FOOD WEB STRUCTURE AND TROPHIC DYNAMICS OF A
SUBTROPICAL PLANKTON COMMUNITY, WITH AN EMPHASIS ON
APPENDICULARIANS

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DEDICATION

This dissertation is dedicated to my family, without whom this work would not have been possible: my mother, Dr. Cynthia Scheinberg; my father and stepmother, Drs. Seymour and Wendy Scheinberg; my sisters, Rachel Birkey and Sandra Scheinberg; my brothers, Daniel Scheinberg and Scott Birkey; my grandparents, Norman and Lucille Trinkle; my uncles, Michael and Paul Trinkle; my aunts, Karen Rodriguez and Patty Steen Trinkle; my hanai aunt, Lietta Wood; and my cousins, Brian Rodriguez, Lisa Garcia and Garrett Trinkle. Thank you for your love and for your support of all of my adventures, scientific and otherwise.
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Food web structure and trophic dynamics of a subtropical plankton community were investigated to assess the ecological importance of metazoan organisms capable of feeding directly on the autotrophic and heterotrophic prokaryotes that dominate tropical waters. Particular emphasis was given to evaluating the efficiency of energy transfer in a short, appendicularian-mediated food chain, and the temporal and spatial variability of appendicularian impact on the microbial community. The feeding capabilities of warm-water appendicularians on natural plankton prey were investigated in Kaneohe Bay and along the northwestern coast of Oahu, Hawai‘i. To provide a specific ecological context for this research, the temporal variability (vertical and spatial) of the plankton community (bacteria to zooplankton) in Kaneohe Bay was also investigated. Assessment of appendicularian rate capabilities and short and long-term variability in plankton community structure, abundance and biomass in Kaneohe Bay allowed for the evaluation of appendicularian importance in a subtropical ecosystem.

Appendicularians play a number of significant roles in the plankton assemblage of Kaneohe Bay – as grazers, competitors and prey – and their importance varies substantially in time and space. Appendicularian grazing impact approaches that of protozoans and is significantly greater than that of copepods. Evidence from long and short term sampling efforts indicates that bottom-up controls may be important, but that predators likely exert the most control on appendicularian populations. Calculations based on appendicularian grazing rates and plankton abundance and biomass in Kaneohe
Bay suggest that even intermittent blooms of appendicularians lead to their dominance of trophic transfer to higher-level consumers (chaetognaths and larval fishes) in these coastal tropical waters.
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CHAPTER 1
INTRODUCTION

Understanding carbon transfer from microbes to top consumers is essential to elucidating food web dynamics, fisheries yield and export production in tropical waters. Tropical pelagic systems typically have complex, multi-level, food webs in which most production originates from, or passes through, photosynthetic or heterotrophic bacteria and is dissipated through multiple levels of a protistan grazing chain (e.g. Azam et al. 1983, Landry & Kirchman 2002). Certain metazoans, like appendicularians, are relatively unique, however, in having the ability to feed directly on bacteria-sized particles, thereby short-circuiting many intermediate trophic links in the microbial food web (e.g., Harbison 1992). These organisms are consequently believed to be disproportionately important in transferring energy to top consumers (Turner et al. 1998).

Appendicularians are small pelagic tunicates that live in mucus houses that they secrete and discard several times daily (Paffenhöfer 1973, Flood & Deibel 1998). The animals beat their tails to pump water through the house and feed on a stream of particles concentrated by a complex filtering structure (Alldredge & Madin 1982, Acuña et al. 1996, Deibel 1998). Their internal filtering structures can retain particles as small as 0.13-μm diameter (Deibel & Lee 1992, Flood et al. 1992). They also have high growth and grazing rates relative to other metazoans (Alldredge & Madin 1982). For instance, in early observations of flow rates using tracer particles, Alldredge (1977) estimated that individuals of the appendicularians Oikopleura longicauda and O. fusiformis could,
respectively, pump 0.86 and 1.7 liters of water through their houses daily. In Caribbean coastal waters, *O. longicauda* has a generation time of about one day and instantaneous growth rates (2.0 ± 0.6 d\(^{-1}\)) that can double biomass in 6 - 12 h (Hopcroft et al. 1998). In contrast, copepods in Kaneohe Bay, Hawaii have maximum clearance rates of only 0.006 l animal\(^{-1}\) d\(^{-1}\) (Calbet et al. 2000) and instantaneous growth rates of 0.05 - 0.41 d\(^{-1}\) (Newbury & Bartholomew 1976).

Appendicularians can also be very abundant, with swarms of up to 3600 ind l\(^{-1}\) (Owen 1966, Alldredge 1982). Although actual clearance rates on naturally occurring prey were unknown prior to the present study, the high flow-rate estimates of Alldredge (1977) suggest that a population density of only 1 animal l\(^{-1}\) would be capable of exerting a grazing impact on bacterial prey populations equivalent to growth rates of one or more cell doublings daily.

This dissertation focuses on appendicularians because of their potential to bridge a relatively wide gap of normally disconnected components of subtropical food webs (i.e. short-circuiting transfer inefficiencies in the microbial community) and the extreme lack of information on these potentially important organisms in tropical waters. The governing hypothesis of this work was that appendicularians serve as a direct link between bacteria-sized primary producers and higher consumers such as chaetognaths and fish, and thus provide an efficient route for the transfer of energy through the food web. Over the course of conducting the research, related subthemes also emerged — such as what regulates the abundance of appendicularians (food and predator relationships)
and how do appendicularians compare to other potentially competing organisms (copepods and protists) as consumers of phytoplankton standing stocks and production?

There are two dominant species of appendicularian in Hawaiian coastal waters, *O. longicauda* and *O. fusiformis*. These species have distinct morphological characteristics with respect to the sizes and structures of their mucus feeding apparatuses. The most remarkable of these differences are the absence of house inlet filters for *O. longicauda* and the larger house to body size ratio of *O. fusiformis*, which is approximately twice that of *O. longicauda*.

While these two species are commonly considered to be warm water species, they (or perhaps closely related sibling species) are found in every ocean except the Arctic, and tend to be among the most abundant appendicularians wherever they occur (Fenaux et al. 1998). In fact, *O. longicauda* is often reported as the dominant appendicularian in both coastal and open-ocean waters (Lohmann & Hentschel 1939, Tokioka 1960, Fenaux 1968b, Fenaux & Dallot 1980, Taguchi 1982, Tomita et al. 2003). In addition to their numerical dominance, Lopez-Urrutia et al. (2003) recently found that *O. longicauda* and *O. fusiformis* dominated appendicularian grazing on phytoplankton in temperate oceanic waters.

Although appendicularians appear to have the potential to play a major trophic role in tropical waters, their relative importance depends both on their abundance and on the composition of the plankton community with which they occur. To provide a specific ecological context for my research, I chose to study the plankton community of Kaneohe Bay, on the island of Oahu, Hawaii. *Oikopleura longicauda* and *O. fusiformis* both occur
in the bay, along with mesozooplanktonic competitors and predators (copepods and chaetognaths), many of which are relatively well known from detailed plankton studies in the 1970s when the bay was impacted by sewage discharge (Newbury & Bartholomew 1976, Peterson 1976, Szyper 1976). The prey assemblage in Kaneohe Bay after the diversion of sewage discharge is typical of tropical coastal systems in the dominance of relatively small cells (Landry et al. 1984, Taguchi & Laws 1987) that fall within the size range readily ingested by appendicularians (Bedo et al. 1993, Fernandez et al. in press). The importance of the microbial community was largely unknown when major plankton studies were conducted in the bay several decades ago. This is, therefore, the first investigation to provide a quantitative analysis of the total plankton community and its variability in the bay using modern methods.

The likely major competitors of appendicularians for food resources in Kaneohe Bay are protozoans and copepods. Protists feed directly on bacteria and small phytoplankton and are known to exert significant grazing pressure in tropical/subtropical systems, including Kaneohe Bay and the adjacent open-ocean (e.g., Landry et al. 1984, Calbet & Landry 1999). Counter to the dominant paradigm regarding copepod feeding, adult copepods in Kaneohe Bay are also able to feed efficiently on small eukaryotic cells within the prey size range consumed by appendicularians (Calbet et al. 2000). Both protists and copepods typically display less temporal and spatial variability than appendicularians, and thus may play a more consistent role in carbon and energy transfer relative to the boom/bust dynamics of appendicularians.
Potential consumers of appendicularians include a wide variety of vertebrate and invertebrate predators. Chaetognaths, commonly referred to as "arrow worms", are a major predator of appendicularians in tropical waters. These ambush predators detect their prey by vibration, making free-swimming appendicularians an excellent target due to the rapid beating of their tails. *Sagitta enflata*, the dominant chaetognath species in Kaneohe Bay, is known to feed selectively on adult copepods and on *O. longicauda*, with a main population mortality impact on the appendicularians (Szyper 1978, Kimmerer 1984). Many temperate and tropical fish also rely on appendicularians as a food source (Last 1978, Clarke 1989, Fenaux et al. 1990).

Changes in habitat quality due to short-term perturbations (e.g., storm runoff) or longer-term climate-related cycles (e.g., warming, drought) may have dramatic effects on the population abundances and ecosystem impacts of appendicularians. Runoff provides significant nutrient inputs to tropical coastal waters, and appendicularians have often been observed to bloom following rain-stimulated periods of increased productivity (E. Parnell pers. comm.). Few studies have investigated the impact of episodic nutrient inputs on tropical coastal plankton communities, but it seems likely that food pulses from nutrient-rich runoff could have significant impacts on appendicularian dynamics because of their explosive growth and reproductive potentials.

The general goal of my dissertation research was to assess the role of appendicularians in the coastal subtropical food web of Kaneohe Bay. I was specifically interested in investigating the rate of energy transfer in a short, appendicularian-mediated food chain, and the temporal and spatial variability of appendicularian impacts. Research
relating to these objectives is presented in five chapters which progress from the study of appendicularian rate capabilities to their roles as grazers, competitors and as prey in a subtropical ecosystem.

In Chapter 2 (Scheinberg and Landry in press), the clearance rates and filtration efficiencies of *Oikopleura fusiformis* on the natural plankton prey assemblage of coastal Oahu, Hawaii are examined in order to evaluate the efficiency of this appendicularian as a trophic intermediary between picoplankton production and higher consumers. Despite its cosmopolitan distribution and its potential to consume the small prey common in tropical systems, very little information was available on its role in tropical marine food webs at the start of the present research (Alldredge 1981, Taguchi 1982, Hopcroft et al 1998, Gorsky et al. 1999). With its small body size and disproportionately large house diameter, *O. fusiformis* may be uniquely adapted to exploit systems characterized by picoplankton dominance and low standing biomass compared to other species that dominate richer temperate and higher latitude systems (e.g., Deibel 1988, Acuña & Keifer 2000, Acuña et al. 2002).

Chapter 3 (Scheinberg et al. accepted) assesses the clearance rates of two co-occurring appendicularian species, *Oikopleura longicauda* and *O. fusiformis*, on microbial prey in Kaneohe Bay, in order to evaluate and compare their roles and to better understand the reasons for their relative abundances in these waters. In addition, this chapter contrasts *O. fusiformis* feeding rates in Kaneohe Bay with rates measured in a more oligotrophic coastal setting (Chapter 2).
Chapter 4 presents data from two annual-scale sampling programs in Kaneohe Bay. These programs were conducted to characterize the major components of the plankton community in the bay, including temporal variability on timescales of weeks to years. In addition, one of the programs sampled along a natural gradient in the bay, from near open-ocean oligotrophy in the north, to coastal mesotrophy in the south (Smith et al. 1981, Laws & Allen 1996, Kinzie et al. 2001), to characterize differences in plankton abundance and composition across a range of trophic conditions. These data provide the background for assessing the relative importance of appendicularians in their natural environment.

Chapter 5 addresses the impact of storm runoff on zooplankton community structure, abundance, and biomass in Kaneohe Bay. Results from long-term sampling in Kaneohe Bay (Chapter 4) suggested that perturbation events, such as storms, might have a significant impact on the zooplankton community, particularly appendicularians. Kaneohe Bay normally is subject to frequent and substantial inputs of new nutrients via storm runoff from the adjacent watersheds (Hoover 2002). The impacts are transient (Ringuet & Mackenzie submitted), however, and traditional long-term sampling methodologies like those employed in Chapter 4 are poorly suited to characterizing short-term changes in plankton community structure and composition. As a result, an intensive sampling program was implemented to investigate the response of the tropical coastal plankton assemblage to inputs of nutrient-enriched storm runoff.

In the final chapter of this dissertation (Chapter 6), elements of chapters 2 through 5 are brought together under three general themes: the regulation of appendicularian
abundance in Kaneohe Bay by food and predators, the relative role of appendicularians as grazers of microbial production, and the relative roles of appendicularians and copepods in trophic transfer to high levels.
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CHAPTER 2
CLEARANCE RATES AND EFFICIENCIES OF OIKOPLEURA FUSIFORMIS
ON THE NATURAL PREY ASSEMBLAGE OF A SUBTROPICAL COASTAL
ECOSYSTEM

INTRODUCTION

Tropical waters have complex multi-level food webs in which most production
originates from, or passes through, photosynthetic or heterotrophic bacteria and is
dissipated through multiple levels of transfer via a protistan grazing chain (e.g., Azam et
al., 1983; Landry and Kirchman, 2002). Certain metazoans, however, with the ability to
feed directly on bacteria-sized particles could short-circuit much of the microbial portion
of the food web, eliminating several inefficient trophic steps (Turner et al., 1998). If so,
such organisms would be disproportionately important to higher level consumers and a
potentially significant energy pathway in tropical plankton systems.

Appendicularians are small pelagic tunicates that might have such capabilities. Their
mucus houses include complex internal filtering structures made of fine glycoprotein and
carbohydrate filaments, which can retain particles as small as 0.13 \( \mu \text{m} \) in diameter (Flood
et al., 1992; Deibel, 1998). Appendicularians are themselves subject to predation by a
wide variety of vertebrates and invertebrates, such as larval fishes and chaetognaths
(Shelbourne, 1962; Kimmerer, 1984; for review see Purcell et al., this volume). The
potential therefore exists for appendicularians to bridge a relatively wide size range of
normally disconnected components of plankton food webs. In addition, appendicularians
contribute to the transport of microbial carbon via discarded houses and rapidly sinking fecal pellets (Gorsky et al., 1984; Fortier et al., 1994; Gorsky et al., 1999).

While previous studies have investigated the rate at which appendicularians can clear particles from seawater, few have reported the efficiencies with which they can retain submicron and micron-sized prey (Deibel and Lee, 1992; Flood et al., 1992; Bedo et al., 1993; Gorsky et al., 1999). Furthermore, most studies have typically utilized proxies for natural prey, such as latex beads, ink particles or cultured phytoplankton. The goal of the present study was to quantify the potential of a subtropical appendicularian, *Oikopleura fusiformis*, in efficient energy transfer of picoplankton production by investigating its clearance rates and filtration efficiencies on the natural community of pico- and nanoplankton. This study is the first to determine the feeding rates of a pelagic tunicate on the broad size spectrum of its natural prey.

*Oikopleura fusiformis*, one of the dominant appendicularians in coastal waters off Oahu, Hawaii (R. Scheinberg, unpubl. data), is primarily a warm-water species, although it has been documented in every ocean but the Arctic (Fenaux et al., 1998). Despite widespread distributions and the potential to consume small particles, very little information is available on its role in the tropical marine food web (Alldredge, 1981; Taguchi, 1982; Hopcroft, 1998a,b; Gorsky et al., 1999). With its small body size and disproportionately large house diameter, *O. fusiformis* may be uniquely adapted to exploit systems characterized by picoplankton dominance and low standing biomass compared to other species that dominate richer temperate and higher latitude systems, such as *O. dioica* and *O. vanhoeffeni* (e.g., Deibel, 1988; Acuña and Keifer, 2000; Acuña et al.,
METHODS

Design of feeding experiments. Grazing impact and particle-retention efficiency of Oikopleura fusiformis were investigated at two sites along the northern and western shores of Oahu, Hawaii (21°28'N, 158°13'W; 21°40'N, 158°03'W) during September 2001. Field experiments were conducted to determine clearance rates on heterotrophic bacteria, Prochlorococcus spp., Synechococcus spp. and autotrophic eukaryotes. At each of the experimental sites, individual appendicularians were gently captured in situ in 265-ml wide-mouth, polycarbonate bottles, approximately 20-m offshore. For each experimental set of 4 bottles with appendicularians, two additional control bottles were collected with ambient seawater without appendicularians. All bottles were incubated onshore in plastic chambers maintained at ambient temperature (28 ± 1° C) by the periodic addition of fresh seawater. Incubation times ranged from 30 to 120 min. Prior to incubation, initial 1-ml aliquots were taken from each bottle, preserved in cryogenic tubes with paraformaldehyde (PFA; 5% final concentration) and frozen in liquid nitrogen for later analysis. Approximately every 30 min., or until the animal no longer maintained a steady feeding current in an inflated house, additional 1-ml aliquots were taken from each bottle. After the experiments were terminated, the bottles were placed on ice and brought back to the laboratory for immediate measurement and identification of animals.

Appendicularians were identified according to Bückmann and Kapp (1975) and Fenaux (1993) and measured to the nearest 20 μm using a Wild M5A stereomicroscope.
with an ocular ruler. The biomass of each appendicularian was estimated from the ash-free dry weight ($W, \mu g$) to trunk length ($TL, \mu m$) relationship of Hopcroft (1998b), $\log W = 4.21 \log TL - 11.35$, as calculated from Alldredge (1976). Biomass was converted to carbon (C) using a C:W ratio of 0.52 (Alldredge, 1981).

**Measurement of clearance rates.** Clearance rates were determined from changes in cell densities relative to controls as measured over the incubation period using flow cytometry (FCM). Frozen 1-ml aliquots from the grazing experiments were thawed and spiked with a mixture of Polysciences Fluoresbrite YG 0.57- and 0.98-\(\mu\)m visible beads to normalize light scatter signals to consistent size references. Subsamples of 400 \(\mu l\) were taken from each 1-ml aliquot prior to bead addition for the enumeration of heterotrophic bacteria. These subsamples were incubated at 37° C for 15 min following the addition of detergent (Triton X-100; 0.1 % final concentration) to increase the permeability of the cell membrane. The aliquots were then stained for 4 h with SYTOX green (5 \(\mu M\) final concentration) so that stained heterotrophic bacteria could be detected at 488 nm (Lebaron et al., 1998). Subsamples of 50 \(\mu l\) from the original 1-ml aliquot and 100 \(\mu l\) from the 400-\(\mu l\) subsample were enumerated individually on a Coulter EPICS 753 flow cytometer equipped with an argon laser and MSDS II automatic sampling. The laser was tuned to 488 nm at 1.3 W to excite the pigments of autotrophic cells and stained heterotrophic bacteria. Picoplankton populations were distinguished from one another by differences in light scatter and fluorescence emission. Heterotrophic bacteria (Hbact) were identified by strong green fluorescence (530 ± 40 nm). *Prochlorococcus* spp. (Pro) were identified primarily by strong red fluorescence (680 ± 40 nm) due to divinyl
chlorophyll a, *Synechococcus* spp. (Syn) were separated by the presence of orange (575 ± 40 nm) fluorescence (phycoerythrin), and autotrophic eukaryotes (Aeiks) were identified by higher forward and right angle light scattering (indices of cell size) and enhanced red fluorescence compared to Pro.

**Prey carbon estimates.** Picoplankton prey abundance was converted to biomass equivalents using cellular carbon values of 11, 32 and 101 fg cell⁻¹ for Hbact, Pro and Syn, respectively (Garrison et al., 2000). The abundance of autotrophic picoeukaryotes (<2 μm in diameter) was calculated by subtracting the total number of >2-μm cells determined by epifluorescence microscopy from the total number of Aeiks in the flow cytometry counts. The biomass of <2-μm eukaryotic cells was estimated by assuming an average cell diameter of 1.5 μm and converting biovolumes (BV, μm³) to biomass (pg C) using the relationship of Eppley et al. (1970): \( \log_{10}(\text{pg C}) = 0.94 \log_{10}(\text{BV}) - 0.60 \) for flagellates. We set an upper limit of 13 μm (maximum diameter) for prey of *Oikopleura fusiformis*, based on the house inlet filter pore size of this species (Flood and Deibel, 1998). Total available prey biomass was therefore determined as the sum of the carbon estimates for picoplankton categories plus those from microscopic analyses of autotrophic and heterotrophic nanoplankton (2 - 13 μm). Nanoplankton subsamples (50ml) from the field experiments were preserved with PFA (4 % final concentration) and stained with the fluorochromes proflavin and DAPI (e.g., Verity and Sieracki, 1993). These samples were then filtered onto 1.0-μm black Poretics polycarbonate membrane filters, mounted on glass slides using low-fluorescence immersion oil and frozen at -80°C C for later analysis. Slides were viewed with a Zeiss epifluorescence microscope coupled
via a ZVS 3-chip CCD video camera connected to a computer. Blue excitation was used to visualize proflavin-stained cytoplasm and autofluorescence, and UV excitation was used for DAPI-stained nuclei. The images were processed using Zeiss Image Pro Plus software to facilitate counting and sizing of all 2 – 13 μm heterotrophic and autotrophic organisms. Cells greater than or equal to 1.9 μm were included in the 2 – 13 μm size range of prey based on reported estimates of cell shrinkage due to preservation and staining (28 - 44 % volume equivalent to 3.5 % cell diameter for nanoflagellates preserved with gluteraldehyde or formalin; Booth, 1987; Børshiem and Bratbak, 1987; Verity et al., 1992). Approximately 100 cells were enumerated and sized at 40X for each sample.

