carbon (C; pg C cell^{-1}), using the relationship $C = 0.216 \times BV^{0.939}$, which applies well to taxonomically diverse protists (Menden-Deuer & Lessard 2000). Averages of cell abundance and biomass from the triplicate CC measurements were binned into 5 prey size groupings (2–5, 5–10, 10–15, 15–20, and 20–35 µm), chosen based on their relevance to known prey sizes in Kane'ohe Bay and also due to use in prior studies of adult copepod grazing in Kane'ohe Bay (Calbet et al. 2000). The binned, averaged data for initial and final time points for each control and treatment bottle were used to calculate carbon ingestion (I; ng C nauplius^{-1} h^{-1}) and clearance rates (F, ml nauplius^{-1} h^{-1}) on each prey size group using the equations of Frost (1972) (see 'Data analyses' below).

Photosynthetic eukaryotes (flow cytometry)

Flow cytometry (FCM) samples (1.5 ml) for photosynthetic eukaryote (PEUK) abundance were preserved in 0.4% paraformaldehyde (final concentration), flash-frozen in liquid nitrogen and transferred to a −80°C freezer until processing. Preserved, frozen FCM samples were thawed in batches, stained with the DNA dye Hoechst 34442 (1 µg ml^{-1}, final concentration) (Campbell & Vaulot 1993, Monger & Landry

1993), and analyzed using a Beckman-Coulter Altra flow cytometer for phytoplankton population abundances using fluorescence signals from DNA, phycoerythrin and chl a. Data were grouped into relevant populations using FlowJo (Treestar). PEUK cell abundances were converted to biomass using data from parallel microscopy samples, which showed that the eukaryotic phytoplankton in these samples were dominated by 2–3 µm ESD spherical cells, with an average biomass of 1.55 pg C cell^{-1} (biomass conversions as in Menden-Deuer & Lessard 2000).

Nano- and microplankton abundance and biomass (microscopy)

Initial and final samples for nano- and microplankton abundance by epifluorescence microscopy (EPI) were preserved (0.4% paraformaldehyde, final concentration), and kept in the dark and cold (4°C) until filtered within 24 to 48 h. EPI samples (25 or 50 ml) were stained with 0.5 nM proflavin (1 to 2 h prior to filtration), then filtered onto 0.8 µm black polycarbonate filters (Midland Scientific), stained with 4′,6-diamidino-2-phenylindole (DAPI) for 2 min and mounted on a slide. These slides were frozen at −80°C until digitally imaged within 2 mo of collection.

Digital images of the slides were taken using a color camera (Olympus U-LH100HGAPO) attached to an epifluorescence microscope (Olympus Model BX51 TRF, 400× total magnification), and the software program Microfire™ (Optronics). For each slide, 3 sequential digital images were taken of 30 random fields, using 3 different excitation/emission filters; one each to illuminate chl a/proflavin (EX450-480; DM500, EM≥515), phycoerythrin (primarily due to *Synechococcus*), and DNA (EX330-385, DM400, EM>420) fluorescence. Living cells were distinguished from dead cells and debris by the presence of nuclei, and autotrophic and heterotrophic cells were distinguished by the presence of chl a.

Images were analyzed by sizing, counting, and identifying autotrophic and heterotrophic cells 2–10 µm in size until >100 cells were characterized. In 2 cases, slide and image quality was poor, and as few as 60 cells were characterized (i.e. 31 May *B. similis*: 60 cells counted; 31 May *P. crassirostris*: 95 cells counted). To evaluate the large (>10 µm) cell abundance and biomass, all >10 µm cells on ¼ of one randomly selected control and treatment slide from each experiment were counted, identified as an autotroph or heterotroph, and measured using a calibrated ocular micrometer. Cell dimensions were used to

estimate biovolume (oblate spheroid), and converted to biomass (Menden-Deuer & Lessard 2000). Diatoms >10 µm were also quantified, but they were never abundant during our experiments (see Table 2).

Ciliate biomass and abundance was estimated by inverted microscopy on samples preserved with a 1/20 dilution of acid Lugol's solution (Throndsen 1978), and kept in the dark at room temperature until analysis (~1 yr later) by the Utermöhl technique (Sherr & Sherr 1993). Aliquots of 28 ml from randomly selected control and experimental treatments were settled and their entire contents examined (18 to 87 total cells in sample volume, median 55) with a Zeiss inverted microscope (400× magnification), with digital images taken (Moticam camera and software) for subsequent dimensional analyses. The measured length and width of each cell was converted to biovolume based on the appropriate geometric shapes, and converted to carbon biomass (Menden-Deuer & Lessard 2000). Note that larger dinoflagellates were removed by pre-screening, and the acid Lugol's technique does not allow differentiation between autotrophs and heterotrophs; thus we focused on ciliates here.

Chl *a* determinations

For chl *a*, triplicate 305 ml samples were filtered onto GF/Fs (Whatman), flash-frozen (LN_2), and kept at −80°C freezer until measurements were made 4 mo later. Chl *a* (and phaeopigment) was measured using a Turner Designs (model 10AU) fluorometer, using the standard extraction and acidification technique (Yentsch & Menzel 1963, Strickland & Parsons 1972).

Data analyses

Hourly clearance rates on each prey size or type were calculated from cell abundance (Frost 1972), and converted to biomass ingestion by multiplying cell ingestion by the biomass estimate per cell for that prey group. Prey type ingestion rates were calculated on ciliates, PEUKs (FCM), and 2–5, 5–10, and >10 µm size classes of autotrophs and heterotrophs (EPI). Negative clearance rates of prey were omitted from further analysis. According to Gifford (1993) and Båmstedt et al. (2000), the change in prey concentration within grazing experiment treatment bottles must be 20 to 40% so that the variation in count replicates (CV often up to 20%) is less than the difference between initial and final counts. Thus, the percent reduction of prey abundance within the experi-

mental bottles between initial and final time points is reported in Tables S1 & S2 in the Supplement at www.int-res.com/articles/suppl/m572p057_suppl.pdf).

Specific ingestion rates were calculated using the carbon biomass of mid-stage nauplii measured directly for a cohort of *P. crassirostris* nauplii (Exeter Analytical CE 440) (Gordon 1969, Sharp 1974). These measurements (Jungbluth 2016) agreed well with naupliar biomass based on equations using nauplius body length (White & Roman 1992). Given this agreement, and the dimensional similarity of *P. crassirostris* and *B. similis* mid-stage nauplii (McKinnon et al. 2003), the measured value of 0.057 ± 0.019 µg C nauplius^{-1} (mean ± SE) was applied to calculate weight-specific ingestion rates for both species on the different prey types.

Corrections for microzooplankton grazing

Naupliar grazing rates on total phytoplankton (as total chl *a*) and PEUKs were corrected according to the general method as described in Nejstgaard et al. (2001, Eqs. 1–4) to account for trophic interactions in the bottles. This method uses the change in biomass of potentially competing micrograzers (e.g. ciliates, dinoflagellates, etc.) over the course of the experiment, as well as the community microzooplankton grazing impact on specific prey populations determined from parallel seawater dilution experiments, to correct the estimates of naupliar grazing. Results of the concurrent community microzooplankton grazing experiments were used to derive these correction factors for total chl *a* and PEUKs (see Table S3 in the Supplement) with methods as in Selph et al. (2005), except 1 l incubation bottles were used. Note that whole seawater was used for these experiments. Corrections for changes in non-nauplii microzooplankton during the incubation were made for ciliates and other heterotrophs (>2 µm heterotrophs, EPI) by summing these 2 groups, with dinoflagellate abundances included in the EPI data since these data allow us to distinguish between autotrophs and heterotrophs. Results of this correction are reported as 'corrected rates' in comparison with 'uncorrected rates', and the corrected rates are further used in calculations of specific ingestion rates and resulting grazing impacts on these prey types.

Selectivity on prey

To assess whether the nauplii were selecting for or against specific prey items according to the availabil-

ity of prey in the environment, the percent of prey in the environment was compared with the percent of prey in the diet for each species (Chesson 1978). To do this, biomass ingestion rates were compared with the initial prey biomass separately for 2 prey groups: prey size groups (CC), and prey types in different autotroph and heterotroph prey size groups (EPI). The percent contribution to the total initial prey biomass was calculated for each non-overlapping prey type (or size group) and compared with the percent contribution of that taxon (or size group) in the nauplius diet (% diet). This measure was used to indicate a selection for (above 1:1 line) or against (below 1:1 line) a prey type compared with the availability of that prey item. In some cases, there were cell types that were abundant in the environment, but nauplii had negative grazing rates on these potential prey items. These prey were included in this analysis since the prey type was abundant in the environment, and the percent in the nauplius diet was considered to be zero.

In addition to the above comparison, an electivity coefficient was calculated to assess for selective feeding on prey in the different prey quantification groups using prey clearance rates, according to the electivity index ($E^\star$) in Vanderploeg & Scavia (1979):

$$E^\star = \frac{W_i - \dfrac{1}{n}}{W_i + \dfrac{1}{n}} \qquad (1)$$

where n is the number of categories of prey and W_i is defined by:

$$W_i = \frac{F_i}{\sum F_i} \qquad (2)$$

with F_i as the clearance rate (ml nauplius^{-1} h^{-1}) on prey type i and $\sum F_i$ as the sum of clearance rates on all non-overlapping potential prey types in the experiment. This index was chosen because it gives an accurate measure of electivity in environments where different prey types are rare or of variable abundance (Lechowicz 1982). Electivity was calculated for the 2 prey quantification groups (size, type) described above. Electivity > 0.5 suggests strong positive selection for a prey item, and electivity < −0.5 suggests strong selection against a prey item. Negative clearance rates were omitted from this analysis.

Estimates of *in situ* naupliar abundance

Naupliar abundances of the 2 target species *in situ* were estimated using a quantitative polymerase chain reaction (qPCR)-based method (Jungbluth et al. 2013), as well as microscopic counts of calanoid and cyclo-

poid nauplii. The qPCR-based method allows application of individual species grazing rates to *in situ* abundances to estimate the total potential grazing impact of each species. Samples were collected by duplicate vertical microplankton net tows (0.5 m diameter ring net, 63 µm mesh) from near bottom (10 m depth) to the surface with a low speed flow meter (General Oceanics). The contents of each net were split quantitatively. One half was size-fractionated through a series of 5 Nitex sieves (63, 75, 80, 100, and 123 µm) to separate size groups of nauplii from later developmental stages, and each was preserved in 95% non-denatured ethyl alcohol (EtOH). The second half of the sample was preserved immediately in 95% EtOH for counts of total calanoid and total cyclopoid nauplii, which were used for comparison to the qPCR-based results of the abundance of each calanoid species. All samples were stored on ice in the field until being transferred to a −20°C freezer in the laboratory. EtOH in the sample bottles was replaced with fresh EtOH within 12 to 24 h of collection to ensure high-quality DNA for analysis (Bucklin 2000).