Carbon estimates were derived from measured cell dimensions, appropriate geometric formulae and carbon-to-volume ratios. Biovolumes of autotrophic eukaryotes (BV, μm³) were converted to biomass (pg C) based on Eppley et al. (1970): \( \log_{10}(\text{pg C}) = 0.76 \log_{10}(\text{BV}) - 0.29 \) and \( \log_{10}(\text{pg C}) = 0.94 \log_{10}(\text{BV}) - 0.60 \) for diatoms and flagellates, respectively. These equations do not take into account cell shrinkage due to preservation and thus yield minimum estimates of cell carbon content. In addition to prey abundance and biomass, total chlorophyll \( \text{a} \) concentrations were determined from water collected during the field experiments. Triplicate 25-ml water samples were filtered onto Whatman GF/F glass-fiber filters for standard fluorometric analysis. Filters were extracted in 5 ml of 90 % acetone in the dark at -20° C for 24 h. Chlorophyll \( \text{a} \) was quantified using a TD 700 fluorometer calibrated against HPLC-determined concentrations (Holm-Hansen & Riemann 1978).
Clearance and ingestion rate calculations. Clearance rates were calculated according to the equations of Frost (1972). Clearance rates, defined as the volume of water effectively cleared of a particular particle size by both the house and the animal over time (Deibel, 1998), were determined from the rates of disappearance of cells in experimental incubations relative to controls. Prey populations were divided by size into four groups: Hbact (0.3 – 0.7 μm), Pro (0.6 – 0.8 μm), Syn (0.8 – 1.0 μm), and Aeuks (1.0 - 13.0 μm = sum of pigmented pico- and nanoplanktonic eukaryotes). Clearance rates for heterotrophic eukaryotes (Heuks) were assumed to be equivalent to the measured clearance rates on similarly sized Aeuks, and the biomass components of auto- and heterotrophs were combined (Euks = total < 13-μm eukaryotes) to calculate ingestion rates. Statistically significant differences between mean cell density changes in control and experimental bottles were determined using paired t-tests at α = 0.05. Error was calculated as 95% confidence intervals. Coefficients of variation of replicate FCM abundance estimates averaged 1.5% for natural bacterial concentrations (Monger and Landry, 1993). Experiments with less than a 2% change of any prey category between initial and final samples were considered to have a net change equal to 0.

Ingestion of prey cells by Oikopleura fusiformis was determined indirectly using measured clearance rates and assuming that 30 ± 5% of the particles cleared were retained on the filtering apparatus and not ingested, as determined by Gorsky (1980) and Gorsky et al. (1984) in experimental work with Oikopleura dioica fed Isochrysis galbana and Thalassiosira pseudonana, respectively. In addition, it was assumed that
Synechococcus spp. passed through the appendicularian gut intact (Gorsky et al., 1999) and thus was returned directly to the environment and did not contribute to the nutrition of the animal.

The particle retention efficiency (RE, %) of Oikopleura fusiformis was defined as the clearance rate (volume time$^{-1}$) divided by the filtration rate (volume of water pumped through the house, volume time$^{-1}$) (Deibel, 1998). RE was calculated according to the following equations (adapted from Deibel and Lee, 1992; based on Jacobs, 1974): $RE_p = (VF_p / VF_{tot}) / (VS_p / VS_{tot})$ and $RE = RE_p / RE_{max} \times 100 \%$, where $VF_p$ and $VS_p$ are the concentrations of prey ‘p’ filtered and in suspension, and $VF_{tot}$ and $VS_{tot}$ are the concentrations of total prey filtered and in suspension. $RE_p$ is the particle retention efficiency for a given prey type and $RE_{max}$ is the maximum RE calculated for an individual animal on any one of the prey categories (= 9) enumerated. Statistical significance was determined from paired t-tests comparing the mean particle retention efficiencies on each prey type. These equations assume that RE does not vary with differences in the initial abundances of prey cells. The relationships between trunk length and RE on each prey type were determined for two size groups of O. fusiformis, 0.5 – 0.75 mm and 0.75 – 1.00 mm. Non-paired t-tests were used to determine statistical significance by comparing the means of the RE of each size group for each prey type.

**RESULTS**

**Plankton community composition.** Microscopic identification confirmed that all experimental animals ($n = 50$) were Oikopleura fusiformis. Their trunk lengths ranged
from 0.54 to 1.0 mm over the experimental period, with a mean of 0.72 ± 0.11 mm and an equivalent mean biomass of 2.99 ± 0.65 μg C. Abundance and biomass estimates of appendicularian prey during the experimental period are reported in Table 1. Heterotrophic bacteria dominated the plankton community in both abundance and biomass, with a mean biomass of 11.7 ± 0.1 μg C l⁻¹. The mean biomass values for Pro, Syn and Euks were 0.73 ± 0.11, 0.44 ± 0.05 and 4.53 ± 0.47 μg C l⁻¹, respectively. Margins of error represent the 95% confidence intervals for the means of each prey group over the experimental period (n = 50). Aeucks represented 89% of the total eukaryotic biomass. Chlorophyll a concentration ranged from 0.23 to 0.34 μg l⁻¹, with a mean (standard deviation) of 0.28 ± 0.06 μg l⁻¹.

**Clearance rates and filtration efficiencies.** Clearance rates of *Oikopleura fusiformis* did not differ significantly (p ≤ 0.05) during the experiments and were consistently highest on the largest prey size fraction (Aeucks) (Fig. 1). There was no significant change in prey abundance in the controls over the experimental period (t-test, p ≥ 0.05). The \( RE_{\text{max}} \) clearance rate estimates on Aeucks ranged from 107 to 182 ml indiv⁻¹ h⁻¹, with a mean of 144 ± 21 (n = 50), and were significantly greater than clearance rates on Hbact, Pro and Syn (p = 0.0001, paired t-test; Fig. 2). Clearance rates on the autotrophic prokaryotes, Pro and Syn, were not significantly different (p = 0.5) from one another, with means of 29 ± 11 ml indiv⁻¹ h⁻¹ (3 – 58 ml indiv⁻¹ h⁻¹) and 29 ± 10 ml indiv⁻¹ h⁻¹ (14 – 45 ml indiv⁻¹ h⁻¹), respectively. *Oikopleura fusiformis* cleared the smallest size fraction of prey, heterotrophic bacteria, at a significantly lower rate than the other prey groups (p
Clearance rates on this size fraction ranged from 1 – 9 ml indiv\(^{-1}\) h\(^{-1}\), with a mean of 6 ± 3 ml indiv\(^{-1}\) h\(^{-1}\). The particle retention efficiency of \textit{O. fusiformis} increased with increasing prey size, with a maximum RE of 97 ± 4 \% for 1.0 – 13.0 \(\mu\)m autotrophs. Accordingly, the particle retention efficiency for Euks was significantly greater than for all other prey \((p = 0.0001, \text{Fig. 3})\). Hbact (0.3 – 0.7 \(\mu\)m), which determined total prey abundance, was retained with the lowest efficiency (5 ± 2 \%), compared to the largest and less abundant prey Pro, Syn and Euks. Retention efficiencies for Pro and Syn (26 ± 9 \% and 25 ± 9 \%, respectively) were not statistically different \((p = 0.88, \text{paired } t\text{-test})\).

Particle retention efficiencies calculated using the clearance rate ratio method of Bedo et al. (1993) yielded similar results, with mean REs equivalent to 4, 20, 23 and 97 \% for Hbact, Pro, Syn and Euks, respectively. The limited range in trunk lengths of experimental animals prevented us from determining definitive relationships between appendicularian size and RE. Nonetheless, RE of \textit{Oikopleura fusiformis} was significantly related to size for \textit{Synechococcus} spp. \((p = 0.04, \text{non-paired } t\text{-test})\), and an increasing trend with size was evident for \textit{Prochlorococcus} spp. as well. No relationships were found between trunk length and RE for Hbact or Euks.

**Ingestion rates.** \textit{Oikopleura fusiformis} ingested the majority of its carbon from the largest and least abundant size fraction of planktonic prey, 1 – 13 \(\mu\)m Euks. Both ingestion rates and weight specific ingestion rates on each size fraction were significantly higher for eukaryotic prey on all experimental days \((p = 0.0001, \text{Fig. 4})\). Mean ingestion rates on all prey size fractions ranged from 0.35 – 0.60 \(\mu\)g C indiv\(^{-1}\) h\(^{-1}\), with an overall
mean of $0.48 \pm 0.09 \mu g \text{ C indiv}^{-1} \text{ h}^{-1}$. These rates did not vary significantly within the assumed range of $30 \pm 5\%$ particulate adhesion to the mucus house. The mean daily ration of \textit{O. fusiformis} was $5.0 \pm 1.2 \mu g \text{ C } \mu g \text{ C}^{-1} \text{ d}^{-1}$ (Fig. 5).

**DISCUSSION**

**Clearance rates on nanoplankton.** We report the first clearance rates for the feeding of \textit{Oikopleura fusiformis} on a naturally occurring assemblage of pico- and nanoplankton prey. Alldredge (1977) has previously estimated the clearance rate of \textit{O. fusiformis} from flow rate calculations, and Sato et al. (this volume) has determined feeding rates based on the disappearance of $6-\mu m$ plastic beads. Intuitively, one would expect that these other approaches, with relatively large hydrophobic beads (e.g., Bedo et al., 1993; Monger et al., 1999), and particularly the flow-based estimates, would set an upper bound to the clearance rates for this species. Nonetheless, our estimates for the largest of our prey categories (Aeiks) are more than 2-fold higher than previously reported. The reason for this difference is unknown, but could in part result from the elevated temperature of Hawaiian coastal waters ($3 - 6^\circ \text{ C}$ higher than temperatures in prior experiments) and/or to modest differences in the sizes of experimental animals. The possible ability of appendicularians to taste their food and reject undesirable particles also may impact clearance rates of non-natural proxies for prey (Deibel, 1998). Alternatively, there may be some as yet uncharacterized ecotypical adaptation to enhance food gathering capabilities of local animals under chronically oligotrophic conditions. The clearance rates of \textit{O. fusiformis} are also much higher than previously reported for two other
similarly sized warm-water species, *O. dioica* and *O. longicauda* (Table 2). These disparities could be due to morphological differences in house size. For example, while *O. fusiformis*, *O. dioica* and *O. longicauda* have similar mature trunk lengths (Alldredge, 1977, 1981), the *O. fusiformis* house diameter is approximately twice that of the other two species (Lohmann, 1899; Alldredge, 1977). This difference is approximately equivalent to a 4-fold increase in internal filter diameter for animals of a given size, which might allow for more efficient particle capture.

**Clearance rates on picoplankton.** In addition to high maximum clearance rates, our mean clearance rate data also show much higher rates on sub-micron prey for *Oikopleura fusiformis* than those reported for other appendicularians. At 29° C, *O. fusiformis* cleared total bacterioplankton (≤1.0-μm Hbact, Pro and Syn) from the water column at a mean rate of 29 ± 11 ml indiv⁻¹ h⁻¹, an order of magnitude greater than rates determined for *O. dioica* at 15.0° C by King et al. (1980) using <1.0-μm radiolabeled bacterioplankton. Clearance rates on the individual sub-micron prey groups, Pro (0.6 – 0.8 μm) and Syn (0.8 – 1.0 μm), were also an order of magnitude greater than those calculated for 0.75-μm beads at 13.5° C (Bedo et al., 1993). These differences can be explained by the rejection of non-natural prey by the appendicularian, large differences in experimental temperatures, the disproportionately large house size of *O. fusiformis*, or some combination of these three factors. The clearance rates of *O. fusiformis* on ≤1.0-μm cells are comparable to those of solitary and aggregate forms of the small salp species, *Thalia democratica* (2 – 10 mm), feeding on 0.6 x 2.6 μm heterotrophic bacteria at 30° C (Mullin, 1982). One would expect that the differences in house size and experimental
temperatures discussed above would work to enhance filtration rates of *O. fusiformis* on all prey sizes uniformly. However, the feeding rates of *O. dioica* on 0.2-μm beads at 13.5°C reported by Bedo et al. (1993) are quite similar to our rates on 0.3 – 0.7 μm prey. It could be that physio-chemical properties of 0.2 μm particles, such as hydrophobic and London-van der Waals forces, greatly influence capture or retention efficiencies of submicron particles (see Monger et al., 1999). If that is the case, the bead or ink proxies commonly used to determine feeding rates on bacterial-sized particles may exaggerate the relative importance of natural bacteria as food for pelagic tunicates. While this is likely to some extent, filtration efficiency results considered below suggest that large discrepancies between proxy and natural prey rates are more the aberration than the rule.

**Particle retention efficiency.** The present estimates for the particle retention efficiencies of *Oikopleura fusiformis* on natural prey are consistent with the results of studies of *O. dioica* and *O. vanhoeffeni*, using latex microspheres (Deibel and Lee, 1992; Fernández et al., this volume) (Table 3). Assuming that clearance rates (particles ingested plus particles captured in the house) and retention efficiencies (particles that are both captured and ingested) have comparable size-dependencies, both the large cold-water appendicularian, *O vanhoeffeni*, and its smaller warm-water counterparts appear to be equally efficient in retaining >1.0-μm cells and equally inefficient in retaining submicron particles. In fact, the retention efficiencies of several appendicularian species follow a similar pattern with prey size, with efficiencies typically exceeding 80% for >1.0-μm cells (Table 3). Among larger salp species, there is some indication of a steeper drop-off in retention efficiencies with particle size (e.g., Caron et al., 1989), but the
results are not remarkably different overall from the means for appendicularians. However, none of the studies of salp feeding have directly estimated the clearance efficiencies of particles in the average size range of oceanic Hbact.

In contrast to the many studies describing a strong positive relationship between RE and prey size, Bedo et al. (1993) reported that the retention efficiencies of *Oikopleura dioica* were not size sensitive down to 0.2-µm. This conclusion was based on equivalent clearance rate estimates determined for 0.2 and 0.75-µm beads and >0.9-µm phytoplankton cells. However, their actual data on the retention efficiencies for phytoplankton prey (their Figure 2, p. 6) suggests that <1.0-µm cells were in fact retained with a much lower efficiency (~30 %) than those > 6.0-µm (>80 %) (Table 3). In addition, 0.2-µm beads were ingested with efficiencies 34 - 62 % of those 0.75-µm in size. Thus, based on a more discriminating look at the Bedo et al. (1993) data, it appears to be generally consistent with the trend observed in all other studies.

The similarities between results from the present study with natural picoplankton prey and previous studies with latex microspheres suggest that bead surface chemistry does not grossly alter the probabilities of particle retention. However, more subtle effects could still be possible. For example, for the smallest size category (<0.5 µm) in Table 3, studies with beads typically show efficiencies 2 – 5 fold higher than the 5 % estimates for Hbact in the present study. Moreover, the bead (or ink particle) sizes that were utilized in these studies (0.13 or 0.2 µm) are often much smaller than the nominal mean equivalent diameter (0.5 µm) of oceanic Hbact. Thus, the rate discrepancies (2 - 5X higher for smaller particles) could be substantial when extrapolated to biomass of Hbact consumed
or grazing impact on the bacterial community. Such potential inaccuracies in using non-natural prey proxies are worthy of additional scrutiny. The similarities in retention efficiencies of *Oikopleura fusiformis* and *O. vanhoeffeni* are noteworthy because these species have very different trunk lengths and house sizes. If indeed clearance rates and retention efficiencies provide comparable indices of size selectivity, their similar efficiencies support the notion that the pharyngeal filters retain particles by direct interception onto filter fibers, rather than sieving (Deibel and Lee, 1992). This concept is further supported by the relatively uniform pore sizes of food concentrating filters among species (Flood and Deibel, 1998).

Our short-term experiments could result in underestimates of the particle retention efficiencies of *Oikopleura fusiformis* due to disturbance or increased rate of house abandonment in experimental bottles. Most experimental animals built two new houses per h during the incubations, an elevated rate compared to the 1.14 houses h\(^{-1}\) previously determined for this species at 26° C (Sato et al., 2001). If the experiments had been conducted in larger vessels, we may have seen lower house production rates and an increase in sub-micron retention due to particle aggregation, as described by Deibel (1986) and Flood et al. (1992). These studies reported that particles passing through the pharyngeal filter were not lost from the house, but transferred from the spiracles to the tail chamber and subsequently recycled through the food-concentrating filter. This process would lead to the retention of particles of infinitely small size due to their eventual contact with the fibers on the filter apparatus (Deibel and Lee, 1992). However, recent experiments conducted to determine the particle size retention efficiency of
*Oikopleura dioica* revealed that particles are not recirculated. Instead, particles exit the house via the exit chamber (D. Fernández, A. López-Urrutia, A. Fernández, J. L. Acuña and R. Harris, pers. comm.). This observation, also supported by Fenaux (1986), suggests that our experiments did not underestimate particle retention efficiency despite an increased rate of house abandonment.

**Daily ration.** Weight-specific ingestion estimates for *Oikopleura fusiformis* give a mean consumption rate of ~21% of body carbon h$^{-1}$, or 504% d$^{-1}$. This daily ration is roughly comparable to the 350% d$^{-1}$ calculated for *O. dioica* at the lower temperature of 17° C (Gorsky, 1980). Given Sato et al.’s (2001) house production rate of 1.14 ± 0.15 houses h$^{-1}$ and a house carbon to body carbon ratio of 9.2 ± 0.6% at 26° C, our ingestion rate estimates would leave *O. fusiformis* ~10% of its body carbon h$^{-1}$ for growth, respiration and reproduction. Such an intake could support a biomass doubling time of less than 24 h, as described by Hopcroft and Roff (1995) for appendicularian growth at 29° C, but only if the gross growth efficiency is relatively high (= 40%). However, it is possible that our ingestion rates underestimated carbon consumption due to inaccurate carbon conversion factors, additional sources of particulate food (e.g., detritus), or more efficient consumption of filtered particles than we assumed (i.e., we assumed that only 70 ± 5% of the particles cleared from the water were actually consumed). Thus, while our clearance rates for *O. fusiformis* are relatively high, they yield modest growth estimates relative to the known capabilities of these animals in other tropical systems.

**Implications for carbon transfer through a subtropical food web.** *Oikopleura*
*O. fusiformis* appears to ingest the majority (86%) of its carbon from ≥1-μm eukaryotic cells even though sub-micron cells represent a potential food resource 3-fold higher in biomass. Nonetheless, because they are so abundant, the least efficiently grazed prey category (Hbact) provides 8% of daily carbon intake, the second largest contribution of the four prey groups. Although *O. fusiformis* does not feed as efficiently on heterotrophic bacteria, *Prochlorococcus* spp. and *Synechococcus* spp. as it does on eukaryotic cells, one animal in a liter of seawater can still consume 16% of bacterial standing stock daily. Given that abundances often exceed 2 animals l⁻¹ in coastal Hawaiian waters, *O. fusiformis* could potentially remove over one-third of the bacterial biomass from the water column on a daily basis. Thus, although inefficient grazers on bacterioplankton, they can still function significantly as a direct trophic link from microbes to higher-level consumers. Perhaps not surprisingly given the relative importance of picoplankton in tropical and subtropical oceans, *O. fusiformis* can clear naturally occurring prey in this size range from seawater at rates substantially higher than those of comparably sized appendicularians studied to date, and vastly exceeding the rates of co-occurring copepods (Calbet et al., 2000). When abundant, therefore, *O. fusiformis* may divert significant quantities of picoplankton production around multiple levels of food web inefficiency to higher-level consumers (chaetognaths and larval fishes). Nevertheless, if ingestion correlates with clearance rate, then *O. fusiformis* must derive most of its nutrition from the eukaryotic phytoplankton and thus enjoys no substantial adaptive advantage, relative to temperate species, for enhanced feeding efficiencies on picoplankton.
LITERATURE CITED


Bückmann, A. and Kapp, H. (1975) Taxonomic characteristics used for the distinction of


Fenaux, R., Bone, Q. and Deibel, D. (1998) Appendicularian distribution and


Table 1 Abundances (cells ml\(^{-1}\)), size ranges (\(\mu m, n \geq 100\)), and biomass concentrations (\(\mu g C l^{-1}\)) of appendicularian prey.

<table>
<thead>
<tr>
<th>Prey type</th>
<th>Size</th>
<th>Abundance</th>
<th>Biomass</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hbact</td>
<td>~ 0.5</td>
<td>1.1 (\times) (10^6) ± 1.0 (\times) (10^4)</td>
<td>11.7 ± 0.1</td>
</tr>
<tr>
<td>Pro</td>
<td>~ 0.7</td>
<td>23,000 ± 3,500</td>
<td>0.7 ± 0.1</td>
</tr>
<tr>
<td>Syn</td>
<td>~ 0.9</td>
<td>4,300 ± 490</td>
<td>0.4 ± 0.1</td>
</tr>
<tr>
<td>Aecks</td>
<td>1.0 – 13.0</td>
<td>1,700 ± 230</td>
<td>4.0 ± 0.1</td>
</tr>
<tr>
<td>Euks</td>
<td>1.0 – 13.0</td>
<td>1,800 ± 340</td>
<td>4.5 ± 0.5</td>
</tr>
</tbody>
</table>

Hbact = heterotrophic bacteria, Pro = Prochlorococcus spp., Syn = Synechococcus spp., Aecks = autotrophic eukaryotes, Euks = total autotrophic and heterotrophic eukaryotes
Table 2  Comparison of estimated clearance rates (ml indiv$^{-1}$ h$^{-1}$) for pelagic tunicates (highest to lowest maximal rates) at temperatures $>$ 20° C. Length (mm) represents trunk length for appendicularians, total length for salps and doliolids.

<table>
<thead>
<tr>
<th>Species</th>
<th>Length (mm)</th>
<th>Clearance rate</th>
<th>Temp</th>
<th>Method</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Stegosoma magnum</em></td>
<td>2 - 5.1</td>
<td>627*</td>
<td>23 - 24</td>
<td>Beads (2-15 µm)</td>
<td>Alldredge, 1981</td>
</tr>
<tr>
<td><em>Oikopleura rufescens</em></td>
<td>1.52 ± 0.29</td>
<td>144 - 480</td>
<td>25</td>
<td>Visual - dye</td>
<td>Alldredge, 1977</td>
</tr>
<tr>
<td><em>Oikopleura cornutogastra</em></td>
<td>1.3 ± 0.28</td>
<td>177 - 243</td>
<td>25</td>
<td>Visual - dye</td>
<td>Alldredge, 1977</td>
</tr>
<tr>
<td><em>Oikopleura fusiformis</em></td>
<td>0.72 ± 0.11</td>
<td>107 - 182</td>
<td>29</td>
<td>Natural prey (FCM)</td>
<td>This study</td>
</tr>
<tr>
<td><em>Oikopleura rufescens</em></td>
<td>NA</td>
<td>158*</td>
<td>26</td>
<td>Beads (6-µm)</td>
<td>Sato et al., this vol.</td>
</tr>
<tr>
<td><em>Oikopleura fusiformis</em></td>
<td>NA</td>
<td>78*</td>
<td>26</td>
<td>Beads (6-µm)</td>
<td>Sato et al., this vol.</td>
</tr>
<tr>
<td><em>Oikopleura fusiformis</em></td>
<td>0.55 ± 0.12</td>
<td>72</td>
<td>25</td>
<td>Visual - dye</td>
<td>Alldredge, 1977</td>
</tr>
<tr>
<td><em>Dolioletta gegenbauri</em></td>
<td>2.8 - 6.5</td>
<td>4.8 - 58</td>
<td>26.5</td>
<td>Cultured PP</td>
<td>Gibson &amp; Paffenhöfer, 2000</td>
</tr>
<tr>
<td><em>Oikopleura longicauda</em></td>
<td>NA</td>
<td>39*</td>
<td>26</td>
<td>Beads (6-µm)</td>
<td>Sato et al., this vol.</td>
</tr>
<tr>
<td><em>Oikopleura longicauda</em></td>
<td>1.06 ± 0.21</td>
<td>36</td>
<td>25</td>
<td>Visual - dye</td>
<td>Alldredge, 1977</td>
</tr>
<tr>
<td><em>Thalia democratica</em></td>
<td>2.0 - 8.0</td>
<td>2 - 30</td>
<td>30 - 32</td>
<td>$^{14}$C-PP</td>
<td>Mullin, 1982</td>
</tr>
<tr>
<td><em>Oikopleura dioica</em></td>
<td>0.6 - 1.3</td>
<td>12.5*</td>
<td>23 - 24</td>
<td>Beads (2-15 µm)</td>
<td>Alldredge, 1981</td>
</tr>
</tbody>
</table>

* denotes maximum rate, PP = phytoplankton
Table 3 Comparison of the retention efficiencies (%) of pelagic tunicates on sub-micron and micron-sized prey.

<table>
<thead>
<tr>
<th>Species</th>
<th>Particle Size (μm)</th>
<th>Method</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>&lt; 0.5</td>
<td>0.5 – 1.0</td>
<td>1.0 – 5.0</td>
</tr>
<tr>
<td><em>Oikopleura fusiformis</em></td>
<td>5</td>
<td>25</td>
<td>97</td>
</tr>
<tr>
<td></td>
<td>(0.2-0.5)</td>
<td>(0.75)</td>
<td>(1.0-2.0)</td>
</tr>
<tr>
<td><em>Oikopleura dioica</em> **</td>
<td>10-25</td>
<td>43</td>
<td>54-80</td>
</tr>
<tr>
<td></td>
<td>(0.2-0.5)</td>
<td>(0.75)</td>
<td>(1.0-2.0)</td>
</tr>
<tr>
<td><em>Fritillaria borealis</em></td>
<td>10-25</td>
<td>43</td>
<td>54-80</td>
</tr>
<tr>
<td></td>
<td>(0.2-0.5)</td>
<td>(0.75)</td>
<td>(1.0-2.0)</td>
</tr>
<tr>
<td><em>Oikopleura dioica</em></td>
<td>7-36</td>
<td>58</td>
<td>87</td>
</tr>
<tr>
<td></td>
<td>(0.2-0.5)</td>
<td>(0.75)</td>
<td>(1.0-2.0)</td>
</tr>
<tr>
<td><em>Fritillaria borealis</em></td>
<td>10-25</td>
<td>43</td>
<td>54-80</td>
</tr>
<tr>
<td></td>
<td>(0.2-0.5)</td>
<td>(0.75)</td>
<td>(1.0-2.0)</td>
</tr>
<tr>
<td><em>Oikopleura vanhoeffeni</em></td>
<td>10</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>58-88</td>
<td>91</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(1.3)</td>
<td>(0.6 - 7.0 μm)</td>
</tr>
<tr>
<td><em>Pegea bicaudata</em></td>
<td></td>
<td>&lt;30</td>
<td>60-100</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(&lt;2.5)</td>
<td>(&gt;2.5)</td>
</tr>
<tr>
<td><em>Oikopleura vanhoeffeni</em></td>
<td>10</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>(0.13)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Cyclosalpa affinis</em></td>
<td></td>
<td>&lt;3 – 9</td>
<td>33 - 83</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(0.7-1.0)</td>
<td>(2.0-2.5)</td>
</tr>
<tr>
<td><em>Salpa maxima</em></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Pegea confoederata</em></td>
<td></td>
<td>10 – 40</td>
<td></td>
</tr>
<tr>
<td><em>Cyclosalpa spp.</em></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

() = particle size (μm); * = large; ** = small; Hflag = heterotrophic microflagellates
Fig. 1 Clearance rates of *Oikopleura fusiformis* on heterotrophic bacteria (Hbact), *Prochlorococcus* spp. (Pro), *Synechococcus* spp. (Syn) and 1.0 - 13.0 μm autotrophic eukaryotes (Aeuks) from grazing experiments on Oahu, Hawaii during September 2001. Rates are based on flow cytometric analysis of the rate of cell decline during incubation experiments, and are plotted as daily averages for each prey population. Error bars represent upper 95% confidence intervals of mean estimates. Number of experiments (n) is indicated above each date.

Fig. 2 Mean clearance rates for *Oikopleura fusiformis* on heterotrophic bacteria (Hbact), *Prochlorococcus* spp. (Pro), *Synechococcus* spp. (Syn) and autotrophic eukaryotes (Aeuks). Error bars represent 95% confidence intervals of mean estimates (n = 50). The mean clearance rate for Aeuks was significantly greater than for Syn and Pro (t-test, p < 0.0001), which in turn were significantly greater than for Hbact (t-test, p < 0.0001) but not significantly different from each other (t-test, p > 1.000).

Fig. 3 Mean filtration efficiency (FE) of *Oikopleura fusiformis* on heterotrophic bacteria (Hbact), *Prochlorococcus* spp. (Pro), *Synechococcus* spp. (Syn) and autotrophic eukaryotes (Aeuks). FE was calculated according to the equations for retention efficiency of Deibel and Lee (1992). Error bars indicate upper 95% confidence intervals for the mean estimates (n = 50).
Fig. 4 Ingestion and weight-specific ingestion rates for *O. fusiformis* over the experimental period, in µg C indiv\(^{-1}\) h\(^{-1}\) and µg C µg C\(^{-1}\) h\(^{-1}\), respectively. The mean ingestion rate for the five experiments was 0.48 ± 0.09 µg C indiv\(^{-1}\) h\(^{-1}\) and the mean weight-specific ingestion rate was 0.21 ± 0.04 µg C µg C\(^{-1}\) h\(^{-1}\). Error bars represent upper 95% confidence intervals for the mean estimates (n = 50).

Fig. 5 Mean weight-specific ingestion rates for *O. fusiformis* from grazing experiments on Oahu, Hawaii during September 2001. Heterotrophic bacteria (Hbact), *Prochlorococcus* spp., *Synechococcus* spp. and 1.0 - 13.0 µm eukaryotes were the four prey groups investigated. Eukaryotes are divided into autotrophic (striped) and heterotrophic (solid) categories.
FIGURES

Fig. 1
Fig. 2
Fig. 3
Fig. 5
CHAPTER 3

GRAZING IMPACTS OF TWO WARM WATER APPENDICULARIANS ON THE NATURAL PREY ASSEMBLAGE OF A SUBTROPICAL COASTAL ECOSYSTEM

INTRODUCTION

Subtropical waters have complex multi-level food webs in which most production originates from, or passes through, prokaryotes and is dissipated through multiple levels of transfer via a protistan grazing chain (e.g., Azam et al. 1983, Landry & Kirchman 2002). It has been noted, however, that certain metazoans with the ability to feed directly on bacteria-sized particles could short-circuit much of the microbial portion of the food web, eliminating several inefficient trophic steps (Turner et al. 1998). If so, such organisms would be disproportionately important to higher level consumers and a potentially significant energy pathway in subtropical plankton systems.

Appendicularians are small pelagic tunicates with this short-circuiting potential. Their mucus ‘houses’ include complex internal filtering structures made of fine glycoprotein and carbohydrate filaments, which can retain particles as small as 0.13-\(\mu\)m diameter (Flood et al. 1992, Deibel 1998). They are themselves subject to predation by a wide variety of vertebrates and invertebrates, such as larval fishes and chaetognaths (Shelbourne 1962, Kimmerer 1984, for review see Purcell et al. in press).
Appendicularians therefore bridge a relatively wide size range of normally disconnected components of plankton food webs.

While it has been previously suggested that the large, gelatinous feeding structures of pelagic tunicates are a general adaptation to the low food concentrations of oligotrophic environments (Harbison 1992, Acuña 2001), distinct morphological differences in these structures among appendicularian species (Alldredge 1977) suggest that there may be unique adaptations to particular niches within the oligotrophic environment. Although numerous studies have investigated the rates at which appendicularians can clear and/or ingest particles from temperate, sub-arctic and arctic waters, (Acuña et al. 1999, López-Urrutia et al. 2003, Fernandez et al. in press), only a handful have been conducted with warm water species (Alldredge 1977, 1981; Sato et al. in press) and almost all utilized proxies for natural prey, such as latex beads or ink particles.

The goal of our study was to determine the relative rate capabilities of two common warm water appendicularians, *O. longicauda* and *O. fusiformis*, with respect to grazing on natural prey, and to better understand the reasons for the relative abundances of these two dominant species in oligotrophic, subtropical waters. These species have distinct morphological characteristics, with respect to the sizes and structures of their mucus feeding apparati. The most remarkable of these differences are the unique absence of house inlet filters for *O. longicauda* and the large house to body size ratio of *O. fusiformis*, which is approximately twice that of *O. longicauda* (Figure 1). The comparatively large inlet holes of *O. longicauda* (approximately 1.0 mm, as measured from the scaled line drawing of Alldredge 1977) are almost an order of magnitude greater
in diameter than the 13-μm mesh inlet filters of *O. fusiformis* (Alldredge 1977). *O. longicauda* also possesses a unique mucus hood that covers the entirety of its trunk and may afford it some as yet undetermined advantage during grazing (e.g. Alldredge 1977).

While they are obviously significant structurally, are the morphological differences of these two species functionally important, specifically with respect to their relative feeding capabilities? We would expect that *O. longicauda*’s unobstructed inlets allow them to maximize their feeding rates in extremely oligotrophic environments void of large cells, by reducing potential barriers to water flow and thus the energy required by the animal to pull fresh water into the house. It would then follow that their mucus hood would serve to shield them from the occasional potentially hazardous cell, but would most likely be an insufficient barrier in the instance of a bloom of large plankton. Alternatively, we would imagine that the house inlet filters of *O. fusiformis* function as screens for large cells to prevent internal filter clogging, giving this species an advantage in waters with greater variability in prey size. Ultimately, due to the larger house size and surface area of their internal filters, we would expect the filtering capabilities of *O. fusiformis* to be greater than those of *O. longicauda*. In addition to having a greater surface area for particle collection, the larger filtering apparatus of *O. fusiformis* requires a lower pressure differential across the internal filters, likely neutralizing any resistance to flow caused by the presence of inlet filters, and allowing this species to expend less energy per unit of water cleared than the small-housed, *O. longicauda*. The morphological differences of their houses and their similarities in body size suggest that *O. fusiformis* would be more successful than *O. longicauda* in any body of water.
While these two species are commonly considered to be warm water species, they are found in almost every ocean, with the exception of the Arctic, and tend to be among the most abundant appendicularians wherever they occur (Fenaux et al. 1998). In fact, *O. longicauda* is often reported as the dominant appendicularian in both coastal and open ocean waters (Lohmann & Hentschel 1939, Tokioka 1960, Fenaux 1968b, Fenaux & Dallot 1980, Taguchi 1982, Tomita et al. 2003) and has been observed in swarms of up to 3,600 ind l\(^{-1}\) (Owen 1966, Alldredge 1982). In addition to their numerical dominance, Lopez-Urrutia et al. (2003) recently found that these two species together had the greatest grazing impact among appendicularians on phytoplankton populations in the temperate open ocean. However, based on the relative temporal and spatial distributions of *O. longicauda* and *O. fusiformis*, it appears that they may be maximally successful under different environmental conditions (Acuña & Anadón 1992, Acuña 1994, Fenaux 1998, R. Scheinberg unpubl. data).

We have previously determined that *O. fusiformis*, with its small body size and disproportionately large house, is well adapted for exploiting ultra-oligotrophic waters dominated by picoplankton and with low standing biomass (Chapter 2, Scheinberg & Landry in press). However, despite the success of *O. fusiformis* at our study site along the northwestern coast of Oahu, Hawaii, this species maintains a relatively low abundance compared to *O. longicauda* at other coastal sites. The discrepancy in abundance is particularly apparent in waters with a greater terrestrial influence, higher nutrient concentrations and reduced circulation compared to the open ocean-influenced waters of our previous study. To better understand the ecological importance of these
species in subtropical environments, feeding experiments were conducted in a mesotrophic embayment. Experiments were performed to measure clearance rates on natural picoplankton and nanoplankton prey, and a sampling station was established to determine the temporal variability in relative abundances during the experimental period. Our findings suggest that, contrary to our initial hypotheses, differences in house structure, and possibly tail length, width and musculature, may allow *O. longicauda* to deal better with the higher prey concentrations and episodic blooms characteristic of coastal waters, while *O. fusiformis* appears to be more successful in waters with relatively low concentrations of larger prey. To our knowledge, this is the first study to compare the grazing impacts of these two cosmopolitan, warm-water appendicularians in tropical waters on natural prey assemblages.

**METHODS**

**Study site and sampling protocol.** Figure 2 shows a map of our sampling and experimental study sites in southern Kaneohe Bay, on the northeast coast of Oahu, Hawaii. The sampling station was chosen based on its highly representative nature with respect to the plankton community of the southern Kaneohe basin. Approximately bi-weekly sampling was conducted over a five-month period prior to and following appendicularian feeding experiments (see next section) to determine the relative biomass and abundances of *O. longicauda* and *O. fusiformis*. Gentle vertical tows were made with a 0.5-m diameter (64-μm Nitex mesh) net to minimize damage to the fragile gelatinous animals. The small mesh allowed for the collection of all life history sizes,
beginning with the first developmental stage after hatching (Fenaux 1998). Net tow samples were preserved in the field with borax-buffered formalin (5% final concentration). In the laboratory, a minimum of 100 individuals of each species were measured and counted. Water samples were also collected every 3 m from the surface to 12-m depth using a 1-L Niskin bottle. For chlorophyll-α analyses, 25-ml aliquots were filtered onto Whatman GF/F glass-fiber filters, and filters were extracted in 5 ml of 90% acetone in the dark at -20° C for 24 h. Chlorophyll α was quantified using a TD 700 fluorometer calibrated against HPLC-determined concentrations (Holm-Hansen & Riemann 1978).

**Feeding experiments.** Grazing impacts of *O. longicauda* and *O. fusiformis* were investigated using animals and seawater collected from a deep channel approximately 10 m from shore on Coconut Island in Kaneohe Bay. Field experiments were conducted to determine clearance rates on heterotrophic bacteria, *Synechococcus* spp. and autotrophic eukaryotes. Individual appendicularians were gently captured *in situ* in 265-ml wide-mouth, polycarbonate bottles. Each experiment consisted of 4 bottles with appendicularians and two control bottles with ambient seawater without appendicularians. All bottles were incubated onshore in plastic chambers maintained at ambient temperature (24 ± 1°C) by the periodic addition of fresh seawater to the chamber. Incubation times ranged from 50 to 170 min. Prior to incubation, initial 1-ml aliquots were taken from each bottle, preserved in cryogenic tubes with paraformaldehyde (PFA; 5% final concentration) and frozen in liquid nitrogen for later analysis of picoplankton prey abundance. Additional 1-ml aliquots were taken from each
bottle approximately every 60 min., or until the animal no longer maintained a steady feeding current in an inflated house. Aliquots of 50 mls were also removed from each bottle prior to incubation and upon termination of the experiments. These samples were preserved with PFA (4% final concentration) and stored on ice for later analysis of nanoplankton prey. After the experiments were terminated, the appendicularians were removed from the bottles, preserved (borax-buffered formalin; 5% final concentration) and brought back to the nearby laboratory (Hawaii Institute of Marine Biology) for immediate measurement and identification.

Appendicularians were identified according to Bückmann & Kapp (1975) and Fenaux (1993) and measured to the nearest 20 μm using a Leica MZ 9.5 stereomicroscope with an ocular ruler. Appendicularian trunk length was defined as the distance from the tip of the mouth to the posterior edge of the trunk. Shrinkage of preserved specimens was determined by measuring 50 live animals and then re-measuring them up to one year following preservation. Biomass estimates were determined from the ash-free dry weight (W, µg) to trunk length (TL, µm) relationships of Hopcroft et al. (1998) for O. longicauda, log W = 2.49 log TL - 5.97, and Alldredge (1976) for O. fusiformis, log W = 4.21 log TL - 11.35, as reported in Hopcroft et al. (1998). Biomass was converted to carbon (C) using a C:W ratio of 0.52 (Alldredge 1981). House lengths were estimated using the house length (L) to trunk length (T) relationships of Alldredge (1977), where L = 4.1 T + 1.2 for O. longicauda and L = 9.9 T - 0.4 for O. fusiformis.

**Measurement of clearance rates.** Clearance rates were determined from changes in prey cell densities over the incubation period as measured using flow cytometry (FCM).
Frozen 1-ml aliquots from the grazing experiments were thawed and stained with Hoechst 33342 (0.8 μg ml⁻¹ final concentration) for 30 min before analysis (Monger & Landry 1993). Each aliquot was spiked with a mixture of Polysciences Fluoresbrite YG 0.57- and 0.98-μm visible beads and 0.46-μm UV beads to normalize light scatter signals to consistent size references. Subsamples of 100 μl were enumerated on a Coulter EPICS 753 flow cytometer equipped with dual argon lasers and MSDS II automatic sampling. The lasers were aligned colinearly with the first laser tuned to the UV range to excite Hoechst-stained DNA. The second laser was tuned to 488 nm at 1.0 W to excite the pigments of autotrophic cells. Picoplankton populations were distinguished from one another by differences in light scatter and fluorescence emission. Heterotrophic bacteria (Hbact) showed DNA staining but no red (680 ± 40 nm) fluorescence. *Synechococcus* spp. (Syn) were characterized by the presence of orange (575 ± 40 nm) fluorescence (phycoerythrin), and autotrophic eukaryotes (Aeiks) were identified by higher forward and right angle light scattering (indices of cell size) and enhanced red fluorescence compared to Syn.

**Prey size and carbon estimates.** The size composition of prokaryotic prey was determined using both epifluorescence microscopy and flow cytometry. Subsamples (50-ml) for sizing of Hbact were preserved with PFA (4% final concentration) and stained for 1 min with the fluorochrome acridine orange (AO; 50 μg ml⁻¹ final concentration) (Kirchman et al. 1982). These samples were then filtered onto 0.2-μm black Poretics polycarbonate membrane filters, mounted on glass slides using low-fluorescence immersion oil and viewed with a Zeiss epifluorescence microscope and a ZVS 3-chip
CCD video camera connected to a computer. Blue excitation was used to visualize acridine orange-stained cytoplasm, and the images were processed using Zeiss Image Pro Plus software. To avoid problems in distinguishing Syn from other groups of larger, phycoerythrin-containing cells microscopically, Syn size was determined via FCM analysis of size-fractionated water from Kaneohe Bay. Size and abundances of eukaryotic prey were determined microscopically. Nanoplankton subsamples (50 ml) from field experiments were preserved with PFA (4% final concentration) and stained with the fluorochromes proflavin and DAPI (e.g. Verity & Sieracki 1993). Samples were then filtered onto 1.0-μm black Poretics polycarbonate membrane filters, mounted on glass slides using low-fluorescence immersion oil and frozen at -80°C for later analysis by epifluorescence microscopy. Blue excitation was used to visualize proflavin-stained cytoplasm and autofluorescence, green excitation was used to distinguish phycoerythrin-containing cells and UV excitation was used for DAPI-stained nuclei. The images were processed using Zeiss Image Pro Plus software to facilitate counting and sizing of <13-μm heterotrophic and autotrophic organisms. Cells greater than or equal to 0.9 μm were included in the <13-μm size range of prey based on reported estimates of cell shrinkage due to preservation and staining (28 - 44% volume equivalent to 3.5% cell diameter for nanoflagellates preserved with gluteraldehyde or formalin; Booth 1987, Børsheim & Bratbak 1987, Verity et al. 1992). Approximately 300 cells were enumerated and sized at 400X for each sample.

Picoplankton prey abundance was converted to biomass equivalents using biovolume to carbon conversion factors of 380 fg μm⁻³ (Lee & Fuhrman 1987) and 235 fg μm⁻³.
(Shalapyonok et al. 2001) for Hbact and Syn, respectively. Biovolumes of autotrophic eukaryotes (BV, \( \mu m^3 \)) were converted to biomass (pg C) based on Eppley et al. (1970):

\[
\log_{10}(pg \ C) = 0.76 \log_{10}(BV) - 0.29 \quad \text{and} \quad \log_{10}(pg \ C) = 0.94 \log_{10}(BV) - 0.60
\]

for diatoms and flagellates, respectively. These equations do not take into account cell shrinkage due to preservation and thus yield minimum estimates of cell carbon content. We set an upper limit to prey size of 13 \( \mu \text{m} \) (maximum diameter) for the purposes of a clearance rate comparison between \( O. \ longicauda \) and \( O. \ fusiformis \), based on the house inlet filter pore size of \( O. \ fusiformis \) (Flood & Deibel 1998). This upper limit may have led to an underestimation of the possible impact of \( O. \ longicauda \) on > 13.0-\( \mu \text{m} \) prey. Total available prey biomass was determined as the sum of the carbon estimates for picoplankton categories plus those from microscopic analyses of < 13-\( \mu \text{m} \) autotrophic and heterotrophic nanoplankton.