The 3 smallest plankton size fractions from the net collection were analyzed with qPCR to enumerate *P. crassirostris* and *B. similis* nauplius abundances (Jungbluth et al. 2013). In brief, DNA was extracted from 3 plankton size fractions (63, 75, and 80 µm) using a modified QIAamp Mini Kit procedure (Qiagen). The total number of DNA copies in each sample was then measured using species-specific DNA primers and qPCR protocols (Jungbluth et al. 2013). On each qPCR plate, 4 to 5 standards spanning 4 to 5 orders of magnitude in DNA copy number were run along with the 2 biological replicates of a size fraction for each sampling date along with a no template control (NTC), all in triplicate. A range of 0.04 to 1 ng µl^{-1} of total DNA per sample was measured on each plate ensuring that the range of standards encompassed the amplification range of samples, with equal total DNA concentrations run in each well on individual plates. In all cases, amplification efficiencies ranged from 92 to 102%, and melt-curves indicated amplification of only the target species. The qPCR estimate of each species' mitochondrial cytochrome oxidase *c* subunit I (COI) DNA copy number was converted to an estimate of nauplius abundance using methods described in Jungbluth et al. (2013).

Statistical analysis

Statistical analyses were performed in R using the stats package (R Core Team 2016). For each prey

type that had replicate measurements, 2-tailed *t*-tests of growth rates were run to test for significant differences of growth in control and treatment bottles for each species, which assumes the variances of growth in control and treatment bottles were equal. Analyses of prey growth rates were conducted separately for prey size spectra, chl *a*, and PEUK prey groups (see Tables S1 & S2, underlined values). Two-tailed *t*-tests were also performed to test whether the differences were significant between uncorrected and corrected ingestion rate estimates of chl *a* and PEUKs. To test the null hypothesis that there was no selective grazing behavior by either species, a 1-way ANOVA was run on the electivity data across experiments for each species assuming sample independence, normality, and equality of variances. A Shapiro-Wilks test was used to verify normality of the samples and a quantile–quantile plot was used to assess the equality of sample variances. A post hoc Tukey test was run when the ANOVA was significant.

RESULTS

Environmental conditions

The water temperature (26.4 to 26.6°C) and salinity (34.1 to 34.7 PSU) were stable at the sampling depth across all experimental dates. Further, in the 3 mo leading up to our experiments, the watershed received an average of 0.24 cm of daily rainfall (10.31 cm max., 16 d before E1). Between E1 and E2 (28 May), a storm caused 20.37 cm of rainfall over 24 h. Wind speeds ranged between 11.8 and 15.2 knots, except for 29 May 2013, when wind speeds were 6.9 knots and solar irradiation was relatively low (229 cal cm^{-2}) due to high cloud cover.

In situ community

CC measurements indicated a ~3-fold increase in total biomass of the prey community in Kaneʻohe Bay from E1 (57.11 µg C l^{-1}) to E5 (186.91 µg C l^{-1}), with little change in the relative abundance of most prey size groups (Fig. 1a, Table 1). The size groups contributing the most to the biomass were 2–10 µm, which made up 65 to 78% of the initial prey biomass and 99% of the abundance. Microscopy measurements of the autotrophic and heterotrophic community, despite being lower overall, also showed a ~2-fold increase in biomass during this period (E2:

18.26 µg C l^{-1} to E5: 33.92 µg C l^{-1}), again mostly due to an increase in the 2–10 µm size fractions.

When the total biomass of the prey community is divided into size classes, the 5–10 µm autotrophs contributed 24 to 52% to the available prey biomass (Fig. 1b); whereas the 5–10 µm heterotrophs contributed 32 to 59%. The other prey size classes contributed <13% to the total prey biomass. Peak autotroph biomass and abundance occurred at the start of E4 (20.09 µg C l^{-1}), almost 3-fold higher than the start of E2 (7.12 µg C l^{-1}), while peak heterotroph biomass and abundance occurred at the start of E5 (21.79 µg C l^{-1}), roughly 2-fold higher than E2 (10.74 µg C l^{-1}). Prey type measurements indicate 5–10 µm autotrophs and heterotrophs made up 24 to 59% of the total prey type biomass on the EPI slides (Fig. 1b), suggesting that 2–5 µm prey were not well represented on the EPI slides.

Chl *a* was low (0.26 to 0.33 µg chl *a* l^{-1}; Table 1) in the first 3 experiments (E1 to E3), and increased ~2-fold by E4 and E5 (0.85 and 0.79 µg chl *a* l^{-1}, respectively). Although heavy rainfall can sometimes result in a diatom bloom in the southern region of Kaneʻohe Bay (Cox et al. 2006, Hoover et al. 2006), there was
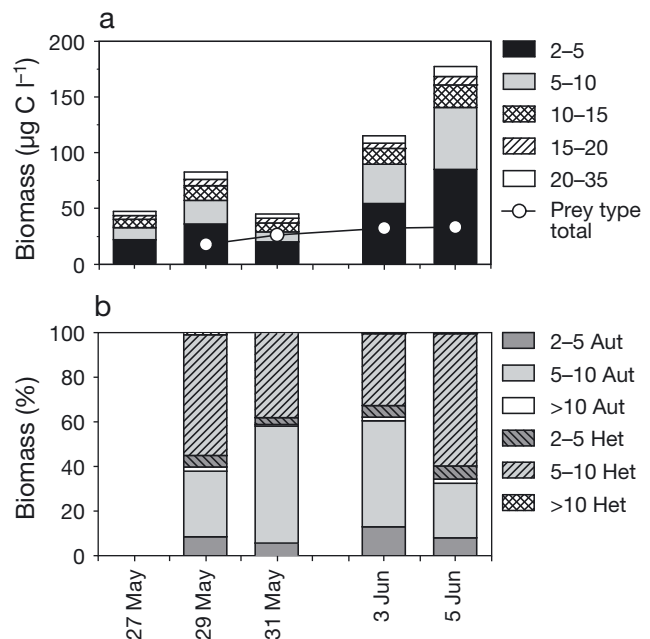

Fig. 1. Biomass (µg C l^{-1}) of the initial prey community on each experimental date from 2 different methodologies: (a) prey size groups by Coulter counter (µm; bars), with epifluorescent microscopy data shown of the combined autotrophic and heterotrophic biomass (open circles), and (b) percent contribution of prey types by epifluorescence microscopy (µm). Aut: autotroph; Het: heterotroph. Experimental days: 27 May = E1; 29 May = E2; 31 May = E3; 3 June = E4; 5 June = E5

Table 1. Initial pico- to microplankton community abundance (Abund; cells ml^{-1}), and biomass (Biom; µg C l^{-1}) by type and size groups (µm) for each grazing experiment. (–) Data not available

Prey category	Size (µm)	Incubation experiments									
		E1 27 May 2013		E2 29 May 2013		E3 31 May 2013		E4 3 Jun 2013		E5 5 Jun 2013	
		Biom.	Abund.	Biom.	Abund.	Biom.	Abund.	Biom.	Abund.	Biom.	Abund.
Chl a[a]		0.33	–	0.35	–	0.26	–	0.85	–	0.79	–
Total particles[b]	2–5	24.79	9411	33.35	11115	21.82	7118	52.85	17009	86.57	27730
	5–10	12.24	513	17.63	803	8.65	389	33.45	1487	59.26	2629
	10–15	10.57	79	8.95	69	6.50	51	13.03	103	21.85	178
	15–20	4.86	14	3.26	9	3.59	10	4.58	13	8.41	24
	>20	4.64	6	5.37	5	4.05	5	6.34	6	10.83	10
	Total	57.11	10024	68.55	12002	44.61	7573	110.25	18618	186.91	30571
Ciliates[c]	<10	0.02	0.3	0.02	0.4	0.01	0.1	0.02	0.4	0	0.1
	10–20	0.14	0.8	0.22	1.2	0.11	0.6	0.14	1.0	0.14	0.6
	20–30	0.10	0.2	0.16	0.4	0.02	0.1	0.25	0.5	0.26	0.4
	>30	0	0	0	0	0.19	0.0	0.62	0.6	0.36	0.5
	Total	0.26	1.4	0.40	2.0	0.33	0.8	1.03	2.5	0.76	1.6
Diatoms[d]	10–35		–	0.20	6	0	1	0.20	5	0.1	8
Autotrophs[d]	2–5		–	1.53	403	1.52	402	4.16	835	2.64	662
	5–10		–	5.23	314	13.81	586	15.39	725	8.08	499
	>10		–	0.36	11	0.15	3	0.54	15	0.65	22
	Total			7.12	728	15.48	991	20.09	1575	11.37	1183
Heterotrophs[d]	2–5		–	0.91	206	0.80	175	1.69	480	2.00	530
	5–10		–	9.66	659	10.00	644	10.41	583	19.63	807
	>10		–	0.17	6	0.07	2	0.17	2	0.16	6
	Total			10.74	871	10.87	821	12.27	1065	21.79	1343

[a]Measured by fluorometry (µg chl a l^{-1}). [b]Measured by Coulter counter. [c]Measured by inverted microscopy. [d]Measured by epifluorescence microscopy

no evidence of a large-celled phytoplankton bloom within the 10 d sampling period, as indicated by the minor increase in the community of >10 µm autotroph and diatoms. Instead, there was close correspondence between the increase in chl a and an increase in 2–10 µm autotrophs, suggesting that the response to the rainfall-induced perturbation occurred primarily in the nanophytoplankton (Table 1). Abundance of FCM-derived PEUKs (including eukaryotic autotrophs ~<5 µm) generally followed the same trend as the 2–5 µm autotrophs found with microscopy, and support the inference of an increase in the smaller cell sizes following the storm. PEUK biomass increased more than 3-fold from E1 to E5, peaking at the start of E5 (33.9 µg C l^{-1}).

Ciliates and diatoms did not contribute significantly to prey community biomass or abundance (Table 1). However, ciliate biomass increased by 4-fold from E1 to the peak during E4 (1.03 µg C l^{-1}), while their abundance increased by less than 2-fold, peaking at 2.5 cells ml^{-1}, because there was a shift in the *in situ* community from smaller to larger ciliates.