**Clearance and ingestion rate calculations.** Clearance rates were calculated according to the equations of Frost (1972). Clearance rates, defined as the volume of water effectively cleared of a particular particle size by both the house and the animal over time (Deibel 1998), were determined from the rates of disappearance of cells in experimental incubations relative to controls. Successful experiments were defined as those with actively feeding appendicularians, interpreted as experiments that resulted in a measurable decrease (> 2%) in the largest prey size fraction, Aeucks. This size fraction was chosen as the determining factor based on the results of previous experiments on \( O. \ fusiformis \), which showed that the large cells were retained with maximum efficiency (Chapter 2, Scheinberg & Landry in press). The number of houses produced during the
incubation period was multiplied by the house production time (approx. = 1 min; R.
Scheinberg unpubl. data) and subtracted from the total incubation time to determine net
grazing time. Clearance rates for heterotrophic eukaryotes (Heuks) were assumed to be
equivalent to the measured clearance rates on similarly sized Aeufs, and the biomass
components of auto- and heterotrophs were combined (Euks = total < 13-µm eukaryotes)
to calculate ingestion rates. Statistically significant differences between mean cell
density changes in control and experimental bottles were determined using paired t-tests
at p = 0.05. Coefficients of variation of replicate FCM abundance estimates averaged
1.5% for natural bacterial concentrations (Monger & Landry 1993). Experiments with <
2% change of any prey category between initial and final samples were considered to
have zero net change. Relative clearance efficiency was calculated assuming a maximum
efficiency of 100 % for > 1.0-µm prey (Chapter 2, Scheinberg & Landry, in press).

Ingestion rates of O. longicauda and O. fusiformis were determined indirectly using
measured clearance rates and assuming that 30 ± 5 % of the particles cleared were
retained on the filtering apparatus and not ingested, as determined by Gorsky (1980) and
Gorsky et al. (1984). This number is close to the mean of the range of 0 – 80%
particulate adhesion reported in the literature (Acuña & Keifer 2000). The fraction of
cleared cells that adhere to the mucus house and do not enter the pharynx of the animal
was assumed to be size-independent. In addition, it was assumed that Synechococcus
spp. passed through the appendicularian gut intact (Gorsky et al. 1999) and were thus
returned intact to the environment in fecal pellets without contributing to the nutrition of
the animal. All margins of error represent the 95% confidence intervals for the mean estimates.

RESULTS

**Prey community composition.** Abundance, size and biomass estimates of appendicularian prey during the grazing experiments are reported in Table 1. The smallest size fraction of prey, Hbact, dominated in abundance and was the only prey type for which abundance varied significantly over the experimental period (Figure 3; \( p < 0.001 \)). Although least abundant, small eukaryotes dominated in terms of biomass, with a mean of \( 8.0 \pm 1.1 \, \mu g \, C \, l^{-1} \) for autotrophs and \( 6.6 \pm 2.2 \, \mu g \, C \, l^{-1} \) for heterotrophs. Net growth rates of \( 0.06 \pm 0.02 \, d^{-1} \) and \( 0.03 \pm 0.01 \, d^{-1} \) were measured in experimental controls for heterotrophic bacteria and *Synechococcus* spp., respectively. There was no significant change in prey abundance in the controls for Aeiks (\( p > 0.1 \)). Epifluorescence microscopy and FCM analyzed size-fractionation experiments were used to determine the size range of each prey category in Kaneohe Bay. Prey size varied little over the experimental period. The mean equivalent spherical diameters (ESDs) of \(< 13-\mu m\) eukaryotes were \( 3.6 \pm 0.3, 4.0 \pm 0.1 \) and \( 3.3 \pm 0.2 \) on February 20 (experimental day 1), February 21 (experimental day 2) and March 11 (experimental day 3), respectively. Hbact had a mean ESD of \( 0.5 \pm 0.03 \, \mu m \) and approximately 93% of all Syn in Kaneohe Bay were between 0.8 and 1.0 \( \mu m \) in diameter.

Chlorophyll \( a \) concentrations ranged from \( 0.2 - 1.6 \, \mu g \, l^{-1} \) in Kaneohe Bay between January and May, with a mean of \( 0.8 \pm 0.3 \, \mu g \, l^{-1} \) (\( n = 9 \)). The maximum coincided with
blooms of the large, chain-forming diatom, *Chaetocerus* spp. and the dinoflagellate, *Ceratium* spp. on experimental days 1 and 2, respectively. Experimental day 3 had a comparable chl *a* concentration of 1.3 μg C l⁻¹.

**Appendicularian community composition.** Microscopic analyses confirmed the presence of either *O. longicauda* or *O. fusiformis* in each of the experimental bottles. In addition, analyses of net collected samples from southern Kaneohe Bay established that they were the only two appendicularian species in the bay over the 5-mo sampling period. Details on the size, biomass and abundance of these species are given in Table 2. *O. longicauda* dominated the appendicularian community in terms of abundance and biomass during all three grazing experiments and on 9 out of 10 sampling days (Figure 4). Peak appendicularian abundance (2.6 ind l⁻¹) occurred on experimental day 3. Sizes of the experimental animals, as indicated by trunk length (TL), were representative of the general appendicularian population, with a range of 0.4 – 0.9 mm (mean = 0.7 ± 0.2) and 0.4 – 1.0 mm (mean = 0.8 ± 0.2) for *O. longicauda* and *O. fusiformis*, respectively. Mean biomass was 2.8 ± 1.9 μg C ind⁻¹ for *O. longicauda* and 4.2 ± 2.6 μg C ind⁻¹ for *O. fusiformis*. Despite similarities in TL, adult *O. longicauda* had a wider and more muscular tail than *O. fusiformis* and the mean house length of *O. fusiformis* was almost 2 times larger than that of *O. longicauda* (7.0 ± 1.3 vs. 3.9 ± 0.5 mm).

**Clearance rates.** Appendicularians exhibited feeding during 61% of the total grazing experiments. Failure of the animal to build a new house following capture was the most common reason for the lack of feeding. Only the results of successful experiments are expressed here, i.e., those in which the animal built a new house following capture and
was observed to maintain a steady feeding current. Clearance rates were variable, but did not differ significantly between experimental dates for *O. longicauda* (*n* = 10) and *O. fusiformis* (*n* = 7) (Figure 5) or for either individual or weight-specific rates (*p* > 0.1). Clearance rates are not reported for *O. longicauda* on experimental day 3 due to terminal bloom conditions of this species that prevented the collection of healthy, feeding individuals. *O. longicauda* exhibited their highest clearance rates on the largest size fraction of prey, Aeus, with a maximum rate of 85 ml ind⁻¹ h⁻¹ (mean = 34 ± 18 ml ind⁻¹ h⁻¹), and cleared both Aeus and Syn at significantly higher rates than they cleared Hbact (mean = 12 ± 7 ml ind⁻¹ h⁻¹; *p* < 0.05). Mean clearance rates on Aeus and Syn were not statistically different (*p* > 0.1). In contrast, *O. fusiformis* cleared all prey at statistically indistinguishable rates (range = 6 – 78 ml ind⁻¹ h⁻¹, mean = 30 ± 9 ml ind⁻¹ h⁻¹, *p* > 0.1) (Figure 6). While *O. longicauda* demonstrated a difference in clearance rates depending on prey size, *O. longicauda* and *O. fusiformis* did not clear any of their prey fractions at a statistically different rate from each other (*p* > 0.05). A *p*-value of 0.07 for the comparative clearance rate of Hbact by both species suggests that this smallest size fraction may be an exception. The single rate measured for *O. fusiformis* on experimental day 3 fell within 2 standard deviations of the mean for all prey types on the first two experimental days and is thus included in our results. Our small sample size of small appendicularians did not allow us to determine a significant relationship between trunk length and clearance rate for either species.

**Ingestion estimates.** Based on measured clearance rates and our assumptions regarding size-independent cell losses to house adhesion, *Oikopleura longicauda* and *O. fusiformis*...
*fusiformis* ingested the majority of their carbon from the largest and least abundant size fraction of planktonic prey, 1 – 13 μm Euks. For both species, ingestion rates of eukaryotic prey were significantly higher than for all other prey combined on all experimental days (p < 0.05, Figure 7). Mean rates did not differ significantly over the experimental period (p > 0.1) or between species (p > 0.5). *O. longicauda* had an overall mean ingestion rate of 0.4 ± 0.2 μg C ind⁻¹ h⁻¹, while *O. fusiformis* ingested prey at a mean rate of 0.5 ± 0.2 μg C ind⁻¹ h⁻¹. Weight-specific ingestion rates of *O. longicauda* were lower on the second experimental day, but were not statistically different between days (p > 0.1, n = 10). Specific ingestion rates of *O. fusiformis* did vary significantly, ranging from 0.04 ± 0.03 to 0.3 ± 0.1 μg C μgC⁻¹ h⁻¹ (p < 0.05; n = 7). Nonetheless, the mean daily rations of *O. longicauda* and *O. fusiformis* were not significantly different over the experimental period, at 4.6 ± 2.6 μg C μgC⁻¹ d⁻¹ and 3.7 ± 2.4 μg C μgC⁻¹ d⁻¹, respectively (p > 0.5; Figure 8).

**DISCUSSION**

Our findings represent the first comparative feeding study of small, warm water appendicularians on their natural prey assemblages. In addition, we report the first directly measured clearance rates of one of the most globally abundant oikopleurids, *O. longicauda*, on naturally occurring nano- and picoplankton prey. *O. longicauda* comprised > 80% of the abundance and biomass of appendicularians in Kaneohe Bay over the experimental period. Yet, despite the relative abundances and morphological differences between the two species, *O. longicauda* and *O. fusiformis* cleared their largest
and most nutritious size fraction of prey at statistically indistinguishable rates ($p > 0.5$; Fig. 6) and ingested comparable amounts of total prey carbon per body weight ($p > 0.5$; Fig. 8). It thus appears that their abundance differences might be explained by factors other than their relative feeding capabilities.

**Clearance rates on nanoplankton.** The few studies that have investigated the feeding capabilities of both *O. longicauda* and *O. fusiformis* vary both in methods and results (Alldredge 1977, López-Urrutia et al. 2003) (Table 3). López-Urrutia et al. (2003) found that *O. longicauda* and *O. fusiformis* ingested phytoplankton at comparable rates in temperate waters with chlorophyll $a$ concentrations similar to those in Kaneohe Bay. In contrast, based on *in situ* estimates of water flow-through rates, Alldredge (1977) noted that *O. fusiformis* could process water at twice the rate of *O. longicauda* under warm temperatures and low food conditions. We have similarly found discordant results for these species. In the present experiments in Kaneohe Bay, their feeding rates are obviously very similar. In previous experiments with *O. fusiformis* from the northwest coast of Oahu, however, their clearance rates were substantially higher than in Kaneohe Bay (Chapter 2, Scheinberg & Landry in press).

When normalized for temperature and body size differences, our clearance rate estimates for *O. longicauda* are consistent with previous rate inferences, which appear to vary little among studies (Table 3). In contrast, the normalized clearance rate estimates for *O. fusiformis* vary by almost 5 fold. Of the two species, therefore, at least *O. fusiformis* appears to exhibit variable feeding capability with potentially some dependence on environmental or experimental conditions.
The virtually identical methods employed in our present and previous experiments and the differences in plankton size structure and biomass between our two study sites offer some potential insight into the responses of *O. fusiformis* to environmental conditions. The plankton community of Oahu's northwest coast is typically dominated by sub-micron cells in terms of abundance and biomass, and chlorophyll *a* concentrations are comparable to ultra-oligotrophic waters of the central North Pacific subtropical gyre (mean = 0.23 µg l⁻¹; Chapter 2, Scheinberg & Landry in press; Karl et al. 2002). In contrast, our experiments in Kaneohe Bay were conducted at yearly maximum chlorophyll *a* concentrations (1.6 µg l⁻¹; R. Scheinberg, unpubl. data) during a bloom of relatively large, chain-forming diatoms (*Chaetocerus* spp.) and dinoflagellates (*Ceratium* spp.). Both chlorophyll *a* concentrations and the biomass of < 13.0-µm cells were approximately 4-fold higher in Kaneohe Bay than during our previous study.

Three possible scenarios may have led to the comparatively reduced clearance rates of *O. fusiformis* on large cells in Kaneohe Bay: 1) sub-optimal prey size, 2) feeding saturation at high food concentrations or 3) reduced inlet filter porosity due to clogging. Fernandez et al. (in press) determined that there is an upper limit to the maximum retention efficiency of appendicularians with respect to prey size. In their experiments, retention efficiencies of small and medium *O. dioica* decreased as bead size increased for their largest size range of beads. They explained this result as size selection by the animal's incurrent filter, which at 9 and 12 µm for small and medium animals, respectively, is close to the 13-µm inlet filter of *O. fusiformis*. In the present case, the difference in mean sizes of > 1.0-µm cells between our two study sites (3.5 µm in
Kaneohe Bay vs. 2 μm in Northwest Oahu) was rather small, perhaps contributing to but unlikely to account, in full, for the 3-fold difference in measured clearance rates.

Consequently, prey density may have a greater impact than prey size on the clearance capabilities of *O. fusiformis*. Comparable ingestion rate estimates for *O. fusiformis* between Kaneohe Bay and northwest Oahu, based on like assumptions regarding particle adhesion and digestion, suggest that this species is responding behaviorally to high prey concentrations in Kaneohe Bay. Tiselius et al. (2003) and Acuña & Kiefer (2000) observed such a functional response in the clearance and ingestion rates of *O. dioica*, respectively. While during their experiments this species presented saturation in subarctic and temperate waters at prey concentrations over 3 times those observed during our study, when fit to a Michaelis Menten curve their saturated filtration and ingestion rates (FR\text{max} & I\text{max}) are remarkably similar to *O. fusiformis* ingestion rates in Kaneohe Bay and northwest Oahu (0.16 & 0.20 vs. 0.15 & 0.21 μg C μg C\text{−1} h\text{−1}, respectively). Actual ingestion rates of *O. fusiformis* at both study sites are likely to be even greater as our estimates do not take into account indigestible Syn, which would impact gut processing time, or the utilization of other food sources, such as detritus. Thus, if food-processing constraints are similar between species, it appears that the clearance rate of *O. fusiformis* is limited by a maximum rate of ingestion. However, if our assumptions are unrealistic and differences do exist between Kaneohe Bay and northwest Oahu with respect to particle adhesion and detritus concentration, *O. fusiformis* ingestion rates may not be at a maximum in Kaneohe Bay. While we imagine that the concentration of un-quantified edible particulate organic matter would be greater in mesotrophic Kaneohe Bay, the
percentage of particles that adhere to *O. fusiformis* houses and escape ingestion may be higher in this environment as well. Therefore, although *O. fusiformis* appears to be food saturated in our present study, further research is needed to understand the influence of prey concentration on the feeding rates of this species.

As an alternative explanation, inhibition of appendicularian feeding by large diatoms has been observed by both Acuña et al. (1999) and Knoechel & Steel-Flynn (1989), who found negative relationships between diatom abundance and gut fluorescence and clearance rates, respectively. This inhibition phenomenon was also observed by Lopez-Urrutia et al. (2003) for *O. fusiformis* feeding during periods of increased large cell abundance in temperate waters within the chlorophyll *a* ranges of our experiments. Moreover, *O. fusiformis* was the only species in their study for which the abundance of > 30-μm cells had a strong, statistically significant effect when total ingestion was considered (their Fig. 6). Selander & Tiselius (2003) further determined that behavioral responses to the abundance of large cells, such as a decrease in tail beats min⁻¹ and an increase in tail arrests min⁻¹ (the periodic arrest of tail motion during feeding), contributed to reduced clearance rates. The possibility of filter clogging due to large diatoms and dinoflagellates is consistent with the fact that the dominant genera, *Chaetocerus* spp. and *Ceratium* spp., both have particularly spiny cells or form chains that may stick to, and thus clog, the mucus inlet filters of appendicularians (Tiselius et al. 2003). We thus suggest that the lower clearance rates of *O. fusiformis* on > 1.0-μm cells in Kaneohe Bay, compared to those previously measured off of the northwestern coast of
Oahu, are, at least in part, a consequence of the abundance of large, sticky cells in Kaneohe Bay.

**Clearance rates on picoplankton.** While both *O. longicauda* and *O. fusiformis* clear subtropical waters of a wide range of prey sizes, the efficiency with which they remove different size fractions varies (Fig. 4). Of the two species in Kaneohe Bay, only *O. longicauda* exhibited significantly different clearance efficiencies relative to prey size, retaining Hbact at approximately 36% of the rate for Aeiks (p < 0.05). The variability in prey removal efficiencies cannot be explained by animal size, prey abundance or prey size. The most significant relationships observed were on the experimental day with the highest number of replicates (*O. longicauda*, experimental day 2). These results are similar to what we found previously for *O. fusiformis* in oligotrophic waters off northwest Oahu (n = 50; Chapter 2, Scheinberg & Landry in press), as well as in studies of *O. dioica* and *O. vanhoeffeni* using latex microspheres (Deibel & Lee 1992, Fernández et al. 2003).

The mean clearance rates of *O. fusiformis* and *O. longicauda* on total sub-micron cells in Kaneohe Bay were not significantly different; however, a p value of 0.07 suggests that *O. fusiformis* cleared Hbact at a higher rate. It thus appears that, despite its larger house size, *O. fusiformis* is at least equally efficient as *O. longicauda* at retaining its smallest prey. Deibel & Powell (1987) noted that the pore size of the internal food-concentrating filter tends to be related to trunk length, rather than to house size. The coarser, pharyngeal filters (Deibel & Lee 1992) may also have a similar porosity if particles that pass through this filter are not re-circulated (Fernandez et al. in press).
Since *O. fusiformis* did feed on submicron prey at relatively high rates in Kaneohe Bay, the lack of a clear size-efficiency trend may be less a consequence of altered behavior and more due to physical clogging. While filter clogging may have a direct negative impact on the removal of larger cells from the water column, it may not significantly alter the rate at which *O. fusiformis* feeds on sub-micron prey. Moreover, the cohesive properties of diatom and dinoflagellate exudates may enhance the retention efficiencies for this prey type beyond what would otherwise be expected given their small size. Elevated clearance rates on Hbact in Kaneohe Bay could also reflect a larger size of heterotrophic bacteria or differences in physio-chemical properties of the cells (e.g., Monger et al. 1999). Consequently, it appears that during bloom conditions when large cells are abundant, *O. fusiformis* might have a disproportionate impact on prokaryotes compared to their impact on larger, eukaryotic prey.

**Relative species abundance.** Despite similar feeding capabilities in terms of net food intake, the persistently low abundances of *O. fusiformis* in Kaneohe Bay compared to *O. longicauda* suggest that growth and reproduction of the former is disproportionately impacted by conditions at this site. One possible explanation is predation. *O. fusiformis* may experience a higher mortality rate due to visual predators, such as larval fish, as a result of its larger house size. Further, if its house production time is longer than *O. longicauda*’s, it may spend disproportionately more time out of its house and vulnerable to tactile predators such as chaetognaths. One might imagine, however, that the large house size of *O. fusiformis* would be more effective in protecting its inhabitant from small tactile predators than the relatively tiny house of *O. longicauda*. Overall, therefore,
it is difficult to say for certain whether large house size would be a benefit or a liability with regard to predators.

The daily energy expenditure on house production should also differ for these appendicularian species. Tiselius et al. (2003) found that the addition of large algae during appendicularian feeding experiments resulted in significantly higher rates of house production in *O. dioica*, an appendicularian with an incurrent filter mesh similar to that of *O. fusiformis* (15μm x 15μm) (see also Sato et al., 2001). They concluded that algal blooms may thus negatively impact population growth due to a lifetime increase in energy expenditure for house production with consequently less for gonad size and reproductive output. This energy allocation hypothesis is supported by Troedsson et al. (2002), who found that appendicularians reproduce at smaller sizes and produce fewer oocytes at sub-optimal food conditions. The higher abundance of large cells in Kaneohe Bay, compared to our previous study site in northwest Oahu, may have thus lead to an increase in house production rates for *O. fusiformis*. We examined this hypothesis as a possible explanation for the low abundances of *O. fusiformis* relative to *O. longicauda* by looking at the daily ration of each species.

**Daily ration.** Based on our assumptions with respect to particulate adherence and prey digestion, we estimate that *O. longicauda* and *O. fusiformis* ingest 460% and 370% of body carbon d⁻¹, respectively. These daily rations are similar to the 500% d⁻¹ previously calculated for *O. fusiformis* at 29°C along the northwest coast of Oahu (Chapter 2, Scheinberg & Landry, in press), and the 350% d⁻¹ calculated for *O. dioica* at 17°C (Gorsky 1980). Their similar ingestion rates suggest that basic differences in the
daily carbon expenditures of *O. longicauda* and *O. fusiformis* due to house production may account for the higher relative abundance of *O. longicauda*. Sato et al. (2003) determined that these two species produce approximately the same number of houses per day at these temperatures (21 vs. 24 houses d\(^{-1}\) for *O. longicauda* and *O. fusiformis*, respectively) but that *O. fusiformis* has a house carbon to body weight ratio approximately twice that of *O. longicauda* (10.2 vs. 5.7%). Therefore, under comparable conditions, *O. fusiformis* spends twice as much weight-specific carbon on house production as *O. longicauda*. Where weight-specific ingestion rates of these two species are similar, as in this study, *O. fusiformis* operates at a distinct disadvantage energetically (Fig. 10). If house production rates also increase disproportionately for this species during a phytoplankton bloom, the presence of large, non-consumable prey would have a disproportionately negative effect on *O. fusiformis*. This rough estimate of daily carbon consumption and expenditure illustrates that the cost of house production for *O. fusiformis* could explain its low abundances in mesotrophic coastal waters, such as Kaneohe Bay.

While particle adhesion may not be identical for these species, the larger house and internal filtering structures of *O. fusiformis* make it unlikely that they experience a lower percentage of house-captured prey than *O. longicauda*. House “stickiness” has also been invoked as a reason for the higher accumulation of non-ingested particles in houses of *O. fusiformis* compared to those of *O. longicauda* (Sato et al. 2003).

Given the arguments above, *O. fusiformis* appears to be optimally adapted to a more oligotrophic environment than Kaneohe Bay. Based on their high clearance rates and low
energetic costs, we would expect *O. longicauda* to also thrive in the more oligotrophic waters off northwest Oahu, yet they do not (R. Scheinberg, unpubl. data). An explanation for this may be found in the limited clearance rate capabilities of this small appendicularian. Low variability observed in clearance rates for *O. longicauda* (Table 3) at food concentrations equal or below those in Kaneohe Bay, suggests that this species may have been feeding at maximum or near-maximum rates during our study. The resistance inherent in pulling water through a small area must limit their clearance abilities, despite their more muscular tail. This feeding limitation would explain their inability to dominate the more oligotrophic coastal waters of Hawaii and imply that *O. longicauda* are optimally adapted to mesotrophic waters due to possible filtering constraints at lower prey concentrations.

**Relative grazing impact on the natural prey assemblage of subtropical waters.**

*Oikopleura longicauda* and *O. fusiformis* both ingest the majority of their carbon from > 1-μm eukaryotic cells even though sub-micron cells dominate subtropical waters in abundance. Nonetheless, because they are so abundant, their smallest prey category (Hbact) provides 12 and 22% of daily carbon intake for *O. longicauda* and *O. fusiformis*, respectively, the second largest contribution of the three prey groups. Although *O. longicauda* does not feed as efficiently on heterotrophic bacteria as it does on eukaryotic cells, at the common abundance of one animal per liter of seawater, it can still remove 30% of bacterial standing stock daily. Given that abundances often exceed 2 animals l\(^{-1}\) in Hawaiian coastal waters, *O. longicauda* can potentially clear over 60% of the bacterial biomass from the water column on a daily basis. While *O. fusiformis* is typically less
numerous than *O. longicauda* in Kaneohe Bay, they still reached approximately 1 ind l\(^{-1}\) during our five month investigation. During such a peak, *O. fusiformis* can remove an almost equivalent amount (> 50%) of bacterial standing stock daily compared to *O. longicauda* because of its somewhat higher mean clearance rates on this size fraction. Thus, although both are considered inefficient grazers on bacterioplankton with respect to their maximum clearance rate capabilities, they can still function significantly as a direct trophic link from microbes to higher-level consumers, as well as a rich source of particulate organic material in the marine environment.

Perhaps not surprisingly given the relative importance of picoplankton in tropical and subtropical oceans, *O. longicauda* and *O. fusiformis* can clear naturally occurring prey in this size range at rates substantially higher than those of comparably sized appendicularians studied to date (King et al. 1980, Alldredge 1981, Bedo et al. 1993, Acuña & Kiefer 2000, Broms & Tiselius 2003, López-Urrutia et al. 2003, Fernandez et al. in press), and vastly exceeding the rates of co-occurring copepods (Calbet et al. 2000). However, in coastal subtropical waters, the consistently high abundances of *O. longicauda* makes them a more likely candidate than *O. fusiformis* for transferring significant quantities of picoplankton production around multiple levels of food web inefficiency to higher-level consumers (chaetognaths and fishes).

**CONCLUSIONS**

Acuña (2001) argued that the relatively large mucus houses of appendicularians are an adaptation for survival in oligotrophic waters. The fine incident filters of *O.
fusiformis, which prohibit the entrance of extremely small, > 13-μm cells, further illustrates this point. However, our results indicate that adaptations beneficial in ultra-oligotrophic waters may also serve as a hindrance to survival in less oligotrophic environments. By lacking incumbent filters and allocating very little carbon (as percent body weight) to house production, O. longicauda appears to be better adapted to exploiting the episodic increases in prey abundance and biomass in subtropical coastal waters, such as Kaneohe Bay, which are subject to variable nutrient loading and associated phytoplankton blooms. In contrast, with their more fragile and costly houses, O. fusiformis appears to be specifically adapted for extremely oligotrophic conditions.
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Table 1 Mean abundances (cells ml\(^{-1}\)), size ranges (\(\mu m\), \(n \geq 100\)), and biomass concentrations (\(\mu g C l^{-1}\)) of appendicularian prey. The abundance and biomass of Hbact was significantly different (\(p < 0.001\)) over two of the experimental days and is thus presented as a range of values. Error terms represent the 95\% confidence intervals for mean estimates.

<table>
<thead>
<tr>
<th>Prey type</th>
<th>Size</th>
<th>Abundance</th>
<th>Biomass</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hbact</td>
<td>0.3 – 0.7</td>
<td>2.5 – 4.6x10(^5)</td>
<td>2.7 – 8.7</td>
</tr>
<tr>
<td>Syn</td>
<td>0.8 – 1.0</td>
<td>1,330 ± 890</td>
<td>1.4 ± 0.1</td>
</tr>
<tr>
<td>Aeuks</td>
<td>1.0 – 13.0</td>
<td>1,720 ± 120</td>
<td>8.0 ± 1.1</td>
</tr>
<tr>
<td>Heuks</td>
<td>1.0 – 13.0</td>
<td>1,970 ± 760</td>
<td>6.6 ± 2.2</td>
</tr>
</tbody>
</table>

Hbact = heterotrophic bacteria, Syn = *Synechococcus* spp., Aeuks = autotrophic eukaryotes, Heuks = heterotrophic eukaryotes
Table 2  Size distribution, weight, house diameter, filter mesh, abundances and biomass of the two species of appendicularian in Kaneohe Bay, Hawaii. Mean values with 95% confidence limits are given in parentheses. For size and abundance, n ≥ 100. Tail width represents the tail muscle band. Individual biomass and house lengths were calculated from trunk length (TL) according to the relationships of Alldredge 1976 and 1977, respectively.

<table>
<thead>
<tr>
<th>Species</th>
<th>Oikopleura longicauda</th>
<th>Oikopleura fusiformis</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Trunk length (mm)</strong></td>
<td>0.4 – 0.9</td>
<td>0.4 – 1.0</td>
</tr>
<tr>
<td></td>
<td>(0.7 ± 0.1)</td>
<td>(0.8 ± 0.2)</td>
</tr>
<tr>
<td><strong>Tail width (mm)</strong></td>
<td>0.07 – 0.5</td>
<td>0.04 – 0.3</td>
</tr>
<tr>
<td></td>
<td>(0.2 ± 0.03)</td>
<td>(0.2 ± 0.04)</td>
</tr>
<tr>
<td><strong>Tail length (mm)</strong></td>
<td>0.8 – 6.0</td>
<td>0.8 – 5.1</td>
</tr>
<tr>
<td></td>
<td>(2.7 ± 0.5)</td>
<td>(2.4 ± 0.6)</td>
</tr>
<tr>
<td><strong>Body weight (μg C ind⁻¹)</strong></td>
<td>0.9 – 8.8</td>
<td>0.2 – 9.2</td>
</tr>
<tr>
<td></td>
<td>(2.8 ± 2.0)</td>
<td>(4.8 ± 2.6)</td>
</tr>
<tr>
<td><strong>House diameter (mm)</strong></td>
<td>2.7 – 5.0</td>
<td>3.6 – 9.3</td>
</tr>
<tr>
<td></td>
<td>(3.9 ± 0.5)</td>
<td>(7.0 ± 1.3)</td>
</tr>
<tr>
<td><strong>Inlet filter mesh</strong></td>
<td>Absent</td>
<td>13 x 13¹</td>
</tr>
<tr>
<td>(length x width; μm)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Food concentrating</strong></td>
<td>610 X 150²</td>
<td>No available data</td>
</tr>
<tr>
<td><strong>filter mesh</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(length x width; μm)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Abundance (ind L⁻¹)</strong></td>
<td>0.01 – 2.6</td>
<td>0.00 – 0.9</td>
</tr>
<tr>
<td></td>
<td>(1.2 ± 0.5)</td>
<td>(0.3 ± 0.2)</td>
</tr>
<tr>
<td><strong>Biomass (μg C L⁻¹)</strong></td>
<td>0.02 – 3.7</td>
<td>0.00 – 1.4</td>
</tr>
<tr>
<td></td>
<td>(1.8 ± 0.1)</td>
<td>(0.4 ± 0.1)</td>
</tr>
</tbody>
</table>

¹Alldredge 1977, ²Deibel and Powell (1987); * Represents lower filter screen.
Table 3  Comparison of estimated clearance rates (ml ind⁻¹ h⁻¹) for *Oikopleura longicauda* and *O. fusiformis* (highest to lowest mean rates). Clearance rates are normalized to 25 °C, assuming a $Q_{10} = 1.8$ (Broms & Tiselius 2003), and to a trunk length of 1.0 mm assuming an exponential relationship between clearance rate and trunk length. Prey size is given in parentheses. Trunk length is in millimeters.

<table>
<thead>
<tr>
<th>Study</th>
<th>Method</th>
<th>Temperature</th>
<th>Trunk length</th>
<th>Measured Clearance Rate</th>
<th>Normalized Clearance Rate</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Oikopleura longicauda</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>This study</td>
<td>Natural prey</td>
<td>24</td>
<td>0.7 ± 0.1</td>
<td>34</td>
<td>37</td>
</tr>
<tr>
<td></td>
<td>(FCM; &gt; 1.0 μm)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>López-Urrutia et al. 2003</td>
<td>Gut chlorophyll content</td>
<td>13 - 15</td>
<td>1.00</td>
<td>20*</td>
<td>36</td>
</tr>
<tr>
<td></td>
<td>(0.7 – 30 μm)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alldredge 1977</td>
<td>Visual - dye</td>
<td>25</td>
<td>1.1 ± 0.2</td>
<td>36+</td>
<td>36</td>
</tr>
</tbody>
</table>

| *Oikopleura fusiformis*       |                                           |             |              |                         |                          |
| This study                    | Natural prey                              | 24          | 0.8 ± 0.1    | 38                      | 41                       |
|                               | (FCM; > 1.0 μm)                           |             |              |                         |                          |
| Scheinberg & Landry, in press | Natural prey                              | 29          | 0.7 ± 0.1    | 143                     | 116                      |
|                               | (FCM; > 1.0 μm)                           |             |              |                         |                          |
| López-Urrutia et al. 2003     | Gut chlorophyll content                   | 13 - 15     | 1.00         | 14*                     | 25                       |
|                               | (0.7 – 30 μm)                             |             |              |                         |                          |
| Alldredge 1977                | Visual - dye                              | 25          | 0.6 ± 0.1    | 72+                     | 77                       |

* calculated assuming their mean gut passage time of 14 min; * represents a single measurement; FCM = flow cytometry
FIGURE LEGENDS

Fig. 1 Houses of (a) *Oikopleura longicauda* and (b) *O. fusiformis*. (T) Trunk; (Ta) Tail; (OI) Incurrent opening. Line drawings modified from Alldredge (1977) and images courtesy of A. Alldredge.

Fig. 2 Map of the study site in southern Kaneohe Bay, O'ahu. The routine sampling station (SB) is marked with a black dot. The location of the Hawaii Institute of Marine Biology (HIMB) on Coconut Island is also indicated.

Fig. 3 Mean abundances of appendicularian prey in Kaneohe Bay, O’ahu, Hawaii over an experimental period of three days. Heterotrophic bacteria (0.3 – 0.7 μm), *Synechococcus* spp. (0.8 – 1.0 μm), and eukaryotes (1.0 – 13 μm) were the three prey groups investigated. Error bars represent upper 95% confidence intervals of the mean (n = 26).

Fig. 4 Mean abundances of *Oikopleura longicauda* and *O. fusiformis* in southern Kaneohe Bay, Oahu, Hawaii over a 5-month period. Each date represents a sampling point. *Oikopleura longicauda* was dominant appendicularian in Kaneohe Bay on 9 out of 10 of our sampling days, with a peak of 2.6 ind l⁻¹ on March 11 2002.

Fig. 5 Mean clearance rates of *Oikopleura longicauda* and *O. fusiformis* on heterotrophic bacteria (Hbact), *Synechococcus* spp. (Syn) and < 13.0-μm autotrophic eukaryotes
(Aeuks) on three experimental days. Grazing experiments were conducted on O'ahu, Hawaii during February and March 2002. Rates are based on flow cytometric analysis of the rate of cell decline during incubation experiments, and are plotted as daily averages for each prey population. Error bars represent upper 95% confidence intervals of mean estimates.

Fig. 6 Mean clearance rates for *Oikopleura longicauda* and *O. fusiformis* on heterotrophic bacteria (Hbact), *Synechococcus* spp. (Syn) and autotrophic eukaryotes (Aeuks). Error bars represent 95% confidence intervals of mean estimates (n = 17). The mean clearance rate of *O. longicauda* for Aeuks and Syn was significantly greater than for Hbact (t-test, p < 0.0001). *O. fusiformis* did not clear any of its prey at a significantly different rate (t-test, p > 0.1). Mean clearance rates were not significantly different for each species (t-test, p > 0.1).

Fig. 7 Mean weight-specific ingestion rates for *Oikopleura longicauda* and *O. fusiformis* in Kaneohe Bay, Oahu, Hawaii. Heterotrophic bacteria (Hbact), *Synechococcus* spp. and < 13.0-μm eukaryotes were the three prey groups investigated. Error bars represent upper 95% confidence intervals of mean estimates.

Fig. 8 Mean daily ration of *Oikopleura longicauda* and *O. fusiformis* on the total prey assemblage from grazing experiments in Kaneohe Bay, O'ahu, Hawaii during February and March 2002 (n = 17). We estimated that *O. longicauda* and *O. fusiformis* ingest 460
% and 370 % of body carbon d⁻¹, respectively, assuming that approximately 30 % of particles cleared adhere to the house and that Synechococcus spp. is not digestible by appendicularians.

Fig. 9 Mean clearance rates for Oikopleura fusiformis on heterotrophic bacteria (0.3 – 0.7 μm), Synechococcus spp. (0.8 – 1.0 μm) and autotrophic eukaryotes (1.0 – 13.0 μm) in Kaneohe Bay, Oahu, Hawaii and northwest (NW) Oahu. Error bars represent 95% confidence intervals of mean estimates for experiments performed in Kaneohe Bay (n = 7) and northwest Oahu (n = 50). O. fusiformis cleared < 13-μm eukaryotes at a significantly lower rate in Kaneohe Bay than in NW Oahu (t-test, p < 0.01). Mean clearance rates were not significantly different for 0.8 – 1.0 μm cells (t-test, p > 0.5). Clearance rates on 0.3 – 0.7 μm prey were significantly higher in Kaneohe Bay (p < 0.05).

Fig. 10 The surplus daily carbon available (% body carbon d⁻¹) following house carbon expenditure for O. longicauda and O. fusiformis over a range of house production rates. The house production rates of O. longicauda (21 houses d⁻¹; Sato et al. 2003) and O. fusiformis (24 houses d⁻¹; Sato et al. 2003) at 24 °C are indicated on the x-axis. Based on their ingestion rates at these temperatures, O. longicauda and O. fusiformis had a daily carbon surplus of 325 % and 112 %, respectively. This is indicated with a dashed arrow. Daily house carbon expenditure was calculated using the house carbon to body ratios of Sato et al. (2003) (5.7 % and 10.2 % for O. longicauda and O. fusiformis, respectively).
Fig 2
Fig 3
Fig 4
Fig 5

Oikopleura longicauda

Oikopleura fusiformis

Experimental date
Fig 6
Fig 7
Fig 8
Fig 9

Clearance rate (ml indiv⁻¹ h⁻¹)

Prey size (μm)

0.3 - 0.7  0.8 - 1.0  1.0 - 13.0

Kaneohe Bay
NW Oahu
Fig 10
CHAPTER 4

TEMPORAL VARIABILITY OF A SUBTROPICAL PLANKTON COMMUNITY
ACROSS A COASTAL OCEAN GRADIENT

INTRODUCTION

Tropical and subtropical waters have traditionally been described as steady-state, oligotrophic systems with very little temporal or spatial variability in plankton community structure or abundance (e.g., Heinrich 1962, McGowan & Hayward 1978, Hayward et al. 1983). However, studies conducted over the past decade, primarily the Joint Global Ocean Flux Study (JGOFS) long-term ocean monitoring in the subtropical Atlantic and North Pacific, have shown that the subtropical open ocean exhibits both significant interannual variability and marked seasonality in nutrient concentrations and microbial and net-plankton abundances, and that the seasonality differs from that in temperate open-ocean systems (Letelier et al. 1993, Venrick 1995, Winn et al. 1993, Campbell et al. 1997, Landry et al. 2001).

Despite the resulting shift in our perception of the dynamics of the tropical ocean, we are only beginning to understand the factors governing plankton community structure and trophic relationships in warm waters (e.g. Landry 2002). Long-term ecological studies in the open ocean present formidable challenges, but many of the answers to the questions concerning the external (physical) and internal (biotic) controls on the spatial and
temporal variability of tropical plankton communities may be found in coastal waters. Although tropical embayments and estuaries can range from eutrophic to oligotrophic, coastal waters in the tropics typically are relatively oligotrophic compared to their temperate counterparts (Corredor et al. 1999, Pinckney et al. 1999). Tropical coastal waters thus provide an opportunity to examine plankton community structure and dynamics under trophic conditions similar to those at more open-ocean sites, as well as under a range of less oligotrophic conditions.

How might tropical coastal plankton communities compare with their open-ocean counterparts? The waters of the open subtropical North Pacific are characterized by low nutrient concentrations, low biomass, and dominated by prokaryotic and protistan autotrophs and heterotrophs (Campbell & Vaulot 1993, Letelier et al. 1993, Campbell et al. 1997). We would expect to see similar structure in oligotrophic coastal waters, with an increase in the relative abundance of eukaryotes to prokaryotes as conditions become more eutrophic. In addition, based on the observed response of open-ocean systems to transient nutrient perturbations, we might expect to see larger components of the community added to a relatively stable base of smaller organisms as more nutrient resources become available (e.g. Landry et al. 2000). We would also expect to see a gradient in the relative importance of different food web components. The planktonic food web in the oligotrophic North Pacific open ocean is characterized by a tightly coupled predatory chain between the dominant pico- and nanoplanckton, with a more diffuse relationship between larger size fractions (Calbet & Landry 1999). In these
waters, mesozooplankton play only a minor role in pico- and nanoplanckton dynamics, but their importance likely would increase in more eutrophic coastal waters.

The Hawaiian Islands provide a unique environment in which to study the gradient from land to open ocean-influenced oligotrophic coastal waters and their relative variability over time. Located in the middle of the North Pacific subtropical gyre, the Hawaiian archipelago is the most isolated group of islands in the world. The proximity of the shelf break (generally < 15 km) and the current structure around the islands result in coastal waters that are highly influenced by the ultra-oligotrophic oceanic waters of the tropical North Pacific (Flament et al. 1996). Kaneohe Bay, located on the island of Oahu, is the largest sheltered water body in these islands. This embayment is relatively oligotrophic compared to temperate coastal waters, with low nutrient concentrations, low freshwater input relative to exchange time, and little stratification throughout the year. However, water in the more enclosed southern sector of the bay has a significantly longer residence time than waters in the central and especially the northern sectors, which are subject to relatively rapid exchange with offshore waters. As a result, Kaneohe Bay provides a natural gradient of trophic conditions from near open-ocean oligotrophy in the north, to coastal mesotrophy in the south (Smith et al. 1981, Laws & Allen 1996, Kinzie et al. 2001).

This study used the natural trophic gradient in Kaneohe Bay to investigate the factors governing food web structure and dynamics in tropical waters. Based on the general expectations outlined above and previous work in Kaneohe Bay, we hypothesized that plankton communities in the Bay would differ along the north-south gradient, exhibiting
tight internal regulation of smaller components of the food web but increasing
fluctuations in larger size fractions at less oligotrophic sites. In addition, based on
temperature-dependent predictions of copepod growth (Huntley & Lopez 1992) and
expected high appendicularian growth rates at bay temperatures (Hopcroft et al. 1998b),
we hypothesized that these components of the mesozooplankton might be capable of
growing at rates comparable to protists in Kaneohe Bay. Hence, variations in
mesozooplankton abundance could significantly impact lower trophic levels, and play an
increasingly important role in food web structure at less oligotrophic stations.

METHODS

Description of Kaneohe Bay

Kaneohe Bay is a subtropical, semi-enclosed embayment of 55 km$^2$ on the
northeastern coast of O‘ahu, Hawaii (Figure 1). Landward and seaward boundaries
consist, respectively, of a narrow coastal plain backed by a near-vertical mountain range
and a barrier reef that extends across the bay mouth. The prevailing winds throughout the
year are trades, blowing into the bay from the Northeast. A reverse in wind direction,
from trades to south- and northwesterly “Kona” winds, signals the start of the wet season.
The tropics are less variable climatologically than temperate regions, generally consisting
of two seasons, wet and dry. The wet season in Hawaii typically runs from November
through April, and the dry season from May through October (Juvik & Juvik 1998). The
long-term average rainfall in the area of Kaneohe Bay is 192 cm yr$^{-1}$, ranging from 85 cm
(dry year) to 365 cm (wet year) (Western Regional Climate Center, Kaneohe Mauka station 781, 1949-1998).

**Study site selection**

Kaneohe Bay was divided into three sectors of varying open-ocean influence based on the predominant circulation patterns in the bay. Tidal exchange and wind-driven currents generally govern circulation (Smith et al. 1981). Circulation in the bay is primarily characterized by a net flow of water along the coast from south to north, with water entering the flow across the barrier reef flat as it moves northward. Oceanic exchange occurs through southern and northern channels. In the more enclosed, southern portion of the bay, this incoming water circulates before moving northward (Figure 2). Southern Kaneohe Bay is thus the sector with the longest residence time (~ 13 d, Bathen 1968), in addition to being the least open-ocean influenced sector of the bay. At the opposite extreme, northern Kaneohe Bay is the most heavily influenced by the open ocean and has a residence time on the order of ~ 5 d (as calculated from Smith et al. 1981). Central Bay is a transition zone between the northern and southern sectors, and is influenced by both oceanic water coming in across the reef flat and circulation from the southern bay.

Freshwater influences on each of these sectors via rainfall and runoff into the bay were determined using data from the United States Geological Survey (USGS) Luluku rain gage, the USGS Kaneohe stream gage and the Hawaii Institute of Marine Biology (HIMG) rain gage on Coconut Island in southern Kaneohe Bay (Figure 1).
Field collection

Two periods were intensively sampled in the bay, one during 1998-99 and one during 2001-02. Beginning in the summer of 1998, four sampling stations were established in the relatively enclosed, southern basin of Kaneohe Bay and visited weekly over an 8-month period to determine baseline conditions in plankton community structure and biomass in the most coastally-influenced sector of Kaneohe Bay. Data from only one of these stations (SB) will be presented here, as the four stations did not differ significantly in any of the measured parameters (t-tests, p > 0.05; R. Scheinberg unpubl.). In 2001, two additional stations were established, one each in the central (CB) and northwest (NB) sectors (Figure 1) to assess plankton community structure and temporal variability along a gradient from coastal to near open-ocean conditions. Biweekly sampling was conducted over 15 months at stations SB, CB and NB. All stations were established along the 14-m isobath (average channel depth) and were marked and revisited using a hand-held GPS (Garmin GPS 12XL, absolute accuracy = 15 m).