Total *in situ* naupliar abundance (count-based) over E1 to E5 ranged from 77 (±8) to 207 (±4) nauplii l^{-1} including all calanoid and cyclopoid nauplii >63 µm. *Parvocalanus crassirostris* was 2- to 11-fold more abundant than *Bestiolina similis* over this time period (qPCR-based), and *P. crassirostris* increased in abundance *in situ* by 10-fold (0.8 to 8.9 nauplii l^{-1}), while *B. similis* increased by 2-fold (0.4 to 0.8 nauplii l^{-1}) from E1 to E5 (Fig. 2a). Over this period, *P. crassirostris* nauplii contributed 0.6 to 7.1% and *B. similis* contributed <1%, respectively, to total nauplius abundance.

Naupliar grazing rates

Application of the Nejstgaard equations to correct for microzooplankton grazing resulted in variable and largely non-significant effects on the mean estimates of chl a and PEUK ingestion rates by nauplii (Fig. 3a–d). The correction was applied to both positive and negative ingestion rates, given the possi-
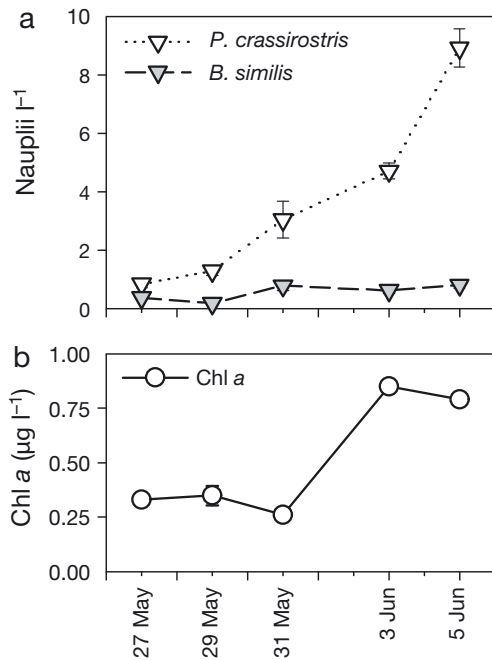
Fig. 2. (a) *In situ* abundances for *Parvocalanus crassirostris* and *Bestiolina similis* nauplii (l^{-1}) in field populations in Kane'ohe Bay on the experimental days, estimated from quantitative PCR, and (b) ambient chl *a* ($\mu g\ l^{-1}$). Error bars: ±SE. Experimental days: 27 May = E1; 29 May = E2; 31 May = E3; 3 Jun = E4; 5 June = E5

bility of decreasing or increasing ingestion rates. The difference between uncorrected and corrected ingestion rates estimated by chl *a* was non-significant for all experiments (*t*-test, p > 0.05), whereas the correction reduced the ingestion rate significantly (33%) for *B. similis* on PEUKs in E2 (*t*-test, p = 0.01; Fig. 3d). The variability observed in the effect of correction is a result of a number of contributing factors: (1) increasing heterotroph availability in the initial prey community from E1 to E5 (2–35 μm EPI heterotrophs plus total ciliates; Table 1), (2) differing ingestion rates by each copepod species on the heterotrophs (Tables S4 & S5 in the Supplement), and (3) differing mortality (*m*) rates of chl *a* and PEUKs removed by the total microzooplankton community across E1 to E5 (*m*; Table S3 in the Supplement). Corrected ingestion rates were used in all subsequent analyses, but negative values were omitted from further analysis.

Ingestion rates on chl *a* and PEUK varied over time from E2 to E6. Ingestion on chl *a* by *P. crassirostris* increased from 0.12 to 0.46 ng chl *a* nauplius^{-1} h^{-1} (corrected ingestion rate; Fig. 3a), while *B. similis* chl *a* ingestion rate increased from 0.06 to 0.46 ng chl *a* nauplius^{-1} h^{-1} (corrected ingestion rate; Fig. 3b). There was no significant trend in PEUK ingestion
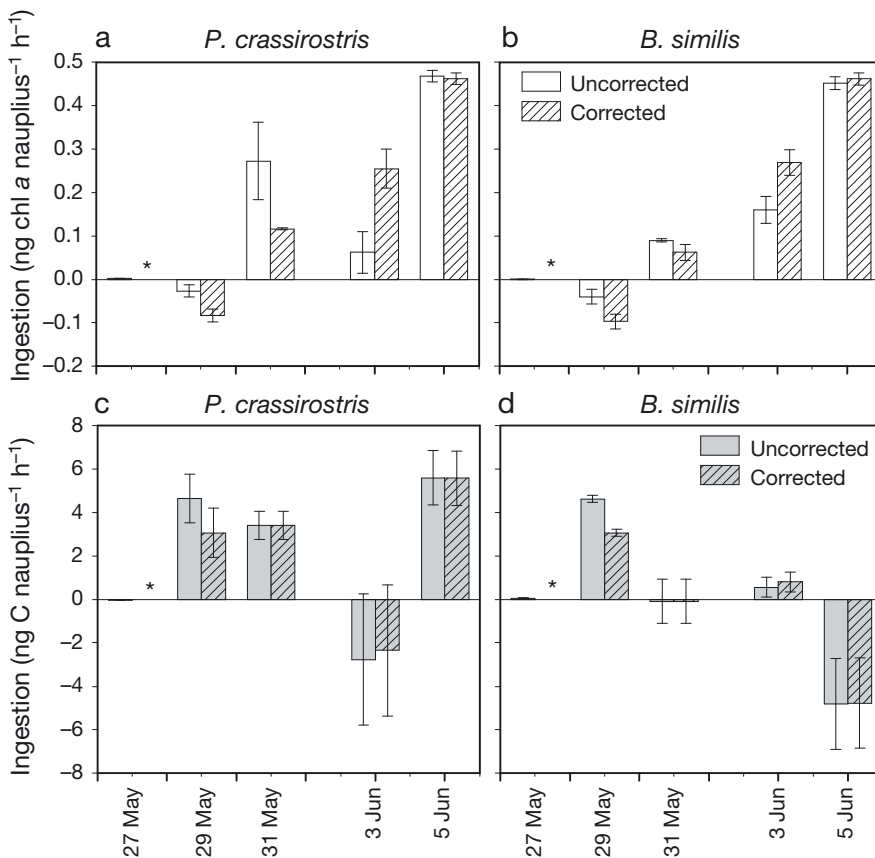

Fig. 3. Average (±SE) ingestion rates of nauplii in each experiment on prey measured as chlorophyll *a* (chl *a*) or on photosynthetic eukaryote carbon (PEUKs) as measured by flow cytometry. Data shown are uncorrected (open) and corrected (hatched) following the method of Nejstgaard et al. (2001). *Parvocalanus crassirostris* (left) and *Bestiolina similis* (right) data on (a,b) ingestion as chl *a* (top, ng chl *a* nauplius^{-1} h^{-1}), and (c,d) ingestion as carbon biomass of PEUKs (bottom; ng C nauplius^{-1} h^{-1}). (*) No correction was available for 27 May, so data are not shown. Experimental days: 27 May = E1; 29 May = E2; 31 May = E3; 3 June = E4; 5 June = E5
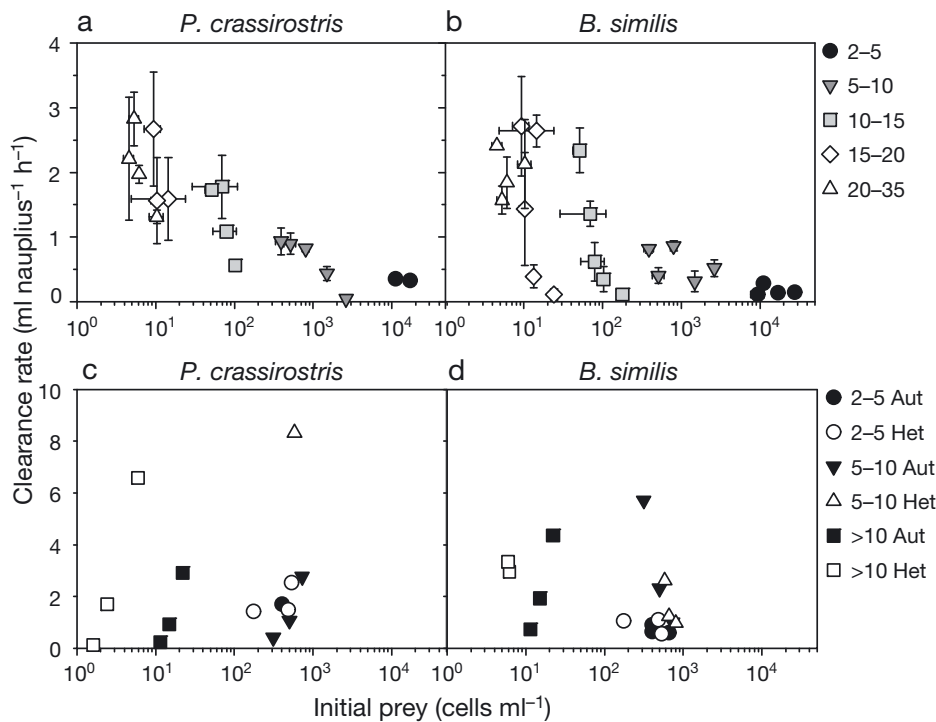
Fig. 4. Average (±SE) clearance rates (ml nauplius^{-1} h^{-1}) for *Parvocalanus crassirostris* (left) and *Bestiolina similis* (right) on (a,b) prey size groups (µm) and (c,d) prey types (Aut: autotrophs, Het: heterotrophs) as a function of initial prey abundance (cells ml^{-1})

over time by *P. crassirostris* (3.06 to 5.57 ng C nauplius^{-1} h^{-1}; Fig. 3c; regression slope = 0, p = 0.99), but *B. similis* ingestion rates showed a significant negative trend (Fig. 3d; regression slope = −0.022, p = 0.04, r^2 = 0.36). These trends give further evidence that nauplii change their prey selection with prey availability.

Clearance rates were generally similar between the 2 species. Mean clearance rates on prey size groups for *P. crassirostris* ranged from 0.04 to 2.83 ml nauplius^{-1} h^{-1} (Fig. 4a) while *B. similis* ranged from 0.11 to 2.71 ml nauplius^{-1} h^{-1} (Fig. 4b), with the highest clearance rates on the largest 20–35 µm prey in both species. Plots of clearance rate as a function of cell abundance in each size fraction resembled the Type 3 functional response (Fig. 4a,b), where clearance rate generally decreases as prey abundance increases within each size group, although prey abundance doesn't approach zero, so we can not determine if a threshold feeding response exists. Clearance rates on prey types generally supported the trends seen in the CC data, however there were instances of high clearance rates on 5–10 µm heterotrophs by *P. crassirostris*, and on 5–10 µm autotrophs by *B. similis* (Fig. 4c,d). By prey types, the maximum average clearance rate measured was slightly higher, up to 8.32 ml nauplius^{-1} h^{-1} for *P. crassirostris* (Fig. 4c) and up to 5.71 ml nauplius^{-1} h^{-1} for *B. similis* (Fig. 4d).