Water samples for pico-, nano- and microplankton and for total chlorophyll analyses were collected from 3-m depth at SB (1998-99) and from 0, 3, 9 and 12 m at all 3 stations (2001-02) using a 1.2-liter Niskin bottle. Samples of 500 ml were stored on ice for later laboratory processing (usually within 90 min of collection). Net tows (0.5-m diameter net, 64-µm Nitex mesh) were conducted at each station to determine micro- and mesozooplankton abundances. Gentle vertical tows pulled by hand minimized damage to fragile gelatinous animals, while the small mesh size allowed for collection of nauplii, juvenile and adult copepods (Hopcroft et al. 1998a), all life history sizes of
appendicularians (Fenaux 1998), as well as collection of early stage chaetognaths (Baier & Purcell 1997). Net tow samples were preserved in the field in 5% (final concentration) borax-buffered formalin. During the 2001-02 sampling period, an additional tow was performed at each station and kept on ice to be processed immediately upon return to the laboratory. The unpreserved net tow sample was sorted into 4 size-classes by gently wet sieving through nested Nitex screens of 1, 0.5, 0.2 and 0.06 mm mesh to produce 4 nominal size classes of 0.06-0.2, 0.2-0.5, 0.5-1 and >1 mm. Each size fraction was concentrated onto a pre-weighed 0.06-mm Nitex screen under low vacuum, rinsed with isotonic ammonium formate solution to remove interstitial sea salt, placed in an individual cryotube and flash frozen in liquid N₂ for later biomass analysis.

In addition to sample collection, water temperature, salinity and turbidity were recorded at each station using a calibrated Horiba U-10 water quality probe. Nutrient data from surface samples at all three stations also were obtained, beginning in the fall of 1998, as part of a long-term monitoring effort by the NOAA/EPA Coastal Intensive Site Network (CISNet) program (Kinzie III et al. 2001).

Chlorophyll

For chlorophyll a analyses, 25-ml seawater aliquots were filtered onto Whatman GF/F glass-fiber filters. Filters were extracted in 5 ml of 90% acetone in the dark at -20°C for 24 h, and chlorophyll a was quantified using a Turner model 10AU (1998-99 samples) or model TD 700 (2001-02 samples) fluorometer calibrated using HPLC measurements of pure pigment standards (Holm-Hansen & Riemann 1978).
Microbial community abundances and biomass

**Picoplankton.** Seawater aliquots (1 ml) from each sampling depth were preserved with 0.2% (final concentration) paraformaldehyde (PFA) and frozen in liquid nitrogen. Frozen samples were thawed and stained with Hoechst 33342 (0.8 μg ml\(^{-1}\) final concentration) for 30 min before analysis by flow cytometry (FCM) (Monger & Landry 1993). Each aliquot was spiked with a mixture of Polysciences Fluoresbrite YG 0.57- and 0.98-μm visible microspheres and 0.46-μm UV microspheres to normalize light scatter signals to consistent size references. Subsamples of 100 μl were enumerated on a Coulter EPICS 753 flow cytometer equipped with dual argon lasers and MSDS II automatic sampling. The lasers were aligned colinearly with the first laser tuned to the UV range to excite Hoechst-stained DNA. The second laser was tuned to 488 nm at 1.0 W to excite the pigments of autotrophic cells. Picoplankton populations were distinguished from one another by differences in light scatter and fluorescence emission. Heterotrophic bacteria (Hbact) showed DNA staining but no red (680 ± 40 nm) fluorescence. *Synechococcus* spp. (Syn) were characterized by the presence of orange (575 ± 40 nm) fluorescence indicating phycoerythrin. Autotrophic picoeukaryotes (Peuks) were identified by higher forward and right angle light scattering (indices of cell size) and enhanced red fluorescence compared to Syn.

The size composition of picoplankton was determined using both epifluorescence microscopy and flow cytometry. Subsamples (50 ml) for sizing of Hbact were preserved with PFA (4% final concentration) and stained for 1 min with the fluorochrome acridine orange (AO, 50 μg ml\(^{-1}\) final concentration) (Kirchman et al. 1982). These samples were
then filtered onto 0.2-μm black Poretics polycarbonate membrane filters, mounted on
glass slides using low-fluorescence immersion oil and viewed with a Zeiss
epifluorescence microscope and a ZVS 3-chip CCD video camera connected to a
computer. Blue excitation was used to visualize AO-stained cytoplasm, and the images
were processed using Zeiss Image Pro Plus software. To avoid problems in
distinguishing Syn from other groups of larger, phycoerythrin-containing cyanobacteria
microscopically, Syn size was determined by FCM analysis of size-fractionated
subsamples. Samples were filtered through a series of membrane filters (pore size = 5, 2,
1, 0.8, 0.6, 0.4 and 0.2 μm) and filtrate of each succession filtration measured for Syn
abundance via FCM.

**Nano- and microplankton.** Cyanobacteria, autotrophic and heterotrophic flagellates,
diatoms, and dinoflagellates in the nano- (2-20 μm) and microplankton (20-200 μm) size
fractions were enumerated and measured using epifluorescence microscopy (Zeiss
Standard). Subsamples (50 ml) from field collections were preserved with PFA (4% final
concentration) and stained with the fluorochromes proflavin and DAPI (e.g. Verity &
Sieracki 1993). These samples were then filtered onto 1.0-μm black Poretics
polycarbonate membrane filters, mounted on glass slides using low-fluorescence
immersion oil, and frozen at -80°C for later analysis by epifluorescence microscopy.
Blue excitation was used to visualize proflavin-stained cytoplasm and autofluorescence,
and UV excitation was used for DAPI-stained nuclei. The images were processed using
Zeiss Image Pro Plus software to facilitate counting and sizing of all heterotrophic and
autotrophic organisms (Verity & Sieracki 1993). Circular cells that contained the
pigment phycoerythrin and fluoresced yellow-orange under blue excitation were identified as *Crocosphaera* spp. (previously referred to as marine *Synechocystis* spp.; Webb et al. 2001, Zehr et al. 2001). Cells ≥ 0.9 μm were included based on reported estimates of cell shrinkage due to preservation and staining (28 - 44% volume reduction equivalent to 3.5% cell diameter reduction for nanoflagellates preserved with gluteraldehyde or formalin; Booth 1987, Børshem & Bratbak 1987, Verity et al. 1992). Approximately 300 cells were enumerated and sized at 400X (nanoplankton) and 250X (microplankton) for each sample.

**Microbial carbon estimates.** Picoplankton abundance was converted to biomass equivalents using biovolume to carbon conversion factors of 380 fg μm$^{-3}$ (Lee & Fuhrman 1987) and 235 fg μm$^{-3}$ (Shalapyonok et al. 2001) for Hbact and Syn, respectively. Approximately 40% of Peuks were > 2.0 μm. These counts were removed from the flow cytometry data to avoid overlap with microscopical counts of autotrophic nanoplankton. The remaining cells (60%) were assumed to comprise the picoeukaryotes. Cell volumes were calculated assuming a mean 1.5-μm diameter. Biovolumes of Peuks and nanoplankton (BV, μm$^3$) were converted to biomass (pg C) based on Eppley et al. (1970): log(pg C) = 0.76 log(BV) - 0.29 and log(pg C) = 0.94 log(BV) - 0.60 for diatoms and flagellates, respectively. These equations do not take into account cell shrinkage due to preservation and thus yield minimum estimates of cell carbon content.
**Zooplankton biomass and abundance**

**Abundance.** Prior to analysis for abundance, preserved net tow samples were washed through a 64-μm mesh sieve to remove preservative, re-diluted to 100 ml with 0.2-μm filtered seawater, and stained with Rose Bengal (in isopropyl alcohol, 0.05% final concentration). A minimum of 100 individuals of each dominant family was counted using a Leica Z9.5 and a Nikon stereomicroscope with an ocular micrometer. All dominant adults, juveniles and nauplii were classified to species and measured to the nearest 20 μm (n ≥ 50). Appendicularian trunk length was defined as the distance from the tip of the mouth to the posterior edge of the trunk. Chaetognaths were separated into three life stages based on gonad development (stages 1, 2 and 3) according to Casanova (1999). Shrinkage of preserved gelatinous specimens was assumed to be 12% (as reported in Chapter 3). The dominant copepods, chaetognaths and appendicularians were identified to species according to Bückmann & Knapp (1975), Fenaux (1993), Bradford-Grieve et al. (1999) and Casanova (1999). Medusae were identified with the help of Bouillon (1999) and Wrobel & Mills (1998). All other organisms were sorted to the group level (polychaetes, bivalves, gastropods, crab larvae, decapod larvae, fish larvae, fish eggs).

**Biomass.** Zooplankton biomass estimates were determined from analyses of size-fractionated net tows. Wet weights (WW) were obtained by thawing frozen Nitex filters, blotting briefly to remove excess water, and weighing on a Mettler Model AE 160 balance. Samples were then dried in a 60°C oven for a minimum of 72 h, cooled in a dessicator and reweighed to obtain dry weights (DW). Zooplankton sample dry biomass
was calculated for each size fraction from total initial wet weights (less filter weight), the 
DW:WW ratios and the fraction of the net hauls that were size-sorted. Dry biomass was 
converted to carbon (C) using a C:DW ratio of 0.37 (Landry et al. 2001).

Statistics

The significance of the temporal and spatial variability of the plankton community in 
Kaneohe Bay was determined using paired and non-paired, two-tailed t-tests. Correlation 
analysis was used to determine relationships between variables and their significance 
established via regression analysis (Moore & McCabe 1993). Unless otherwise noted, 
errors are reported as 95% confidence limits of the mean.

RESULTS

Data from the 2001-2002 sampling period allow characterization throughout the 
entire bay and across a trophic gradient and are presented first. Data from 1998-1999 are 
then presented as the basis for evaluating interannual variability at the SB site.

Inter-station variability in environmental parameters

Despite differences in circulation and hydrology, mean water temperature and salinity 
did not vary significantly between stations SB, CB or NB (p > 0.05), and temperature 
was not significantly different from surface to depth. Salinity also exhibited minimal 
variability with depth, with occasional surface lows (min. 33) during periods of elevated 
rainfall, and a decreasing trend in surface water salinity was evident from station SB to 
NB. The temperature and salinity profiles from this sampling period suggest that all
three stations in Kaneohe Bay are well mixed throughout the majority of the year (Figure 4).

While freshwater runoff is generally higher in the northern sector of the bay due to greater rainfall in northern watersheds, rainfall isohyets suggest that direct rainfall to the bay does not vary as much between sites (Figure 1). Rainfall in 2001 and 2002 was well below average, so for the purposes of this study, the wet season was defined specifically as the period of the year when rainfall and corresponding runoff were elevated significantly above baseline conditions (defined as daily rainfall at the Luluku rain gage below 40 mm – Figure 3). During the 2001-02 sampling period, the wet season lasted approximately 7 months, from November 2001 through May 2002. Rainfall in the spring of 2001, which would normally be considered part of the wet season, was not significantly different from summer 2001 (p > 0.05) and was thus considered part of the dry season. Rainfall ranged from 0.0 to 4.8 mm d\(^{-1}\) at the HIMB Coconut Island rain gage over the course of the sampling period. Solar radiation ranged from 13 (November) to 43 E m\(^{-2}\) (June), with a mean of 29 ± 3 E m\(^{-2}\).

**Nutrients**

Mean nutrient values for the three stations are presented in Table 1. Surface-water nutrient concentrations differed only slightly from south to north (Figure 5), with the exception of nitrate (NO\(_3^-\)+NO\(_2^-\)), which was 5-fold greater at station NB (0.28 µM) than at station SB (0.05 µM). Silicic acid (Si) concentrations were lower at the CB site than either the SB or NB sites. Total dissolved nitrogen (TDN = NO\(_3^-\)+NO\(_2^-\) + ammonia (NH\(_4^+\)) + dissolved organic N (DON)) and Si(OH)\(_4\) were both lower at a station outside
the bay (station OS), while total dissolved phosphorus (TDP = dissolved inorganic phosphorus (DIP) + dissolved organic phosphorus (DOP)) at the OS station was comparable to the highest values inside the bay. All nutrient samples were collected at ~0.5 m and data was obtained courtesy of the CISNet monitoring program in Kaneohe Bay, conducted from 1999 - 2001 (Kinzie III et al. 2001).

Chlorophyll

Chlorophyll a (Chl) concentrations ranged from 0.20 – 4.62 μg l⁻¹ throughout the bay and Chl integrated over the 12-m water column was highest in the most enclosed, southern basin (p < 0.0001; Figure 4). Mean integrated Chl concentrations were 1.32 ± 0.17, 0.99 ± 0.13 and 0.97 ± 0.09 μg l⁻¹ at stations SB, CB and NB, respectively. Chl concentration increased with depth at stations SB and CB (p < 0.001), but remained relatively constant throughout the water column at station NB (p > 0.05). Despite indications of a decreasing trend from Central to North Bay, Chl was not significantly different between these two stations (p > 0.05).

Microbial community abundance and variability

Community composition and size structure. The picoplankton and nanoplankton size fractions in Kaneohe Bay were comprised of the following groups of organisms: heterotrophic bacteria (Hbact), *Synechococcus* spp. (Syn), autotrophic picoeukaryotes (Peuks), >1-μm autotrophic eukaryotes (Aeiks), >1-μm heterotrophic eukaryotes (Heuks) and >1-μm *Crocosphaera* spp. (Crocos). Hbact ranged in size from 0.3 – 0.7 μm (mean = 0.5 ± 0.03). Syn ranged in size from 0.8 – 1.0 μm (mean = 0.9 ± 0.05) based
on the results of the FCM-analyzed size fractionation experiments (approximately 80% of Syn cells passed through a 1.0-µm filter and were retained on a 0.8-µm filter).

*Prochlorococcus* spp. was notably absent from the prokaryotic community throughout the sampling period. Aeukuks, Heuks and Crocos were further divided into four major size fractions (2-5, 5-10, 10-20 and >20 µm) for the purposes of this analysis. The 2-5 and 5-10 µm size fractions were comprised primarily of autotrophic and heterotrophic flagellates, and *Crocosphaera* spp. The 10-20 µm size fraction was comprised of short *Chaetoceros* spp. and *Thalassionema* spp. chains, small pennate diatoms, heterotrophic dinoflagellates and *Crocosphaera* spp. The majority of the >20-µm size fraction was comprised of large pennate diatoms, *Chaetocerus* spp., *Thalassionema* spp. and the dinoflagellate *Ceratium* spp.

**Community abundance and biomass.** Size structure, abundances and biomass of the microbial community in Kaneohe Bay are presented in Table 2. Picoplankton dominated the microbial community in terms of total abundance (mean of 7 ± 1 x 10^5 cells ml^-1) and exhibited a decreasing trend from south to north, with the exception of Syn, which maintained consistent abundances throughout the bay (p > 0.05).

Picoplankton community biomass also decreased from south to north, with mean total picoplankton values of 31 ± 5, 23 ± 4 and 19 ± 5 µg C l^-1 at SB, CB and NB, respectively. This decrease was significant between SB and the other two stations, but was not significant between CB and NB.

As with picoplankton, total nanoplanckton abundance was greater at SB than at the other two stations (p < 0.01), but was not different between CB and NB (p > 0.05).
Individual components of the nanoplankton size fraction followed this same trend, with the exception of Crocos, which were significantly lower in abundance at station CB than at either SB or NB (p < 0.05). Station SB also had the greatest mean biomass in the nanoplankton size fraction, and in total > 20-μm autotrophs and heterotrophs (p < 0.05) (Table 3).

Differences in the biomass of all size fractions and the relative contribution of autotrophs, heterotrophs and cyanobacteria at each station are illustrated in Figure 6. Overall, microplankton community structure and biomass was most comparable to an oligotrophic system at Station NB, where community biomass was inversely related to size. 42% of total biomass at Station NB was due to picoplankton (CB = 30%, SB = 16%). In addition, cyanobacteria (both Syn and Crocos) comprised a significantly greater percentage of the total < 20-μm carbon (approximately 30%) at this station than at either SB (17%) or CB (18%).

**Vertical structure.** Variability in microbial community abundance from near-surface (3 m) to depth (12 m) was most pronounced at station SB, and decreased along a gradient from south to north. In the picoplankton size fraction, station SB and CB exhibited higher abundances at depth of one or more of the dominant groups of organisms (Figure 7). Syn abundance was significantly greater at depth at station SB (p < 0.001) and both Syn and Hbact increased with depth at station CB (p < 0.05). Peuk abundance did not differ vertically throughout the bay. The nanoplankton size fraction exhibited the greatest vertical variability in abundance at station SB. At this station, both Aeuks and Crocos increased with depth (p < 0.05 and 0.01, respectively). The difference
in Croco abundance between 3 and 12 m at each station is illustrated in Figure 8. CB and NB exhibited little difference in nanoplankton abundance from surface to depth, with the exception of an increase in 2 – 5 μm Heuks at 12 m at station NB (p < 0.05).

**Temporal variability.** The temporal variability of the microbial community at all three stations is illustrated in Figures 7 (picoplankton) and 9 (nanoplankton). As with vertical structure, temporal variability in the microbial community was greatest at the SB station and declined successively at the CB and NB stations. At station SB, abundance and biomass of the picoplankton size fraction, and of autotrophs and heterotrophs in the nanoplankton size fraction, decreased significantly from the dry season (March 2001–October 2001) to the wet season (November 2001 – May 2002) (p-values all < 0.05). *Crocosphaera* spp. in the nanoplankton size fraction was the exception at this station, and did not vary seasonally with respect to abundance or biomass. At station CB, Peuks, Aeuks, 2 – 5 μm Heuks and 2 – 5 μm Crocos also decreased in abundance over this time period (dry to wet seasons), although the biomass of these size fractions did not follow the same trend. Of the total Aeuks, only the biomass of small autotrophs (2 – 5 μm Aeuks) decreased. In addition, all Crocos (2 – 20 μm) decreased significantly (p < 0.05). The biomass of 2 – 5 μm Heuks did not decrease. Station NB did not exhibit a significant change in biomass or abundance in any of the size fractions of the microbial community over time (all p-values > 0.05).

Although nanoplankton mean abundance was greater during the dry season at station SB, rainfall explained a significant amount of variability in the SB nanoplankton data during the wet season at rain levels > 2 mm (Figure 10). In the nanoplankton size
fraction at this station, the most significant relationship with rainfall was exhibited by 10
– 20 μm Aeuk (R² = 0.54, p < 0.0005). This relationship was not observed at stations
CB and NB. Syn abundance increased with increasing temperature at all stations, and
was the only component of the plankton community that varied significantly with this
parameter. This relationship also appeared to increase in significance from south to north
(R² = 0.61, p < 0.0001 at station NB; Figure 11).

Zooplankton

Community composition and size structure. A zooplankton species list, with mean
abundances and sizes at each station, is presented in Table 3. The zooplankton
community of Kaneohe Bay was primarily comprised of small copepods, chaetognaths,
appendicularians, hydromedusae, larval molluscs and larval polychaetes. Four dominant
copepod species were observed over the sampling period: the cyclopoids, Oithona
simplex and O. nana, and the calanoids, Parvocalanus crassirostris and Bestiolina
similis. Less abundant copepods included the larger calanoids, Undinula darwinii and
Acartia sp., and several species of harpacticoids. The gelatinous zooplankton community
consisted of one species of chaetognath (Sagitta enflata), two species of appendicularians
(Oikopleura longicauda and O. fusiformis) and two species of cnidarian (an unidentified,
small hydromedusa and Obelia sp.). Due to the rarity of both O. fusiformis and Obelia
sp., abundances are only reported for total appendicularians and total hydromedusae.
Larval molluscs were primarily two unidentified species of gastropod (with occasional
appearances by Limacina sp.) and one unidentified species of bivalve. Three unidentified
species dominated the polychaetes observed. Abundance data is reported for total
molluscs and total polychaetes. Other crustaceans commonly present were *Lucifer chacei*, crab zoea, mysiids and several species of decapod larvae.

Copepods numerically represented approximately 90% of all zooplankton < 1 mm in size, while chaetognaths made up the majority of the > 1 mm size fractions and all of the > 5 mm size fraction at all stations (Figure 12). The 1-5 mm size fraction contained stage 1 chaetognaths, while the > 5 mm fraction contained more mature stages 2 and 3.

**Abundance and Biomass.** Of the three stations, NB had the lowest abundance of all dominant groups of zooplankton (*p* < 0.05). In addition, the majority of the zooplankton groups were more abundant at station SB than at station CB, with the exception of appendicularians, hydromedusae and adult copepods (*p* > 0.05), which did not differ in abundance between the two stations. Small copepods made up the majority of zooplankton at all three stations in terms of abundance, and exhibited a decreasing trend in terms of relative abundance from south to north (Figure 13). Of the small copepods, *O. simplex* comprised greater than 50% of the total copepod members in the bay at all three stations. With the exception of *Acartia* sp., rare copepods were not significantly different between stations (*p* < 0.05). However, rare copepods exhibited an increasing trend from south to north in relative abundance (Figure 14). The abundances of other crustaceans and polychaetes larvae did not vary between stations, while molluscs were significantly lower at station CB throughout the sampling period (*p* < 0.5).

Biomass estimates are illustrated in Figure 15. The 0.06 – 0.2 mm and 0.5 – 1 mm size fractions did not differ significantly between stations (*p* > 0.05), but the 0.2 – 0.5 size
fraction had significantly greater biomass at station SB than at CB or NB. The >1-mm size fraction was also greater at SB than NB (p < 0.001).

Temporal variability. Although the zooplankton community in Kaneohe Bay exhibited high weekly variability, only a few dominant groups appear to follow a bi-yearly trend in abundance. Adult and juvenile copepods were generally more abundant during the dry season than the wet season at all three stations, with the most significant difference between seasons at Station NB (p = 0.07, 0.08 and 0.03 for SB, CB and NB, respectively). Copepod nauplii were also significantly greater in abundance during the dry season at both Central and North Bay stations (p < 0.05), but not at station SB (p > 0.05) (Figure 16). Nauplii were positively correlated with copepod prey populations (> 2 μm Aeuko and Heuko) at stations CB and NB, with equivalent R² values of 0.40 (Figure 17, p < 0.001). Gelatinous zooplankton were generally more abundant during the wet season at all three stations, a trend primarily driven by the appearance of appendicularians in the late fall of 2001. Appendicularian abundances were significantly higher at all three stations during the wet season (p < 0.05), and were inversely related to the abundance of Peus at stations SB and CB (R² = 0.85, 0.31 and 0.11 and p < 0.0001, < 0.05 and > 0.05 at stations SB, CB and NB, respectively) (Figure 18). Other, less abundant groups (polychaetes, molluscs, other crustaceans, fish larvae and fish eggs) did not exhibit statistically significant seasonal variability (Figure 16).

The increase in appendicularian abundance during the wet season was correlated most significantly with the increase in freshwater runoff into Kaneohe Bay (Figure 19; R = 0.73, p < 0.0001). In addition, appendicularians exhibited an opposite seasonal trend to
medusae, appearing for the first time in the bay during the sampling period following a
decline in medusae abundance at the start of the wet season (Figure 20). While
appendicularians and total medusae were not significantly correlated throughout the full
sampling period, appendicularians at station SB exhibited a significant positive
relationship with the larger hydromedusa, *Obelia* sp. (*p* < 0.001). Total medusae were
also positively associated with chaetognaths at stations SB (*R* = 0.40, *p* < 0.05) and CB
(*R* = 0.71, *p* < 0.001) (Figure 21).

**Interannual variability in southern Kaneohe Bay:**

A comparison of results from the 1998-99 and 2001-02 sampling periods

Temporal variability was significant for a number of the parameters measured in
2001-02. Thus, only results from months sampled in both studies (July – March) will be
considered in the following sections.

**Environmental setting**

Environmental parameters followed generally similar patterns in Kaneohe Bay over
the two sampling periods, but with notable difference in direct rainfall to the bay (Figure
22). Water temperature and solar radiation were virtually identical, except for a brief
period of high solar radiation and increased water temperature in late summer/fall 1998.
Cumulative rainfall on Coconut Island (indicative of direct rainfall to the bay) was
approximately 40% lower during the 1998-99 sampling period than during the equivalent
period in 2001-02. The decrease was less dramatic in the watershed, where the Luluku
rain gage recorded approximately 20% lower values for 1998. These results reflect a
significant, island-wide drop in rainfall as indicated by values at Honolulu International
Airport on the leeward side of the island (location illustrated in Figure 1). 1998 was also
the lowest rainfall year between 1997 and 2002 (Figure 23), and was in the lower 10% of
rainfall years in the climatological record at that station (National Weather Service 2002).
While the Luluku rain gage showed a less obvious trend, rainfall over the entire 5-yr
period was well below normal (Figure 23). Although the wet season arrived one month
later in 1998 than in 2001 and rain levels were low throughout the year, mean rainfall
was not significantly different between equivalent 8-mo periods in 1998-99 and 2001-02
(p > 0.05).

Chlorophyll

Mean chlorophyll a concentration was significantly (approximately 2-fold) lower in
1998-99 than during the equivalent time period in 2001-02. Comparison of the datasets
is made difficult by the relatively short period of overlap, however a peak between
November and December of 1998 suggests an increase in Chl during the wet season as
seen in 2001-02 (Figure 24).

Microbial community abundance and variability

Community composition. Microbial community composition did not vary between
the two sampling periods. As in 2001-2002, the dominant planktonic components of the
1998-1999 southern Kaneohe Bay ecosystem were heterotrophic bacteria, *Synechococcus*
spp., autotrophic picoeukaryotes, autotrophic and heterotrophic flagellates, and diatoms
and dinoflagellates (listed from most to least abundant). *Prochlorococcus* spp. were absent from the bay, and *Crocosphaera* spp. were not quantified in 1998-1999.

**Abundance and biomass.** A comparison of the abundance and biomass of the microbial community in southern Kaneohe Bay between sampling periods is presented in Table 4. Heterotrophic bacteria and *Synechococcus* spp. were over 2-fold greater in both biomass and abundance during the 1998-99 sampling period (*p* < 0.0001 for both), while lower, but not statistically significant, values were observed for the Peuks (*p* > 0.05, Figure 25). Total Aeiks were significantly higher in terms of abundance and dropped from a mean of 7600 ± 1500 cells ml⁻¹ in 1998 to 1900 ± 770 cells ml⁻¹ in 2001. This decrease was most evident for the 2-5 μm size fraction. In contrast, due to high weekly variability in this size fraction, Aeuk biomass did not vary significantly during the 8-mo common period between sampling years. Total Heuks did not vary in terms of biomass or abundance (*P* > 0.05). However, the ratio of total picoplankton to their dominant predators, 2-5 μm Heuks (mostly heterotrophic nanoflagellates), was significantly lower in 1998 than in 2001. Figure 26 illustrates Aeuk abundance and biomass and the shift in the proportion of autotrophs to heterotrophs from 1998-99 to 2001-02.

**Temporal variability.** The relative temporal variability of picoplankton and nanoplankton at station SB between the 1998-99 and 2001-02 sampling periods is illustrated in Figures 27 (picoplankton) and 28 (nanoplankton). Heterotrophic picoplankton and nanoplankton exhibited similar trends relative to seasonal rainfall. Hbact and Heuks both decreased significantly in mean abundance and biomass during the
wet season at station SB over the 1998-99 sampling period (p < 0.005). In contrast, AeukS exhibited a positive relationship with direct rainfall, most significantly for the 10 – 20 µm and > 20-µm size fractions (Figure 29; both R² = 0.57, p < 0.001). Direct rainfall was the only environmental parameter that elicited a response from the microbial community in southern Kaneohe Bay during both the 1998-99 and 2001-02 sampling periods.

**Zooplankton**

*Community composition and abundances.* Several changes are evident in zooplankton community structure in southern Kaneohe Bay between 1998 and 2002. While the southern sector of the bay was dominated by small copepods in both 1998-99 and in 2001-02, the relative abundance of the three less dominant copepod species (Figure 30) differed significantly. The cyclopoid, *Oithona nana*, was more abundant in 1998-99 and represented a significantly greater proportion of the total copepods (mean = 1.3 ± 0.3 ind l⁻¹, p < 0.0001). In contrast, the calanoid, *Parvocalanus crassirostris*, was significantly less abundant during this same period (mean = 1.0 ± 0.3 ind l⁻¹, p < 0.0005). *Oithona simplex* dominated the copepod community in 1998 as in 2001, and did not differ significantly in abundance (mean = 8.3 ± 1.8 ind l⁻¹, p < 0.001). Nauplii also did not exhibit a significant difference between the two sampling periods (mean = 30 ± 5 ind l⁻¹ and 34 ± 7 ind l⁻¹ for 1998-99 and 2001-02, respectively; p > 0.05). With respect to the gelatinous zooplankton (Fig. 31), the hydromedusae were comprised of small species with only occasional appearances by *Obelia* sp. during 1998-99, and mean abundances were significantly lower than during 2001-02 (0.03 ± 0.02 ind l⁻¹ and 0.45 ± 0.12 ind l⁻¹
for 1998-99 and 2001-02, respectively; p < 0.005). While not significant, appendicularians also decreased between the two sampling periods (means = 0.77 ± 0.27 ind l⁻¹ and 0.48 ± 0.32 ind l⁻¹ in 1998-99 and 2001-02, respectively; p > 0.1). In addition, *Oikopleura fusiformis* made up a greater proportion of total appendicularian abundance in 1998 than in 2001-02 (11 ± 6% vs 7 ± 6% in 1998-99 and 2001-02, respectively; p < 0.05).

**Temporal variability.** The zooplankton community exhibited less variability over the 8-mo sampling period in 1998 than during 2001-02 (Figure 32). Adult and juvenile copepods were slightly greater in abundance during the drier period of the year, as were chaetognaths and hydromedusae. Appendicularians were also more consistently abundant throughout the 1998 sampling period. Individuals were observed on every sampling day, at abundances ranging from 0.1 to 2.3 ind l⁻¹, while appendicularians were absent from the community during the first 18 of 30 sampling days in 2001-02, yet still ranged in abundance up to 2.8 ind l⁻¹.

During the 2001-02 sampling period, appendicularian abundances in 1998 appeared to be associated with abundance of their prey. Chlorophyll *a* concentrations decreased significantly as appendicularian abundance increased over the sampling period (Figure 33; R² = 0.51, p < 0.0001).
DISCUSSION

The study aimed to utilize the natural gradient in coastal influence in Kaneohe Bay to elucidate factors controlling plankton community structure, abundance and biomass, and to better understand the trophic linkages between microbes and zooplankton. Plankton community structure, abundance and biomass in Kaneohe Bay varied significantly along a gradient from the mesotrophic southern basin to more oligotrophic waters in the north. Variability in total abundance and biomass of the plankton community between sites was correlated with the residence time of oceanic water in each of the sectors, with the southern basin supporting the highest total biomass in the bay. Based on circulation patterns and a decreasing gradient in biomass from south to north, the South Bay appears to function as a source of plankton to the Central and North Bay that is continually diluted by oceanic water as it travels along the coast.

Plankton community structure also reflects this gradient. The observed pattern in the ratio of prokaryotic to eukaryotic microbial plankton biomass (Figure 6) was similar to onshore-to-offshore trends described for the waters of the Arabian Sea, where this ratio was lower at more coastal sites (Garrison et al. 2000). Oceanic species of zooplankton were proportionately more important in the north, as were smaller species. The temporal variability of the plankton community in Kaneohe Bay also varied between stations, with the largest variations in the more coastally-influenced southern basin and the smallest in the north (Figures 9 & 16). These temporal trends corresponded most closely with seasonal changes in temperature and direct rainfall, with the exception of *Synechococcus* spp. The correlation between *Synechococcus* spp. abundance and temperature was highly
significant in Kaneohe Bay and the significance increased in statistical power from south to north. *Synechococcus* spp. growth rates have been strongly linked to temperature in both coastal and oceanic tropical systems (Li 1998, Murrell & Lores 2004, Carpenter & Campbell 1988). The reason for a more consistent temperature correlation from south to north is unknown, but may be due, at least in part, to the lower biomass of microbial predators and thus reduced predation in the northernmost sector.

Despite the high nitrate concentrations in surface waters in the North Bay, Kaneohe Bay is typically considered to be nitrogen-limited (Laws & Allen 1996, Ringuet 2003). The higher NO$_3^-$+NO$_2^-$ concentrations at the North Bay station could be derived primarily from NO$_3^-$+NO$_2^-$-enriched runoff (Hoover 2002) which was only elevated in surface waters. The rapid transport of runoff out of the bay via the northernmost channel may limit NO$_3^-$+NO$_2^-$ uptake by the phytoplankton community.

Significant impacts of nutrient-rich runoff do occur in the southern basin, where the residence time of freshwater runoff from storm events during the sampling period was on the order of days to weeks (Hoover 2002, Ringuet 2003). Nutrient subsidies to the plankton community due to dissolved nutrient loading during storm events and to remineralization of nutrients from sediments deposited by storms are likely more significant in this semi-enclosed sector of the bay.

While water residence time appears to govern total chlorophyll-α and plankton biomass of each sector in the bay, the relative biomass of each major size fraction is likely influenced primarily by factors within the biological community. Our results regarding relative abundance of plankton size fractions at each station and between
stations and temporal and interannual variability of each size fraction of the plankton community allow us to assess possible controls on community structure and trophic linkages.

Plankton community composition

Despite large variations over time and space, pico- and nanoplankton dominate plankton biomass and abundance in all three sectors of Kaneohe Bay. Picoplankton represented 20 - 45% of total community biomass and > 95% of total plankton abundance throughout the bay over both sampling periods. *Synechococcus* spp. (Syn) alone made up 25% of total autotrophic biomass, a value that compares favorably with those previously reported for the contribution of Syn to total Chl in these waters (20-40%, Laws & Allen 1996). Among subtropical systems, pico-, nano- and microplankton biomasses at the SB and CB stations were more similar to the coastal Arabian Sea (Garrison et al. 2000) than to the central North Pacific subtropical gyre (NPSG, Calbet & Landry 1999, Calbet et al. 2001), while the more northern sector of Kaneohe Bay fell within the NPSG range of picoplankton and nanoplankton biomass.

Zooplankton size structure reflected the dominance of small primary producers over the entire 5-yr sampling period, as the community was comprised primarily of small copepods with the ability to feed on pico- and nanoplankton (Calbet et al. 2000, Roff et al. 1995). The dominance of small copepods, particularly *Oithona* spp. and *Parvocalanus crassirostris*, and the numerical dominance of copepod nauplii, has been noted in several other coastal subtropical systems (Murrel & Lores 2004, Hopcroft et al. 1998b). The success of small copepods in tropical coastal waters suggests that they have
an advantage over larger species. A recent study on the grazing capabilities of adult copepods in Kaneohe Bay found that the smallest species, *Oithona simplex*, had the highest weight-specific ingestion rates, while the largest copepod in the study (*Bestiolina similis*, reported as *Acrocalanus inermis* in Calbet et al. 2000), exhibited much lower weight-specific rates. The smaller copepods also exhibited higher growth rates than the largest species (Calbet et al. 2000). The ability of these small copepods to grow at rates equivalent to their protistan prey (Landry et al. 1984) make them well suited to take advantage of event-scale increases in their prey populations.

While copepods numerically dominated micro- and mesozooplankton throughout the study period, gelatinous zooplankton also made up a large percentage of the mesozooplankton and likely played a significant role in structuring the plankton community. The dominant appendicularians in Kaneohe Bay, *Oikopleura longicauda* and *O. fusiformis*, are warm water species associated with both coastal and open ocean waters (Fenaux et al. 1998). Although they are classified as mesozooplankton, *O. longicauda* and *O. fusiformis* in Kaneohe Bay can clear prey ranging in size from 0.5-μm heterotrophic bacteria to > 10-μm nanoplankton at rates that greatly exceed those of co-occurring copepods (Chapters 2 & 3, Scheinberg et al. in press; Calbet et al. 2000). Based on grazing studies described in Chapters 2 and 3, at typical abundances of > 1 ind l⁻¹, appendicularians could clear the entire water column of microbial prey daily. In turn, appendicularians are the preferred prey for the chaetognath, *Sagitta enflata*, in these waters (Kimmerer 1984). The appendicularians in Kaneohe Bay thus are important, both as grazers on a wide range of prey sizes and as a food source for higher trophic levels.
Microbial food web structure

Although picoplankton biomass was substantial throughout the study period, a shift in the importance of picoplankton relative to total plankton biomass in southern Kaneohe Bay occurred from 1998-99 to 2001-02. A significantly higher ratio of picoplankton prey to 2-5 μm heterotrophic predators (HNAN) also occurred in 1998-99. The 2-5 μm size fraction of HNAN represents the dominant predators of picoplankton and has been shown to exert grazing pressure sufficient to control picoplankton prey in tropical waters, despite high growth rates of the latter (Calbet & Landry 1999). Given the mean abundances of HNAN in 1998-99 and assuming a typical clearance rate of 3.5 x 10^5 biovolumes per hour for heterotrophic protists (Landry et al. 2000), grazing by 2-5 μm HNAN should result in picophytoplankton mortality rates of 0.6 - 1.0 d⁻¹, which are comparable to measured mortality rates in these waters (e.g., 0.51-1.09 d⁻¹ for Hbact; Landry et al. 1984). These loss rates do not account for the grazing contributions of appendicularians and copepods and are therefore less than contemporaneous rate estimates of bacterial and picophytoplankton growth (0.87-1.98 d⁻¹; Landry et al. 1984). Since the gross growth rates are large, however, relatively modest differences in net picoplankton growth rates could easily explain the differences in the relative biomass of picoplankton to HNAN biomass between the sampling periods. Specifically, the elevated water temperature in August 1998 may have enhanced picoplankton growth or nutrient remineralization from sediments, leading to higher microbial biomass during this period.

In spite of the interannual variability in the ratio of picoplankton to HNAN, there is both spatial and fine-scale temporal evidence for tight trophic coupling between
picoplankton and then nanoplankton grazers, and between nanoplankton and their micro- and mesozooplankton grazers, throughout the bay. 2-5 μm HNAN appear capable of contributing significantly to grazing regulation of picoplankton in this system. The mean ratio of pico- to nanoplankton biomass and the ratio of picoplankton to their 2-5 μm HNAN grazers did not differ significantly between stations (p > 0.05) despite large differences in total biomass and enhanced nutrient retention in southern Kaneohe Bay.

Biomass ratios did differ, however, for larger size fractions. For example, the North Bay had both the lowest ratio of nano- to microplankton biomass and the highest ratio of micro- to mesozooplankton biomass of the three stations. Size-fraction biomass data suggest that protistan grazing maintains a relative constancy of pico- and nanoplankton populations at all three stations, but that mesozooplankton contributions vary across the trophic gradient. A decrease in abundance and biomass of picoplankton during the wet season further suggests that protists contribute most directly to picoplankton control. At both SB and CB stations, the ratio between pico- and 2-5 μm HNAN was significantly higher during the dry season, when picoplankton biomass was highest.

The unique positive relationship of > 10-μm Aeuks with rainfall indicates that they are uncoupled from the dynamics of the rest of the nanoplankton and the picoplankton size fractions. Although their mean abundances were higher overall during the dry season, increases in > 10-μm Aeuks correlated with direct rainfall in both 1998-99 (both 10 - 20 and > 20-μm Aeuks) and 2001-02 (10 – 20 μm Aeuks). Diatoms and dinoflagellates comprise the majority of this size fraction and have been previously observed to dominate blooms following rain events in Hawaii (Ringuet 2003). Direct
rainfall represents a potentially significant source of new nitrogen to the bay (D. Hoover, pers. comm.) that may be more readily available to this larger size fraction due to grazing regulation of nano- and picoplankton. A significant correlation was not observed between > 20-μm Aeusks and direct rainfall in 2001-02, but statistics for this rather scarce size fraction may not have been robust enough to accurately reflect their variability. The temporal dynamics of rain runoff perturbations are further explored in Chapter 5.

Seasonal differences in microbial biomass were most significant in southern Kaneohe Bay (Stn SB). Increased microbial biomass occurs simultaneously with the appearance of mesoplankton predators (hydromedusae and chaetognaths) and major grazers (appendicularians). The increased abundance of predators may have a positive effect on the abundances of picoplankton by reducing copepod abundance, which would release smaller nanoplankton from copepod grazing pressure (see following sections). The South Bay also differs from other sectors of the bay in the abundance of organically rich sediments (Smith et al. 1981, Smith & McMurtry 1995). Nutrient release from sediments likely provides a significant nutritional subsidy to the South Bay ecosystem, and nutrient release may be greater during warmer months due to faster remineralization rates.

**Zooplankton influence on microbial community structure**

A shift in the ratio of picoplankton to 2-5 μm HNAN, both seasonally and interannually may be explained, at least in part, by the grazing capabilities of copepods in Kaneohe Bay. Adult and juvenile copepod abundances were at a maximum during the warmest months of the year. Based on measured ingestion rates of the dominant adult copepods in Kaneohe Bay (40,000 cells cop⁻¹ d⁻¹ for *Oithona simplex*, 120,000 cells cop⁻¹
for *O. nana*, *Parvocalanus crassirostris* and *Bestiolina similis*; Calbet et al. 2000), these grazers could have consumed up to 40% of 2-5-μm HNAN standing stock daily at maximum abundances during the dry season. This estimate of copepod grazing impact is likely conservative, as it does not take into account grazing by juveniles, which would presumably also consume this size fraction of prey. Coupled with their high potential growth rates at ambient bay temperatures, total copepod grazing could have contributed to the observed seasonal shift in relative abundance between nano- and picoplankton. A strong, positive relationship between nauplii and copepod prey abundances, especially with > 2-μm Aeuks, support increased copepod feeding during the drier period of the year.

Appendicularians also likely played a significant role in seasonal and interannual variations in total pico- and nanoplankton. Appendixularians were the only group of zooplankton to have significantly higher abundances during the wet season, and to exhibit a significant inverse relationship with microbial abundances. Appendixularians covaried negatively with the abundance of picoeukaryotes during the 2001-02 sampling period, explaining over 70% of the variability in Peuk abundance at appendicularian abundances greater than 0.2 ind 1−1. A decline in the ratio of Peuks:Syn during the wet season may also reflect an enhanced appendicularian grazing influence as Syn are ingested but not digested by appendicularians (Gorsky et al. 1999). Like other cyanobacteria, *Synechococcus* spp. cells have an outer coating that allows them to pass through appendicularian digestive systems intact. While Syn abundances remained relatively constant throughout the year, Peuks decreased significantly in the wet season.
A resistance to digestion may also explain, at least in part, the constant Syn abundances between stations. Perhaps *Crocosphaera* spp. were also indigestible and thus had less grazing mortality than other autotrophs of comparable size resulting in their observed lower temporal and spatial variability.

Higher and more consistent appendicularian abundances in 1998-99 compared to 2001-02 may also have influenced the relative proportion of nanoplankton to picoplankton. As developed in Chapters 2 and 3, the dominant appendicularians in Kaneohe Bay, *Oikopleura longicauda* and *O. fusiformis*, clear picoeukaryotes (Peuks) and nanoplankton from the water column at significantly higher efficiencies than the smaller picoplankton components, Hbact and Syn (Scheinberg et al. in press). Size selective grazing by appendicularians may thus result in a disproportionate decrease of nanoplankton, similar to what was observed during the 1998-99 sampling period.

In contrast to the decline in microbial community biomass, mean chlorophyll *a* in 2002 was double that in 1998. While this doubling is consistent with the higher biomass of 10 – 20 μm Aeuks in 2002, this size fraction alone cannot account for the increase in total chlorophyll *a*. Appendicularians were negatively correlated with Chl *a* during the 1998-99 sampling period and their higher abundances during this period likely played a role in maintaining low Chl *a* concentrations. Applying measured clearance rates of appendicularians in Kaneohe Bay to observed appendicularian abundances during this period, appendicularians could have cleared $\geq 75\%$ of the water column daily during over half of the study period and $> 100\%$ during the period of lowest Chl concentrations. The ratio of total autotrophic carbon to Chl was also significantly different between the two
sampling periods, with mean values of 119 and 50 in 1998 and 2002, respectively. Higher solar radiation in 1998 may have led to a decrease in cellular Chl relative to carbon in nano- and picophytoplankton. Higher nutrient availability could also have contributed to higher picophytoplankton growth rates and thus higher C:Chl values in 1998.