Both species showed positive ingestion of prey of a range of sizes and types in the 5 experiments. Total ingestion rates from CC data ranged from 14.4 to 87.8 ng C nauplius^{-1} h^{-1} for *P. crassirostris* and 25.4 to 73.8 ng C nauplius^{-1} h^{-1} for *B. similis* (Table 2). The 2 species generally had similar ingestion rates on prey size groups but the size groups they removed at the highest rates differed across experiments (Table 2, Fig. 5a,b). *P. crassirostris* ingestion rates were highest on 10–15 µm prey during E2 (23.9 ± 5.7 ng C nauplius^{-1} h^{-1}), whereas *B. similis* ingestion rates were highest on 5–10 µm prey during E5 (26.5 ± 7.3 ng C nauplius^{-1} h^{-1}). Total ingestion rates on prey types (EPI) were comparable to the CC data and ranged from 4.4 to 145.4 ng C nauplius^{-1} h^{-1} in *P. crassirostris* and 12.2 to 63.9 ng C nauplius^{-1} h^{-1} for *B. similis* (Table S5). Ingestion rate estimates on individual prey types were within the range of total ingestion observed on prey size groups, but ingestion estimates on >10 µm autotrophs and heterotrophs were lower than that for >10 µm CC prey (Fig. 5c,d, Table S5).

Diet composition and prey preference

The proportion of prey ingested was compared with the proportion available in the environment, and results suggested that there was selective feeding on larger cells by both species (Fig. 6). While the

Table 2. Ingestion rates (I; ng C nauplius^{-1} h^{-1}) and grazing impacts (as % of initial biomass removed by *in situ* population d^{-1}) on the standing stock of prey size groups (µm) by *Parvocalanus crassirostris* and *Bestiolina similis* in each experiment. Total ingestion rate for each experiment (total I; ng C nauplius^{-1} h^{-1}) and the mean ingestion rate by each species on a given prey size (±SE) are also reported (if only 1 point was available for the calculation, then no SE is reported). (–) Negative ingestion rate. Prey quantified by Coulter counter

										Total
	Prey size groups									
2−5 µm		5−10 µm		10−15 µm		15−20 µm		20−35 µm		I (±SE)
I	% d^{-1}	I	% d^{-1}	I	% d^{-1}	I	% d^{-1}	I	% d^{-1}	
Parvocalanus crassirostris										
E1	–		7.7 (±1.2)	1.2	5.6	1.1	4.4 (±2.1)	1.8	–		17.7 (±3.3)
E2	11.5 (±1.0)	1.1	17.1 (±1.0)	3.0	23.9 (±5.7)	8.2	16.4 (±5.0)	15.5	18.9 (±0.8)	10.9	87.8 (±13.5)
E3	–		8.3 (±1.5)	7.0	17.0 (±1.0)	19.1	8.3 (±4.4)	16.9	9.7 (±1.0)	17.5	43.3 (±7.9)
E4	16.3 (±2.8)	3.5	14.9 (±3.8)	5.0	8.2	7.1	–		12.0 (±0.3)	21.4	51.4 (±6.8)
E5	–		2.4	0.9	–		–		12.0 (±0.8)	23.7	14.4 (±0.8)
Mean	13.9		10.1		13.7		9.7		13.2		
Bestiolina similis										
E1	1.9	0.1	4.0 (±0.8)	0.3	6.7 (±4.0)	0.6	12.8 (±4.2)	2.4	–		25.4 (±8.9)
E2	9.3 (±3.0)	0.1	17.8 (±1.2)	0.4	21.6 (±3.4)	1.0	15.6 (±5.3)	2.0	9.5 (±1.4)	0.7	73.8 (±14.4)
E3	–		7.0 (±0.5)	1.5	19.9 (±2.1)	5.7	4.9 (±2.8)	2.5	7.4	3.4	39.2 (±5.4)
E4	6.5 (±1.2)	0.2	10.1 (±4.7)	0.4	4.7 (±2.2)	0.5	2.5 (±1.2)	0.8	11.1 (±1.8)	2.6	35.0 (±11.1)
E5	10.7 (±2.1)	0.2	26.5 (±7.3)	0.9	2.1	0.2	0.8 (±0.5)	0.2	20.3 (±8.7)	3.6	60.4 (±18.6)
Mean	7.1		13.1		11		7.3		12.1		

smallest prey size group (2−5 µm) dominated the biomass of the initial community across all experiments, the proportion of this prey group ingested was much lower than would be expected with indiscriminate grazing. Prey in the 5−10 µm category tended to fall along the 1:1 trend line, whereas with a few exceptions, larger prey (>10 µm) were above the line, suggesting positive selection (Fig. 6).

Electivity index results confirmed that *B. similis* consistently selected against the 2−5 µm prey (Fig. 7b), while *P. crassirostris* was less selective overall (Fig. 7a). Both species had negative electivities on <10 µm prey, while electivity for >10 µm prey varied among experiments: *B. similis* specifically had stronger negative electivity against 10−15 and 15−20 µm prey when these prey types were more abundant than on
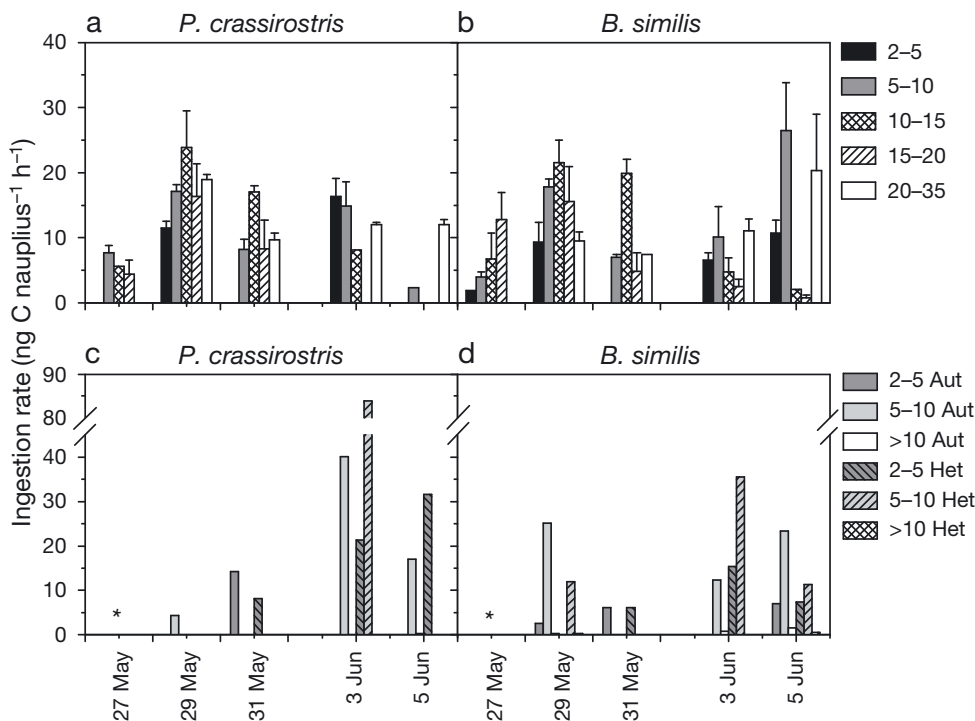

Fig. 5. Average (±SE) ingestion rates (ng C nauplius^{-1} h^{-1}) for *Parvocalanus crassirostris* (left) and *Bestiolina similis* (right) on each experimental date. (a,b) Ingestion on each prey size group (µm) and (c,d) on prey types (Aut: autotroph, Het: heterotroph). (*) Ingestion rates not available for prey types on 27 May. Experimental days: 27 May = E1; 29 May = E2; 31 May = E3; 3 June = E4; 5 June = E5
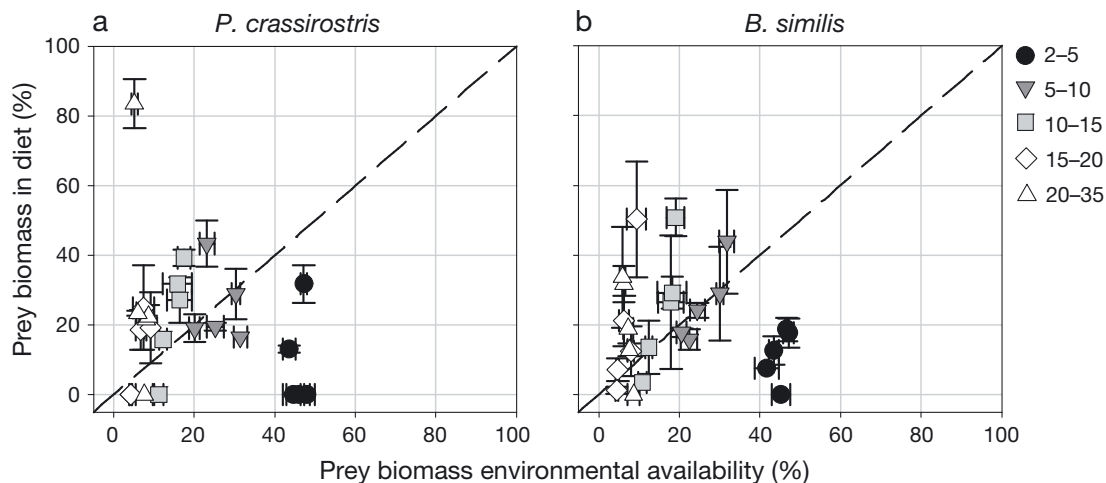
Fig. 6. Average (±SE) percentage of biomass of different prey in naupliar diets (prey in diet; %) compared to the initial prey biomass available in the environment (prey biomass environmental availability; %) by size groups (µm) for (a) *Parvocalanus crassirostris* and (b) *Bestiolina similis.* Compared to their environmental availability, points lying above the 1:1 line indicate selection for the prey item, and those below the 1:1 line indicate selection against a prey item

the other dates (Fig. 7b), while *P. crassirostris* had more neutral electivity on >10 µm prey across all experiments (see Fig. S1a in the Supplement). Selection for or against prey by *B. similis* may change depending on prey availability, as shown by the shift from more positive electivity on 15–20 µm prey in early experiments (E1 and E2) to more positive elec-

tivity on 20–35 µm prey in later experiments (E4 and E5) (Fig. S1b).

An analysis of how electivity indices changed for *B. similis* across experiments showed a significant difference in electivity in the 15–20 (ANOVA; $F_{4,9}$ = 8.94, p = 0.003) and 20–35 µm prey groups across experiments (ANOVA; $F_{3,6}$ = 13.03, p = 0.005). A post
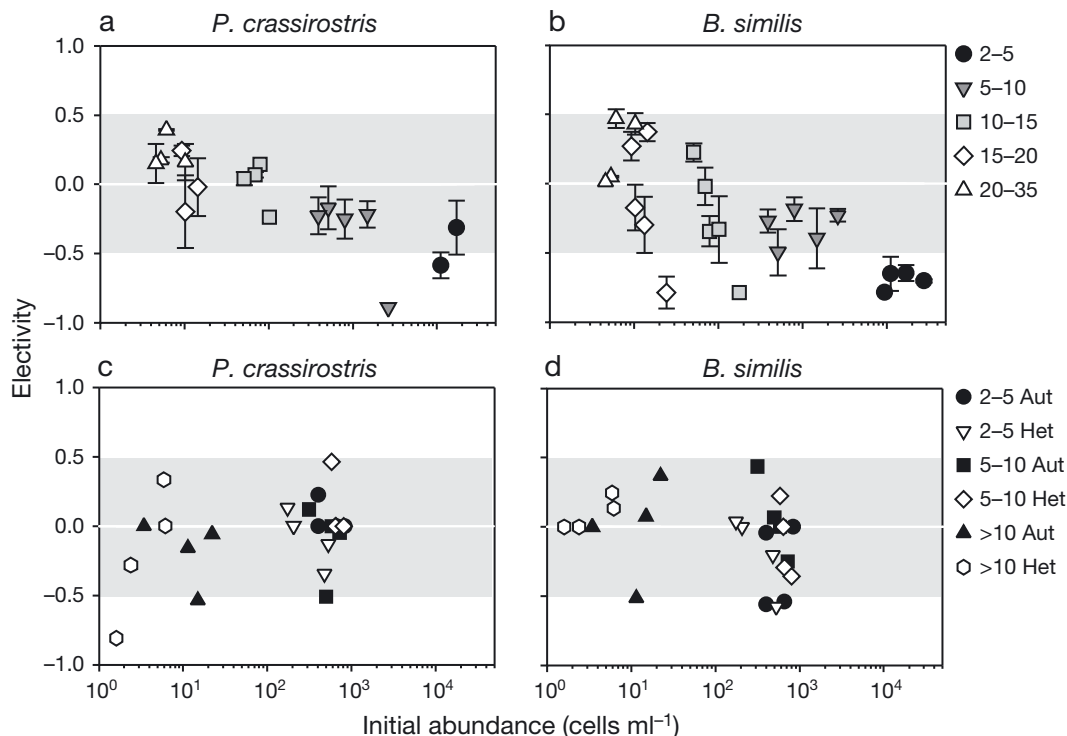

Fig. 7. Electivity index of grazing by each naupliar species compared to prey abundance (cells ml^{-1}). *Parvocalanus crassirostris* (left) and *Bestiolina similis* (right) electivity on (a,b) prey size groups (µm) and (c,d) on prey types (Aut: autotroph, Het: heterotroph). Area outside the shaded grey region: area of strong positive (E > 0.5) or strong negative (E < −0.5) electivity of a prey group (Vanderploeg & Scavia 1979). Error bars: SE

hoc Tukey test found that electivity for the 15–20 μm prey size group was significantly different in E5 versus E2 and E1 (p ≤ 0.007), while electivity of 20–35 μm prey was significantly different in E4 and E5 relative to E2 and E3 (p ≤ 0.04). Despite subtle differences in electivity for prey by *P. crassirostris* (Fig. S1a), there were no significant differences in electivity of any prey size groups over time for this species (ANOVA, p ≥ 0.06)

Results for electivity on prey types (EPI) generally agreed with the results of electivity by prey size (CC). Prey type electivity decreased with increasing initial prey type abundance for <10 μm cells, and electivity increased with increasing cell abundance for >10 μm cells (Fig. 7c,d). Prey type electivity suggested some selection against 2–5 μm prey by *B. similis*, but less strongly than in the prey size data. *B. similis* increased its selection for >10 μm autotrophs over time (Fig. S1d). Both species decreased selection of 2–5 μm heterotrophic prey over time, with the greatest negative electivity during E5 (5 June). Electivity results on prey type also suggested that *P. crassirostris* was less selective than *B. similis*, consistent with results from prey size groups.

Potential grazing impact

Application of ingestion rates to the *in situ* nauplius abundance for each species resulted in a combined total trophic impact for both species ranging from 1.0 to 8.7% of the 2–35 μm prey community across the 10 d experimental period, with the maximum total impact during E3 when initial prey was low relative to E2, E4 and E5 (Table 2). The trophic impact of *P. crassirostris* on total prey was 0.6 to 7.1%, while the impact of *B. similis* was lower, at 0.4 to 1.6%. The greater impact by *P. crassirostris* was primarily due to higher and increasing abundance of this species in the water column over the 10 d period (Fig. 2a). The trophic impact on larger cells was generally higher than that on smaller cells (Fig. 8), particularly by *P. crassirostris*, whose maximum trophic impact was 23.7% of the standing stock of 20–35 μm cells in E5.

The daily trophic impact on prey type standing stocks and on chl *a* was potentially high (Tables S4 & S5). The combined trophic impact by both species on 2–35 μm prey type (EPI) standing stocks from E2 to E5 was 1.7 to 53.5% daily, a majority of which was due to grazing by *P. crassirostris*. The impact of *P. crassirostris* on the total daily prey type biomass was up to 50.6% on E4, while the maximum grazing impact by *B. similis* was 2.9% on E5. The maximum

impact on chl *a* (<35 μm) by *P. crassirostris* was 12.9% during E5 (Table S4, Fig. 8). As expected given their abundance in the water column, trophic impacts on PEUK stocks were relatively low by both species (Table S4). There was no clear trend associating prey size or autotroph versus heterotroph with higher or lower ingestion rates or grazing impacts.

DISCUSSION

When 2 species of copepod nauplii were offered a diverse natural prey assemblage, measured ingestion rates and prey selectivity varied on time scales of 2 to 3 d and differed between the 2 species. These 2 species were shown to be capable of grazing on the same range of prey types and sizes, yet despite being in the same family (Paracalanidae) and having similar body sizes and morphologies, they did not always select for the same prey. When offered the same prey, *Parvocalanus crassirostris* fed more generally, and ingested many prey close to their availability in the environment, while *Bestiolina similis* selected more positively for larger prey and strongly selected against 2–5 μm cells. The 2 species also had very different impacts on prey populations, largely due to the differences in their *in situ* naupliar abundances. The more abundant species, *P. crassirostris*, was capable of removing up to 12.9% of chl *a* standing stock and up to 8.7% of the total 2–35 μm prey. Finally, our results suggest significant trophic impacts of nauplii as grazers in subtropical ecosystems, and future work should include these micrometazoans in studies of food web dynamics in marine systems.

Naupliar ingestion rates

Very few studies of naupliar grazing on natural prey assemblages have been conducted, and in particular, little is known about grazing on diverse prey assemblages such as those found in the subtropics. Previous grazing rate measurements have shown that specific ingestion rates vary widely among species, and that some species impact prey communities significantly, while others do not. Published specific ingestion rates by calanoid copepod nauplii from temperate regions, with cooler water temperatures, slower development times, and less diverse prey range from <0.1 to 2.8 μg-C ingested μg-nauplius C^{-1} d^{-1} (Table 3), most often quantified as grazing on phytoplankton from a mixed assemblage. Our maximum specific ingestion rates on total prey were often
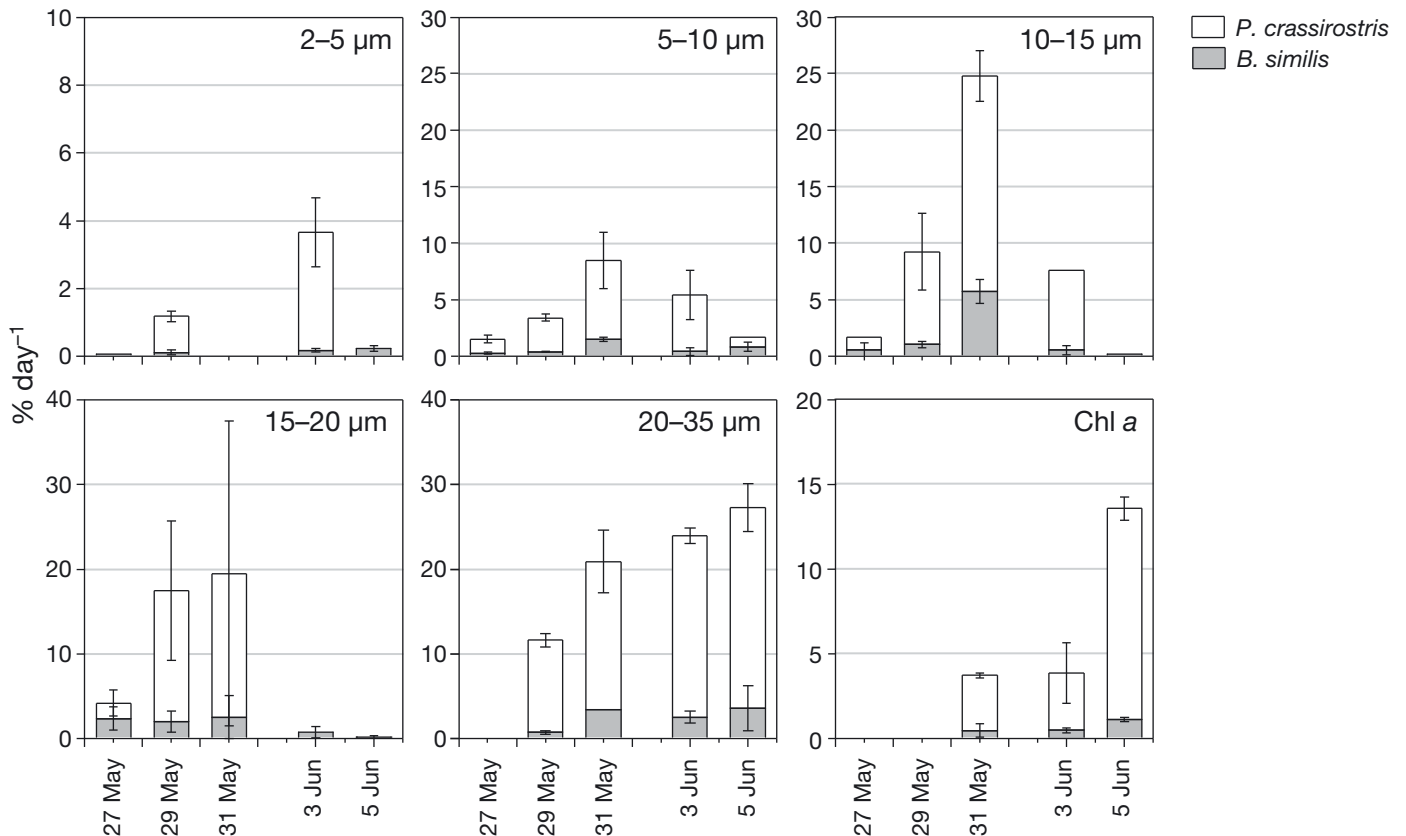
Fig. 8. Daily estimates of prey standing stock removed (% d^{-1}) by *Parvocalanus crassirostris* (white bars) and *Bestiolina similis* (grey bars) nauplii calculated from *in situ* abundance of nauplii and mean ingestion rates measured on each prey size group (data shown in Table 2) and chl *a* (data reported in Table S4 in the Supplement). Note the differing *y*-scales. Experimental days: 27 May = E1; 29 May = E2; 31 May = E3; 3 June = E4; 5 June = E5

higher (Table 3). However, assuming a gross growth efficiency (GGE) of 20%, our rates are only 1.3- to 8-fold higher than expected given metabolic requirements for growth and development based on body carbon, development time, and growth efficiency in this warm-water ecosystem. For instance, *P. crassirostris* N3 and N4 nauplii have a mean carbon content of 57 ng C nauplius^{-1} (Jungbluth 2016), which corresponds well with calculations based on total nauplius length (Berggreen et al. 1988, White & Roman 1992). By length, an N3 nauplius at 50 ng body C requires 155 ng C to grow into an N4 nauplius of approximately 81 ng C (20% GGE; Straile 1997). The stage duration for an N3 to molt to N4 is ~14 h (McKinnon & Duggan 2003), so an N3 nauplius would need to consume 11.1 ng C h^{-1} for 14 h to add 31 ng of carbon to their body weight and molt to the next stage. Our measured rates of total ingestion for *P. crassirostris* (14.4 to 87.8 ng C nauplius^{-1} h^{-1}) and *B. similis* (25.4 to 73.8 ng C nauplius^{-1} h^{-1}; Table 2) modestly exceeded these requirements for growth

and development, despite being high compared to published ingestion rates for nauplii of other species in temperate ecosystems. These calculations suggest that our measured ingestion rates are realistic, and also that nauplii were not food-limited during this study.

There are a number of factors that may also contribute to our comparatively high ingestion rates, many of which are difficult to quantify and not well understood for nauplii. The use of shorter incubation times for our grazing experiments likely resulted in more accurate and higher grazing rate estimates than would have been measured with longer incubations. In a prior study of the impact of incubation time on naupliar ingestion rate estimates, we found that rates measured over 24 h were as much as 75% lower than rates measured over a 6 h incubation (Jungbluth et al. 2017). The significant decline in ingestion rates was attributed to increasingly artificial conditions in bottle incubations due to bottle effects, trophic interactions changing the prey community, and non-feed-

Table 3. Published naupliar specific ingestion (Sp. ingestion) rates on natural prey assemblages, including the results from this study. Total prey biomass (µg C l⁻¹) reported for all prey types quantified. (#) indicates Coulter counter estimates

Nauplius species	Natural prey measured	Total prey biomass ($\mu g\ C\ l^{-1}$)	Sp. ingestion (d^{-1})	Temp (°C)	Incubation time (h)	Source
Calanoids						
Parvocalanus crassirostris	2–35 µm[#]	45–187	6.1–37.2	21	6	This study
Bestiolina similis	2–35 µm[#]	45–187	10.8–31.2	21	6	This study
Parvocalanus crassirostris	2–35 µm	44–67	1.9–61.6	21	6	This study
Bestiolina similis	2–35 µm	44–67	5.2–27.1	21	6	This study
Calanus finmarchicus	Phytoplankton	28–212	0.3	1–12	24	Irigoien et al. (2003)
Calanus spp.	Phytoplankton	5–20	0.0087–0.012	5	23–47	Turner et al. (2001)
Acartia hudsonica	Phytoplankton	300–420	0.8	4–7	0.25–1	White & Roman (1992)
Acartia tonsa	Phytoplankton	300–420	2.8	15–26	0.25–1	White & Roman (1992)
Mixed nauplii	Phytoplankton	15–68	0.1–0.3			Uitto (1996)
Mixed nauplii (50–200 µm)	Whole water	37–950	0.3–1.2	12–24	24	Almeda et al. (2011)
Cyclopoids (*Oithona* spp.)						
O. nana	<125 µm	51–306	0.4–11.1	11–12	24	Böttjer et al. (2010)
O. similis	<100 µm					
O. nana, *O. similis*	<2 µm	14–31	0.5–6.4	11–12	24	Böttjer et al. (2010)

ing during molting. Had the current study used the more common 24 h incubation period, our ingestion rates would have been more similar to prior reports for nauplii but ingestion estimates would not meet the carbon requirements for growth of our species. Other factors that may contribute to higher ingestion rates include sloppy feeding by the nauplii, which may reduce actual ingestion rates by up to 30% (Strom et al. 1997), the potential for feeding at high rates until satiation and then cessation of feeding (e.g. in adults; Mackas & Bohrer 1976, Ishii 1990), or unaccounted-for trophic interactions in the grazing bottles (trophic cascades). Sloppy feeding may be particularly common for nauplii due to their rudimentary feeding appendages.

Natural prey field: methodological considerations

A suite of methods were applied to quantify the potential prey available in the 2–35 µm size range, given the known diversity of potential naupliar prey items in Kaneʻohe Bay (Calbet et al. 2000, Cox et al. 2006). The Coulter counter quantified the full potential suite of prey by size groups from live samples, while the other measurements enabled quantification of predation on other specific prey items (e.g. autotrophs and heterotrophs, ciliates, PEUKs, total phytoplankton as chl *a*) but required preservation prior to quantification, which can result in shrinkage or loss of some types of cells. The limitations to each

method of quantification (Harbison & McAlister 1980, Sherr & Sherr 1993) are the reason why multiple methods were used. One notable discontinuity between live (CC) and preserved methods was that the initial abundance and biomass of prey types in the 2–35 µm prey size range from EPI microscopy were lower than those found with the CC data (Table 1, Fig. 1a). This result was likely due to the loss of small, dim (chlorophyll) cells upon preservation for microscopy, and an inability to accurately distinguish larger prokaryotes from smaller, non-pigmented eukaryotes. FCM data also show that PEUK abundances were an order of magnitude higher (10^4 cells ml⁻¹) than estimates obtained by counting cells on EPI slides (~<10^3 ml⁻¹). Abiotic debris was absent or extremely low in all of the samples, and the CC data are considered to more accurately reflect the total cell counts in our samples.

Estimates of copepod ingestion rate based on bottle incubation experiments are often corrected for microzooplankton grazing by either applying an average microzooplankton grazing rate from the literature (Vargas & González 2004, Huo et al. 2008, Calbet et al. 2009) or by modeling trophic relationships (Klaas et al. 2008; reviewed in Saiz & Calbet 2011). In our study, correction for microzooplankton grazing on chl *a* and PEUKs (FCM; <2 to ~10 µm) was calculated from concurrent seawater dilution measurements, and this correction did not significantly increase or decrease estimates of ingestion on these prey in most experiments. Rather, the correction had variable ef-

fects, increasing or decreasing the ingestion estimates depending on the experiment, likely due to daily changes in the prey community and to different rates of ingestion on, or grazing by, the competing microzooplankton. Therefore, applying an average microzooplankton correction factor for trophic cascades that is derived from literature values is not appropriate for these prey types in Kane'ohe Bay. There is evidence in the CC measurements of microzooplankton grazing that could not be corrected: 4 of the 8 negative ingestion rates we found were on small (2–5 µm) prey (Table 2). It is possible that naupliar feeding on larger microzooplankton released grazing pressure on the small prey, allowing their abundances in treatment bottles to be higher than controls, resulting in negative estimates of ingestion.

Selectivity in naupliar grazing

Selective grazing is well known in adult copepods, but is not well understood in nauplii. Adult copepods can exhibit highly selective feeding behavior, and may select prey based on a range of characteristics, including prey size (Mullin 1963, Marshall 1973, Poulet 1978, Ambler 1986, Hansen et al. 1994), prey motility (Kiørboe et al. 1999, Svensen & Kiørboe 2000), and prey quality (Teegarden 1999, Schultz & Kiørboe 2009) as well as more subtle differences in the nutritional value of prey (e.g. Cowles et al. 1988, Koski et al. 1998). Prior studies of nauplii observed selection for specific prey in a few cases (Fernández 1979, Berggreen et al. 1988, Swadling & Marcus 1994, Meunier et al. 2016), while other studies found no evidence of selectivity in feeding (Allan et al. 1977, Isari et al. 2013). Selectivity by nauplii would be an advantageous trait, because it would allow them to maximize ingestion of nutrients required for growth, which may be critical for survival in these fast-growing subtropical species.

Our results on electivity suggest that both copepod species showed an increase in electivity for large prey as all prey size groups became more abundant in the field, and that *B. similis* was more strongly selective both for and against particular prey than was *P. crassirostris*. The comparison of the proportion of prey in the diet to the prey available in the environment (Fig. 3) also supports the inference of selection for larger prey in both species. The changes in electivity observed across experiments in these 2 species are likely due to switching predation preferences with changing prey availability in the field. The few prior behavioral studies on feeding in nau-

plii suggest 2 different modes of feeding, namely feeding current and ambush feeders, with nauplii exhibiting behaviors broadly similar to adult conspecifics (Titelman & Kiørboe 2003, Henriksen et al. 2007, Bruno et al. 2012). Given the feeding mode of adult *B. similis* and *P. crassirostris*, these nauplii feed using a weak feeding current and would first detect prey when the prey touches the setae on the feeding appendages (Bruno et al. 2012). Such close proximity to prey would enable detection of chemical or size-based cues and facilitate selection for or against prey items by the nauplius. A higher encounter rate with larger cells as they become more abundant could mean that daily caloric requirements could be met by feeding mostly on these preferred cells, thus leading to selection against the abundant small prey.

Comparison of our results to prior studies on adult conspecifics suggests that our nauplii are able to feed on a similar range of prey 2–35 µm in size and have similar selective tendencies as adults under the conditions studied here, particularly in *B. similis*. Calbet et al. (2000) found that ingestion on 2–5 and >5 µm size groups of autotrophic and heterotrophic prey by adults of *P. crassirostris* and *B. similis* (previously reported as *Acrocalanus inermis*) in Kane'ohe Bay also depended on the prey community, and selectivity by adults on prey was stronger for *B. similis* on >5 µm autotrophs when those prey were more abundant, and strong against 2–5 µm heterotrophs despite high abundance of this type (see Fig. 5 in Calbet et al. 2000). Electivity by *P. crassirostris* adults indicated no strong selection against most prey groups, with one event of positive selection for >5 µm autotrophs when overall prey were more abundant. Our results suggest that the general selective or non-selective feeding behavior exhibited in the naupliar stages on 2–35 µm prey continues through to adulthood in these 2 species; however, whether the preferred prey types (e.g. autotroph, heterotroph, or prey species) are the same across development remains unknown. Comparisons of nauplii and adult copepod feeding in other species have found similar selective feeding behavior (Merrell & Stoecker 1998, Finlay & Roff 2004), and similar abilities to feed on a range of prey sizes across developmental stages (e.g. Vogt et al. 2013, Saiz et al. 2014).

Trophic and ecosystem impact of nauplii as grazers

The importance of nauplii as grazers in ecosystems is unclear; previous studies have reported both high and low trophic impacts in a range of environments. For example, *Oithona* spp. nauplii removed up to

54 % of nanoplankton and 21 % of picoplankton off the coast of Chile (Böttjer et al. 2010), and *Eury-temora affinis* nauplii removed up to 56 % of ciliates in Chesapeake Bay (Merrell & Stoecker 1998). A study on micrometazoan community grazing in which the community was dominated by copepod nauplii also found trophic impacts of >40 % on total primary productivity in Great South Bay, Long Island (Lonsdale et al. 1996). However, a number of other studies of this type report insignificant trophic impacts for nauplii (e.g. Verity et al. 1996, Almeda et al. 2011), which were often in environments where phytoplankton standing stocks were much higher than the current study. Low impacts reported for specific naupliar species include *Oithona similis* and *Calanus finmarchicus*, where the copepod populations (including nauplii) had insignificant impacts on prey standing stocks in the Irminger Sea (Castellani et al. 2008), and *Calanus* spp. nauplii, which removed up to only 1.3 % of chl *a* in Disko Bay (Turner et al. 2001).

We found that the trophic impacts of our subtropical nauplii are potentially significant, depending on *in situ* prey densities and naupliar abundance. At the maximum *P. crassirostris* abundance of 8.9 l^{-1} observed during the study, this species removed a significant fraction of the standing stock of some prey: up to 12.4 % of chl *a*, and up to 24 % of 20–35 μm prey during E5. *B. similis*, however, was a less significant grazer, largely due to its lower abundance in the south bay, reaching a maximum abundance of 0.8 nauplii l^{-1} and removing up to 1.1 % of chl *a* (E5) and 5.7 % of the 10–15 μm prey size group (E3). However, the calanoid copepod species studied here made up <1 to 7 % of the total nauplii in Kaneʻohe Bay, with the rest of the naupliar community being dominated by *Oithona* spp. (Jungbluth 2016). Thus, if the ingestion rates and impacts of the cyclopoids are even a small portion of those measured in the current study, the total nauplius population is a substantial contributor to grazing and may exert top-down control on prey populations within Kaneʻohe Bay.

CONCLUSIONS

Nauplii may play particularly important food web roles in subtropical and tropical ecosystems, due to year-round high abundance, rapid rates of growth and development in warm waters, and low standing stocks of phytoplankton. Results reported here show that when copepod nauplii are offered a diverse natural prey assemblage, ingestion rates and prey selectivity can vary on timescales of 2 to 3 d and differ between 2 closely related species, implying complex selectivity in naupliar feeding behavior. Trophic impacts estimated for the 2 calanoid nauplii were also significant, and our results suggest that grazing by the whole naupliar community may exert top-down control on *in situ* prey populations. Future work should include greater consideration of nauplii as potentially important members of the grazer assemblage, and include measurements of abundance and feeding of these micrometazoans.

LITERATURE CITED

Allan JD, Richman S, Heinle DR, Huff R (1977) Grazing in juvenile stages of some estuarine calanoid copepods. Mar Biol 43:317–331

Almeda R, Calbet A, Alcaraz M, Saiz E and others (2011) Trophic role and carbon budget of metazoan microplankton in northwest Mediterranean coastal waters. Limnol Oceanogr 56:415–430

Ambler JW (1986) Formulation of an ingestion function for a population of *Paracalanus* feeding on mixtures of phytoplankton. J Plankton Res 8:957–972

Båmstedt U, Gifford DJ, Irigoien X, Atkinson A, Roman MR (2000) Feeding. In: Harris R, Wiebe PH, Lenz J, Skjoldal HR, Huntley M (eds) ICES zooplankton methodology manual. Academic Press, London, p 297–400

Bendif EM, Probert I, Schroeder DC, de Vargas C (2013) On the description of *Tisochrysis lutea* gen. nov. sp. nov. and *Isochrysis nuda* sp. nov. in the Isochrysidales, and the transfer of *Dicrateria* to the Prymnesiales (Haptophyta). J Appl Phycol 25:1763–1776

Berggreen U, Hansen B, Kiørboe T (1988) Food size spectra, ingestion and growth of the copepod *Acartia tonsa* during development: implications for determination of copepod production. Mar Biol 99:341–352

Böttjer D, Morales CE, Bathmann U (2010) Trophic role of small cyclopoid copepod nauplii in the microbial food web: a case study in the coastal upwelling system off central Chile. Mar Biol 157:689–705

Bruno E, Andersen Borg CM, Kiørboe T (2012) Prey detection and prey capture in copepod nauplii. PLOS ONE 7: e47906

Bucklin A (2000) Methods for population genetic analysis of zooplankton. In: Harris R, Wiebe P, Lenz J, Skjoldal HR, Huntley M (eds) ICES zooplankton methodology manual. Academic Press, London, p 533–570

Calbet A, Landry MR (2004) Phytoplankton growth, microzooplankton grazing, and carbon cycling in marine systems. Limnol Oceanogr 49:51–57

Calbet A, Landry MR, Scheinberg RD (2000) Copepod grazing in a subtropical bay: species-specific responses to a midsummer increase in nanoplankton standing stock. Mar Ecol Prog Ser 193:75–84

Calbet A, Atienza D, Henriksen CI, Saiz E, Adey TR (2009) Zooplankton grazing in the Atlantic Ocean: a latitudinal study. Deep Sea Res II 56:954–963

Campbell L, Vaulot D (1993) Photosynthetic picoplankton community structure in the subtropical North Pacific Ocean near Hawaii (station ALOHA). Deep Sea Res I 40: 2043–2060

Castellani C, Irigoien X, Mayor DJ, Harris RP, Wilson D (2008) Feeding of *Calanus finmarchicus* and *Oithona similis* on the microplankton assemblage in the Irminger Sea, North Atlantic. J Plankton Res 30:1095–1116

Chesson J (1978) Measuring preference in selective predation. Ecology 59:211–215

Cowles TJ, Olson RJ, Chisholm SW (1988) Food selection by copepods: discrimination on the basis of food quality. Mar Biol 100:41–49

Cox EF, Ribes M, Kinzie RA III (2006) Temporal and spatial scaling of planktonic responses to nutrient inputs into a subtropical embayment. Mar Ecol Prog Ser 324:19–35

Drupp P, De Carlo EH, Mackenzie FT, Bienfang P, Sabine CL (2011) Nutrient inputs, phytoplankton response, and CO_2 variations in a semi-enclosed subtropical embayment, Kaneohe Bay, Hawaii. Aquat Geochem 17:473–498

Fernández F (1979) Nutrition studies in the nauplius larva of *Calanus pacificus* (Copepoda: Calanoida). Mar Biol 53: 131–147

Finlay K, Roff JC (2004) Radiotracer determination of the diet of calanoid copepod nauplii and copepodites in a temperate estuary. ICES J Mar Sci 61:552–562

Frost B (1972) Effects of size and concentration of food particles on the feeding behavior of the marine planktonic copepod *Calanus pacificus.* Limnol Oceanogr 17: 805–815

Gifford DJ (1993) Consumption of protozoa by copepods feeding on natural microplankton assemblages. In: Kemp PF, Sherr BF, Sherr EB, Cole JJ (eds) Handbook of methods in aquatic microbial ecology. Lewis Publishers, Boca Raton, FL, p 723–730

Gordon DCJ (1969) Examination of methods of particulate organic carbon analyses. Deep-Sea Res Oceanogr Abstr 16:661–665

Hannides CCS (2007) Seasonal, interannual and decadal variation in zooplankton community structure and function in the North Pacific Subtropical Gyre. PhD dissertation, University of Hawaii at Manoa, Honolulu, HI

Hansen B, Bjornsen PK, Hansen PJ (1994) The size ratio between planktonic predators and their prey. Limnol Oceanogr 39:395–403

Harbison GR, McAlister VL (1980) Fact and artifact in copepod feeding experiments. Limnol Oceanogr 25:971–981

Hart RC (1990) Copepod post-embryonic durations: pattern, conformity, and predictability. The realities of isochronal and equiproportional development, and trends in the copepodid-naupliar duration ratio. Hydrobiologia 206: 175–206

Heinle DR, Harris RP, Ustach JF, Flemer DA (1977) Detritus as food for estuarine copepods. Mar Biol 40:341–353

Henriksen CI, Saiz E, Calbet A, Hansen BW (2007) Feeding activity and swimming patterns of *Acartia grani* and *Oithona davisae* nauplii in the presence of motile and non-motile prey. Mar Ecol Prog Ser 331:119–129

Hoover RS, Hoover D, Miller M, Landry MR, De Carlo EH, Mackenzie FT (2006) Zooplankton response to storm runoff in a tropical estuary: bottom-up and top-down controls. Mar Ecol Prog Ser 318:187–201

Hopcroft R, Roff J (1998) Zooplankton growth rates: the influence of female size and resources on egg production of tropical marine copepods. Mar Biol 132:79–86

Hopcroft RR, Roff JC, Lombard D (1998) Production of tropical copepods in Kingston Harbour, Jamaica: the importance of small species. Mar Biol 130:593–604

Huo YZ, Wang SW, Sun S, Li CL, Liu MT (2008) Feeding and egg production of the planktonic copepod *Calanus sinicus* in spring and autumn in the Yellow Sea, China. J Plankton Res 30:723–734

Irigoien X, Titelman J, Harris RP, Harbour D, Castellani C (2003) Feeding of *Calanus finmarchicus* nauplii in the Irminger Sea. Mar Ecol Prog Ser 262:193–200

Isari S, Ant M, Saiz E (2013) Copepod foraging on the basis of food nutritional quality: Can copepods really choose? PLOS ONE 8:e84742

Ishii H (1990) In situ feeding rhythms of herbivorous copepods, and the effect of starvation. Mar Biol 105:91–98

Jungbluth MJ (2016) Copepod nauplii and their roles in planktonic marine food webs. PhD dissertation, University of Hawaii at Manoa, Honolulu, HI

Jungbluth MJ, Lenz PH (2013) Copepod diversity in a subtropical bay based on a fragment of the mitochondrial COI gene. J Plankton Res 35:630–643

Jungbluth MJ, Goetze E, Lenz PH (2013) Measuring copepod naupliar abundance in a subtropical bay using quantitative PCR. Mar Biol 160:3125–3141

Jungbluth MJ, Selph KE, Lenz PH, Goetze E (2017) Incubation duration effects on copepod naupliar grazing estimates. J Exp Mar Biol Ecol 494:54–62

Kiørboe T, Sabatini M (1995) Scaling of fecundity, growth and development in marine planktonic copepods. Mar Ecol Prog Ser 120:285–298

Kiørboe T, Saiz E, Visser A (1999) Hydrodynamic signal perception in the copepod *Acartia tonsa.* Mar Ecol Prog Ser 179:97–111

Klaas C, Verity PG, Schultes S (2008) Determination of copepod grazing on natural plankton communities: correcting for trophic cascade effects. Mar Ecol Prog Ser 357: 195–206

Koski M, Breteler WK, Schogt N (1998) Effect of food quality on rate of growth and development of the pelagic copepod *Pseudocalanus elongatus* (Copepoda, Calanoida). Mar Ecol Prog Ser 170:169–187

Landry MR, Hassett RP (1982) Estimating the grazing impact of marine micro-zooplankton. Mar Biol 67: 283–288

Lechowicz MJ (1982) The sampling characteristics of electivity indices. Oecologia 52:22–30

Lonsdale DJ, Cosper EM, Kim WS, Doall M, Divadeenam A, Jonasdottir SH (1996) Food web interactions in the plankton of Long Island bays, with preliminary observations on brown tide effects. Mar Ecol Prog Ser 134: 247–263

Mackas D, Bohrer R (1976) Fluorescence analysis of zooplankton gut contents and an investigation of diel feeding patterns. J Exp Mar Biol Ecol 25:77–85

Marshall SM (1973) Respiration and feeding in copepods. Adv Mar Biol 11:57–120

Mauchline J (1998) The biology of calanoid copepods, Vol 33. Academic Press, San Diego, CA

McKinnon AD, Duggan S (2003) Summer copepod production in subtropical waters adjacent to Australia's North West Cape. Mar Biol 143:897–907

McKinnon AD, Duggan S, Nichols PD, Rimmer MA, Semmens G, Robino B (2003) The potential of tropical paracalanid copepods as live feeds in aquaculture. Aquaculture 223: 89–106

Menden-Deuer S, Lessard EJ (2000) Carbon to volume relationships for dinoflagellates, diatoms, and other protist plankton. Limnol Oceanogr 45:569–579

Merrell JR, Stoecker DK (1998) Differential grazing on protozoan microplankton by developmental stages of the calanoid copepod *Eurytemora affinis* Poppe. J Plankton Res 20:289–304

Meunier CL, Boersma M, Wiltshire KH, Malzahn AM (2016) Zooplankton eat what they need: copepod selective feeding and potential consequences for marine systems. Oikos 125:50–58

Meyer B, Irigoien X, Graeve M, Head R, Harris R (2002) Feeding rates and selectivity among nauplii, copepodites and adult females of *Calanus finmarchicus* and *Calanus helgolandicus.* Helgol Mar Res 56:169–176

Monger BC, Landry MR (1993) Flow cytometric analysis of marine bacteria with Hoechst 33342. Appl Environ Microbiol 59:905–911

Mullin MM (1963) Some factors affecting the feeding of marine copepods of the genus *Calanus.* Limnol Oceanogr 8: 239–250

Nejstgaard JC, Naustvoll LJ, Sazhin AF (2001) Correcting for underestimation of microzooplankton grazing in bottle incubation experiments with mesozooplankton. Mar Ecol Prog Ser 221:59–75

Ota AY, Landry MR (1984) Nucleic acids as growth rate indicators for early developmental stages of *Calanus pacificus* Brodsky. J Exp Mar Biol Ecol 80:147–160

Paffenhöfer GA (1971) Grazing and ingestion rates of nauplii, copepodids and adults of the marine planktonic copepod *Calanus helgolandicus.* Mar Biol 11:286–298

Peterson WT (2001) Patterns in stage duration and development among marine and freshwater calanoid and cyclopoid copepods: a review of rules, physiological constraints, and evolutionary significance. Hydrobiologia 453/454:91–105

Poulet SA (1978) Comparison between five coexisting species of marine copepods feeding on naturally occurring particulate matter. Limnol Oceanogr 23:1126–1143

R Core Team (2016) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna

Roff J, Turner J, Webber M, Hopcroft R (1995) Bacterivory by tropical copepod nauplii: extent and possible significance. Aquat Microb Ecol 9:165–175

Roman MR, Rublee PA (1980) Containment effects in copepod grazing experiments: a plea to end the black box approach. Limnol Oceanogr 25:982–990

Saiz E, Calbet A (2007) Scaling of feeding in marine calanoid copepods. Limnol Oceanogr 52:668–675

Saiz E, Calbet A (2011) Copepod feeding in the ocean: scaling patterns, composition of their diet and the bias of estimates due to microzooplankton grazing during incubations. Hydrobiologia 666:181–196

Saiz E, Griffell K, Calbet A, Isari S (2014) Feeding rates and prey:predator size ratios of the nauplii and adult females of the marine cyclopoid copepod *Oithona davisae.* Limnol Oceanogr 59:2077–2088

Schultz M, Kiørboe T (2009) Active prey selection in two pelagic copepods feeding on potentially toxic and nontoxic dinoflagellates. J Plankton Res 31:553–561

Selph KE, Shacat J, Landry MR (2005) Microbial community composition and growth rates in the NW Pacific during spring 2002. Geochem Geophys Geosyst 6:Q12M05

Sharp JH (1974) Improved analysis for 'particulate' organic carbon and nitrogen from seawater. Limnol Oceanogr 19: 984–989

Sherr EB, Sherr BF (1993) Preservation and storage of samples for enumeration of heterotrophic protists. In: Kemp PF, Sherr BF, Sherr EB, Cole JJ (eds) Handbook of methods in aquatic microbial ecology. Lewis Publishers, Boca Raton, FL, p 207–212

Straile D (1997) Gross growth efficiencies of protozoan and metazoan zooplankton and their dependence on food concentration, predator-prey weight ratio, and taxonomic group. Limnol Oceanogr 42:1375–1385

Strickland JD, Parsons TR (1972) A practical handbook of seawater analysis. Fisheries Research Board of Canada, Ottawa

Strom SL, Benner R, Ziegler S, Dagg MJ (1997) Planktonic grazers are a potentially important source of marine dissolved organic carbon. Limnol Oceanogr 42:1364–1374

Svensen C, Kiørboe T (2000) Remote prey detection in *Oithona similis*: hydromechanical versus chemical cues. J Plankton Res 22:1155–1166

Swadling KM, Marcus NH (1994) Selectivity in the natural diets of *Acartia tonsa* Dana (Copepoda: Calanoida): comparison of juveniles and adults. J Exp Mar Biol Ecol 181: 91–103

Teegarden GJ (1999) Copepod grazing selection and particle discrimination on the basis of PSP toxin content. Mar Ecol Prog Ser 181:163–176

Throndsen J (1978) Preservation and storage. In: Sournia A (ed) Phytoplankton manual. UNESCO, Paris, p 69–74

Titelman J, Kiørboe T (2003) Motility of copepod nauplii and implications for food encounter. Mar Ecol Prog Ser 247: 123–135

Turner JT, Tester PA (1992) Zooplankton feeding ecology: bacterivory by metazoan microzooplankton. J Exp Mar Biol Ecol 160:149–167

Turner JT, Levinsen H, Nielsen TG, Hansen BW (2001) Zooplankton feeding ecology: grazing on phytoplankton and predation on protozoans by copepod and barnacle nauplii in Disko Bay, West Greenland. Mar Ecol Prog Ser 221:209–219

Uitto A (1996) Summertime herbivory of coastal mesozooplankton and metazoan microplankton in the northern Baltic. Mar Ecol Prog Ser 132:47–56

Uye Si, Kasahara S (1983) Grazing of various developmental stages of *Pseudodiaptomus marinas* (Copepoda: Calanoida) on naturally occurring particles. Bull Plankton Soc Japan 30:147–158

VanderLugt K, Cooney MJ, Lechner A, Lenz PH (2009) Cultivation of the Paracalanid Copepod, *Bestiolina similis* (Calanoida: Crustacea). J World Aquacult Soc 40:616–628

Vanderploeg HA, Scavia D (1979) Calculation and use of selectivity coefficients of feeding: zooplankton grazing. Ecol Modell 7:135–149

Vargas CA, González HE (2004) Plankton community structure and carbon cycling in a coastal upwelling system. I. Bacteria, microprotozoans and phytoplankton in the diet of copepods and appendicularians. Aquat Microb Ecol 34:151–164

Verity PG, Stoecker DK, Sieracki ME, Nelson JR (1996) Microzooplankton grazing of primary production at 140°W in the equatorial Pacific. Deep Sea Res II 43:1227–1255

Vidal J, Whitledge TE (1982) Rates of metabolism of planktonic crustaceans as related to body weight and temperature of habitat. J Plankton Res 4:77–84

Vogt RA, Ignoffo TR, Sullivan LJ, Herndon J, Stillman JH, Kimmerer WJ (2013) Feeding capabilities and limitations in the nauplii of two pelagic estuarine copepods, *Pseudodiaptomus marinus* and *Oithona davisae.* Limnol Oceanogr 58:2145–2157

Webber MK, Roff JC (1995) Annual structure of the copepod community and its associated pelagic environment off Discovery Bay, Jamaica. Mar Biol 123:467–479

White JR, Roman MR (1992) Seasonal study of grazing by metazoan zooplankton in the mesohaline Chesapeake Bay. Mar Ecol Prog Ser 86:251–261

Yentsch CS, Menzel DW (1963) A method for the determination of phytoplankton chlorophyll and phaeophytin by fluorescence. Deep-sea Res Oceanogr Abstr 10:221–231