**Predation on mesozooplankton**

Although appendicularians are the preferred prey of chaetognaths in Kaneohe Bay (Kimmerer 1984), an alteration in the structure of the gelatinous zooplankton community from 1998 to 2002 suggests that hydromedusae, and not chaetognaths, are more important in controlling appendicularian abundances in this system. Jellyfish are known to be voracious predators of both appendicularians and chaetognaths (Purcell et al. in press), and the gut contents of *Obelia* spp. and a small, unidentified hydromedusa collected in the bay indicate that both were feeding on appendicularians during this study. Appendicularians were consistently higher in abundance in 1998 than in 2001, when hydromedusae abundances were significantly lower. This relationship was also evident in shorter time-scale variability in abundances of these two gelatinous zooplankters.

While appendicularians have the capability to respond rapidly to increases in food, with doubling times typically on the order of 6-12 h (Hopcroft et al. 1998b), the several month lag between the appearance of appendicularians in the 2001-02 data set and the decline in their food suggests that they are being prevented from overwhelming their prey in Kaneohe Bay. As with interannual trends, seasonal variability in the relative abundance of appendicularians and hydromedusae suggest that appendicularians could be
controlled primarily by hydromedusae predation throughout most of the year. Appendicularians only appeared in the bay during 2001-02 in the wet season, coincident with a significant decrease in hydromedusae, and were consistently higher in abundance in 1998-99, when hydromedusae were virtually absent from the bay. While the negative correlation in abundance between these taxa could indicate predator/prey interaction, greater than 50% of the variability in appendicularian abundance can also be explained by freshwater runoff into the bay. It thus appears that appendicularians might be able to take advantage of increasing prey concentration following runoff events and temporarily escape control by predation.

Unlike appendicularians, copepod abundance was not directly related to the abundance of their gelatinous predators. However, the high relative abundance of small copepods may be due to a size-related escape from predation. *Sagitta enflata* feed selectively on larger copepod species in Kaneohe Bay (Kimmerer 1984). Visual predators, such as fish, also likely feed disproportionately on larger copepods. The more frequent occurrence of larger copepods, such as *Acartia* spp. and *Undinula darwinii*, in the North Bay may thus be due to the lower biomass of chaetognaths, and perhaps larval fish, at this station.

**The importance of cyanobacteria**

An unexpected outcome of this investigation was the discovery of significant populations of the N$_2$-fixing cyanobacterium, *Crocosphaera* spp., throughout Kaneohe Bay. *Crocosphaera* spp. occurred in remarkably high abundances at all stations and made up 50% of total cyanobacteria biomass. The presence of *Crocosphaera* spp.
indicates that a significant and previously unknown source of ‘new’ nitrogen may thus exist in these commonly nitrogen-limited waters. *Crocosphaera* spp.-derived nitrogen may help to explain the high standing microbial biomass in this meso- to oligotrophic system, but additional research is needed to better characterize the role and importance of these organisms in Kaneohe Bay.

*Crocosphaera* spp., previously described as marine *Synechocystis* spp., are known to occur in other tropical and subtropical waters (Waterbury & Willey 1988, Zehr et al. 2001). However, little is known about their ecological importance. The size range of the cells in Kaneohe Bay (2 – 20 μm) is typical for these organisms and suggests that several strains were present during this study (Zehr et al. 2001). Mean abundances for *Crocosphaera* spp. were similar to the maximum concentrations observed during bloom conditions in the NPSG (approximately 1000 cells ml⁻¹, Campbell et al. 1997), and ranged from 200 – 70,000 cells ml⁻¹ over the sampling period.

While the actual N₂-fixing activity of *Crocosphaera* spp. in Kaneohe Bay is unknown, we can estimate their potential N₂ fixation rate using the cell-specific estimates of 5 fmol N cell⁻¹ d⁻¹ from Zehr et al. (2001). At this rate, *Crocosphaera* spp. populations in Kaneohe Bay could account for new nitrogen production of 460 – 1100 μmol N m⁻² d⁻¹ (integrated over a 12-m water column). Actual rates could be slightly higher, as *Crocosphaera* spp abundances in Kaneohe Bay increased with depth. Based on a typical primary production rate of 100 mmol C m⁻² d⁻¹ (Ringuet 2003), net production of 15% and a C:N molar ratio of 6.7 (Redfield 1963), N₂-fixation by Crocos could provide on the order of 25-50% of the “new” N requirement in the bay.
The significantly greater abundance of *Crocosphaera* spp. at depth in southern Kaneohe Bay (Figure 8) and generally higher abundances of these organisms at depth in the northern sector, could be due to a number of factors. One possibility is photoinhibition in surface waters. Cells could also accumulate at or near the sediment surface if they remain viable in zooplankton fecal pellets and appendicularian houses exported from the surface layer and/or can be resuspended from the sediment. Lastly, *in situ* growth rate of Crocos could be enhanced near the water-sediment interface if nutrient or trace elements are elevated by remineralization processes. However, it seems unlikely that light conditions would be optimal in the turbid benthic boundary layer.


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and Impacts on Coastal Waters. PhD dissertation, Department of Oceanography, University of Hawaii, Honolulu


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(Synechococcus, Trichodesmium, and Crocosphaera spp.): identification of the IdiA protein. Applied and Environmental Microbiology 67(12):5444-5452


Table 1 Mean (± 1 sd) nutrient concentrations in the southern (SB), central (CB) and northern (NB) sectors of Kaneohe Bay, Oahu, Hawaii. Results from a sampling station offshore (OS) are included as well. Nitrate plus nitrite (NO$_3^-$ + NO$_2^-$), Ammonium (NH$_4^+$), dissolved inorganic nitrogen (DIN, = ((NO$_3^-$ + NO$_2^-$) + NH$_4^+$), dissolved organic nitrogen (DON), dissolved inorganic phosphorus (DIN), dissolved organic phosphorus (DOP) and silicate are reported in micromoles per liter (µM). Data are from Kinzie III et al. (2001). Values in parentheses are numbers of samples analyzed.

<table>
<thead>
<tr>
<th>Nutrients</th>
<th>SB</th>
<th>CB</th>
<th>NB</th>
<th>OS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nitrogen</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NO$_3^-$ + NO$_2^-$</td>
<td>0.053 ± 0.12</td>
<td>0.093 ± 0.28</td>
<td>0.28 ± 0.32</td>
<td>0.053 ± 0.054</td>
</tr>
<tr>
<td>NH$_4^+$</td>
<td>0.12 ± 0.10</td>
<td>0.14 ± 0.16</td>
<td>0.14 ± 0.11</td>
<td>0.12 ± 0.07</td>
</tr>
<tr>
<td>DIN</td>
<td>0.17 ± 0.17</td>
<td>0.23 ± 0.35</td>
<td>0.42 ± 0.36</td>
<td>0.17 ± 0.08</td>
</tr>
<tr>
<td>DON</td>
<td>7.2 ± 1.4</td>
<td>6.9 ± 1.5</td>
<td>6.6 ± 1.4</td>
<td>5.7 ± 1.5</td>
</tr>
<tr>
<td>Phosphorus</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DIP</td>
<td>0.075 ± 0.030</td>
<td>0.064 ± 0.041</td>
<td>0.070 ± 0.041</td>
<td>0.094 ± 0.029</td>
</tr>
<tr>
<td>DOP</td>
<td>0.25 ± 0.05</td>
<td>0.24 ± 0.06</td>
<td>0.23 ± 0.06</td>
<td>0.23 ± 0.07</td>
</tr>
<tr>
<td>Silicate</td>
<td>6.9 ± 1.8</td>
<td>5.0 ± 2.0</td>
<td>7.1 ± 1.7</td>
<td>2.3 ± 0.6</td>
</tr>
</tbody>
</table>

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Table 2: Mean microbial community abundance (cells ml^{-1}) and biomass (µg C l^{-1}) at stations SB, CB and NB in Kaneohe Bay during 2001-02. Picoplankton (< 2 µm) and nanoplankton (2 – 20 µm) were measured via flow cytometry and epifluorescence microscopy, respectively. Errors represent 95% confidence intervals of the mean.

<table>
<thead>
<tr>
<th></th>
<th>SB</th>
<th>CB</th>
<th>NB</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Abundance</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Picoplankton</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hbact</td>
<td>5.8 ± 0.8 x 10^5</td>
<td>4.8 ± 0.9 x 10^5</td>
<td>3.6 ± 0.5 x 10^5</td>
</tr>
<tr>
<td>Syn</td>
<td>1.1 ± 0.1 x 10^5</td>
<td>1.0 ± 0.1 x 10^5</td>
<td>0.86 ± 0.23 x 10^5</td>
</tr>
<tr>
<td>Peuks</td>
<td>7500 ± 2500</td>
<td>5100 ± 1200</td>
<td>3700 ± 900</td>
</tr>
<tr>
<td>Heuks</td>
<td>3500 ± 1100</td>
<td>2300 ± 800</td>
<td>1700 ± 600</td>
</tr>
<tr>
<td>Crocos</td>
<td>230 ± 160</td>
<td>100 ± 60</td>
<td>500 ± 300</td>
</tr>
<tr>
<td>Total picoplankton</td>
<td>7.0 ± 0.8 x 10^5</td>
<td>5.9 ± 1.0 x 10^5</td>
<td>4.5 ± 0.5 x 10^5</td>
</tr>
<tr>
<td><strong>Nanoplankton</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aeuks</td>
<td>2800 ± 800</td>
<td>1600 ± 600</td>
<td>1200 ± 500</td>
</tr>
<tr>
<td>Heuks</td>
<td>5800 ± 1600</td>
<td>3200 ± 1100</td>
<td>3100 ± 1100</td>
</tr>
<tr>
<td>Crocos</td>
<td>2100 ± 600</td>
<td>1100 ± 300</td>
<td>2600 ± 2000</td>
</tr>
<tr>
<td>Total nanoplankton</td>
<td>1.1 ± 0.3 x 10^4</td>
<td>5900 ± 1900</td>
<td>6800 ± 2100</td>
</tr>
<tr>
<td><strong>Biomass</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Picoplankton</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hbact</td>
<td>17 ± 2</td>
<td>13 ± 2</td>
<td>10 ± 1</td>
</tr>
<tr>
<td>Syn</td>
<td>10 ± 1</td>
<td>9.2 ± 1.3</td>
<td>7.7 ± 2.1</td>
</tr>
<tr>
<td>Peuks</td>
<td>3.4 ± 1.2</td>
<td>2.2 ± 0.5</td>
<td>1.6 ± 0.4</td>
</tr>
<tr>
<td>Heuks</td>
<td>1.3 ± 0.4</td>
<td>0.83 ± 0.31</td>
<td>0.81 ± 0.33</td>
</tr>
<tr>
<td>Crocos</td>
<td>0.11 ± 0.07</td>
<td>0.052 ± 0.029</td>
<td>0.24 ± 0.13</td>
</tr>
<tr>
<td>Total picoplankton</td>
<td>31 ± 5</td>
<td>23 ± 4</td>
<td>19 ± 5</td>
</tr>
<tr>
<td><strong>Nanoplankton</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aeuks</td>
<td>54 ± 21</td>
<td>33 ± 18</td>
<td>19 ± 7</td>
</tr>
<tr>
<td>Heuks</td>
<td>45 ± 19</td>
<td>22 ± 9</td>
<td>13 ± 4</td>
</tr>
<tr>
<td>Crocos</td>
<td>13 ± 5</td>
<td>5.7 ± 1.6</td>
<td>13 ± 5</td>
</tr>
<tr>
<td>Total nanoplankton</td>
<td>110 ± 40</td>
<td>60 ± 23</td>
<td>44 ± 13</td>
</tr>
</tbody>
</table>

Table 3 Mean abundance (ind l⁻¹) and size (mm) of the dominant zooplankton at station SB, CB and NB in Kaneohe Bay, Hawaii over the 2001-02 period. Size class for biomass estimates is included for reference. Errors are 95% confidence intervals of the mean. For size and abundance, n ≥ 100. na = not available.

<table>
<thead>
<tr>
<th>Individual Biomass</th>
<th>Abundance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Size Class</td>
<td>SB</td>
</tr>
<tr>
<td>Gelatinous zooplankton</td>
<td></td>
</tr>
<tr>
<td>Sagitta enflata stage 1</td>
<td>2.7 ± 1.3</td>
</tr>
<tr>
<td>Sagitta enflata stage 2</td>
<td>9.7 ± 1.3</td>
</tr>
<tr>
<td>Sagitta enflata stage 3</td>
<td>11 ± 1</td>
</tr>
<tr>
<td>Total Chaetognaths</td>
<td>0.37 ± 0.07</td>
</tr>
<tr>
<td>Oikopleura spp. juveniles</td>
<td>0.32 ± 0.13</td>
</tr>
<tr>
<td>Oikopleura spp. adults</td>
<td>0.70 ± 0.06</td>
</tr>
<tr>
<td>Total appendicularians</td>
<td>0.37 ± 0.23</td>
</tr>
<tr>
<td>Hydromedusa</td>
<td>0.31 ± 0.07</td>
</tr>
<tr>
<td>Obelia spp.</td>
<td>2.1 ± 0.4</td>
</tr>
<tr>
<td>Total Medusa</td>
<td>0.28 ± 0.15</td>
</tr>
<tr>
<td>Copepods</td>
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<tr>
<td>Nauplii</td>
<td>0.10 ± 0.02</td>
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<tr>
<td>Calanoid juvenile</td>
<td>0.27 ± 0.07</td>
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<tr>
<td>Cyclopoid juveniles</td>
<td>0.21 ± 0.03</td>
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<tr>
<td>Total juvenile copepods</td>
<td>0.49 ± 0.01</td>
</tr>
<tr>
<td>Bestiolina similis</td>
<td>0.36 ± 0.00</td>
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<td>Parvocalanus crassirostris</td>
<td>0.90 ± 0.02</td>
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<tr>
<td>Acartia sp.</td>
<td>1.8 ± 0.1</td>
</tr>
<tr>
<td>Undinula darwinii</td>
<td>0.25 ± 0.01</td>
</tr>
<tr>
<td>Oithona simplex</td>
<td>0.34 ± 0.01</td>
</tr>
<tr>
<td>Total adult calanoids</td>
<td>2.2 ± 0.3</td>
</tr>
<tr>
<td>Total adult cyclopoids</td>
<td>6.8 ± 1.3</td>
</tr>
<tr>
<td>Harpacticoids</td>
<td>0.29 ± 0.02</td>
</tr>
<tr>
<td>Total adult copepods</td>
<td>9.0 ± 1.5</td>
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<tr>
<td>Other zooplankton</td>
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<tr>
<td>Crab zoea</td>
<td>0.80 ± 0.11</td>
</tr>
<tr>
<td>Decapods, Lucifer chacei</td>
<td>2.7 ± 0.8</td>
</tr>
<tr>
<td>Total other crustaceans</td>
<td>26 ± 8E⁻³</td>
</tr>
<tr>
<td>Molluscs</td>
<td>0.26 ± 0.03</td>
</tr>
<tr>
<td>Polychaetes</td>
<td>0.16 ± 0.04</td>
</tr>
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</table>
Table 4 Mean microbial community abundances (cells ml\(^{-1}\)) and biomass (µg C l\(^{-1}\)) in southern Kaneohe Bay, Oahu, Hawaii from July through March during two sampling periods (1998-99 and 2001-02). Picoplankton (< 2 µm) and nanoplanckton (2 – 20 µm) were measured via flow cytometry and epifluorescence microscopy, respectively. Errors represent 95% confidence intervals of the mean.

<table>
<thead>
<tr>
<th></th>
<th>1998-99</th>
<th>2001-02</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Abundance</strong></td>
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<td></td>
</tr>
<tr>
<td><em>Picoplankton</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hbact</td>
<td>16 ± 2x10(^5)</td>
<td>5.4 ± 0.1x10(^5)</td>
</tr>
<tr>
<td>Syn</td>
<td>2.2 ± 0.3x10(^5)</td>
<td>1.0 ± 0.2x10(^5)</td>
</tr>
<tr>
<td>Peuks</td>
<td>4300 ± 2700</td>
<td>6000 ± 1400</td>
</tr>
<tr>
<td>Total picoplankton</td>
<td>18 ± 2x10(^5)</td>
<td>6.9 ± 1.4x10(^5)</td>
</tr>
<tr>
<td><em>Nanoplankton</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aeuks</td>
<td>7600 ± 1500</td>
<td>1900 ± 800</td>
</tr>
<tr>
<td>Heuks</td>
<td>4800 ± 1200</td>
<td>3800 ± 1600</td>
</tr>
<tr>
<td>Total nanoplanckton</td>
<td>1.2 ± 0.2x10(^4)</td>
<td>0.57 ± 0.22x10(^4)</td>
</tr>
<tr>
<td><strong>Biomass</strong></td>
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<td></td>
</tr>
<tr>
<td><em>Picoplankton</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hbact</td>
<td>42 ± 4</td>
<td>16 ± 8</td>
</tr>
<tr>
<td>Syn</td>
<td>19 ± 2</td>
<td>9.3 ± 1.6</td>
</tr>
<tr>
<td>Peuks</td>
<td>1.9 ± 1.2</td>
<td>3.1 ± 0.7</td>
</tr>
<tr>
<td>Total picoplankton</td>
<td>63 ± 7</td>
<td>25 ± 6</td>
</tr>
<tr>
<td><em>Nanoplankton</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aeuks</td>
<td>59 ± 11</td>
<td>40 ± 19</td>
</tr>
<tr>
<td>Heuks</td>
<td>22 ± 6</td>
<td>26 ± 13</td>
</tr>
<tr>
<td>Total nanoplanckton</td>
<td>82 ± 13</td>
<td>66 ± 31</td>
</tr>
</tbody>
</table>

Hbact = heterotrophic bacteria, Syn = *Synechococcus* spp., Peuks = autotrophic picoeukaryotes, Aeuks = autotrophic nanoeukaryotes, Heuks = heterotrophic nanoeukaryotes.
FIGURE LEGENDS

Fig. 1  Map of Kaneohe Bay, Oahu, Hawaii. Samples for zooplankton analyses were collected at stations in the northern (NB), central (CB) and southern sectors (SB). Topographic contours are at 100-ft intervals. Locations of the Luluku rain gage and Kaneohe stream gage in the watershed (green shaded area) and the rain gage at Honolulu International Airport (HIA, inset map) are shown for reference. Rainfall isohyets on inset map are in m y$^{-1}$.

Fig. 2  Dominant circulation patterns in Kaneohe Bay, Hawaii (after Smith et al. 1981).

Fig. 3  Water temperature (A), solar radiation (B) and freshwater input (C) in Kaneohe Bay over the 15-mo sampling period from 2001-02. Rainfall is reported for the USGS Luluku rain gage (top) and the NOAA rain gage on Coconut Island in southern Kaneohe Bay (middle) and stream discharge is reported for Kaneohe Stream (bottom).

Fig. 4  Vertical profiles of A) temperature ($^\circ$ C), B) salinity and C) total chlorophyll $a$ concentration ($\mu$g l$^{-1}$) at stations SB (south bay), CB (central bay) and NB (north bay) over the 2001-02 sampling period. Plots represent data from depths of 0, 3, 6, 9 and 12 m.
Fig. 5  Nutrient concentrations at southern (SB), central (CB), northern (NB) and offshore (OS) stations in Kaneohe Bay, Oahu, Hawaii during the 2001-02 sampling period. Data are from Kinzie et al. 2001.

Fig. 6  Mean microbial community biomass in southern (SB), central (CB) and northern (NB) Kaneohe Bay during 2001-02. Autotrophs (green), heterotrophs (blue) and cyanobacteria (yellow) are divided into five size fractions, <2, 2-5, 5-10, 10-20 and >20 μm. Size fractions include both prokaryotes and eukaryotes.

Fig. 7  Picoplankton abundance (cells ml⁻¹) at stations SB (south bay), CB (central bay) and NB (north bay) over the 2001 sampling period. Autotrophic picoeukaryotes (A), *Synechococcus* spp. (B) and heterotrophic bacteria (C). Data is reported from depths of 0, 3, 6, 9 and 12 m.

Fig. 8  Mean biomass of cyanobacteria (μg C l⁻¹) at 3 m (yellow) and 12 m(gray) in southern (SB), central (CB) and northern (NB) Kaneohe Bay during the 2001-02 sampling period. Error represents 95% confidence intervals of the mean. High error in the 2 – 5 and 5 – 10 μm size fraction at station NB was due to two days of bloom conditions at depth during the sampling period. Note the variation in y-axis scales between stations.
Fig. 9 Nano- and microplankton abundance over the 2001-02 sampling period at stations SB (left), CB (center) and NB (right). Autotrophic eukaryotes (A), heterotrophic eukaryotes (B) and Crocosphaera spp. (C) are divided into four size classes, 2-5 \( \mu m \) (green), 5-10 \( \mu m \) (blue), 10-20 \( \mu m \) (gray) and > 20 \( \mu m \) (red). Note the difference in y-axis scales between plots A, B and C.

Fig. 10 Relationship between 10-20 \( \mu m \) autotrophic eukaryotes (red; Aeusks), total cells in the nanoplankton size fraction (black) at station SB and rainfall measured at the USGS Luluku weather station (\( R^2 = 0.54 \) and 0.45, respectively; \( p < 0.0005 \)) in 2001-02.

Fig. 11 Relationship between *Synechococcus* spp. (cells ml\(^{-1}\)) and temperature (\( ^\circ C \)) at station SB, CB and NB in Kaneohe Bay, Oahu, Hawaii. \( R^2 \) values are 0.23, 0.40 and 0.61 and \( p \)-values were < 0.02, 0.001 and 0.0001 for stations SB, CB and NB, respectively.

Fig. 12 Percent abundance of major groups of meso- and microzooplankton in size fractions corresponding to those used to determine biomass (Figure 15). Data represent geometric mean averages. A minimum of 100 individual animals of each size class in each group were measured.

Fig. 13 Mean relative abundance of the dominant groups of zooplankton in Kaneohe bay during the 2001-02 sampling period at stations SB, CB and NB.
Fig. 14 Mean relative abundance of copepod species at stations SB, CB and NB over the 2001-02 sampling period.

Fig. 15 Mean zooplankton biomass at three stations in Kaneohe Bay. Biomass is divided into 4 size fractions, 0.06-0.2 mm (red), 0.2-0.5 mm (green), 0.5-1.0 mm (yellow) and > 1.0 mm (blue).

Fig. 16 Zooplankton community abundances (ind l⁻¹) over the 2001-02 sampling period. Copepods (A), gelatinous zooplankton (B) and other zooplankton (C). Abundances are presented for South Bay (left), Central Bay (center) and North Bay (right).

Fig. 17 Relationships between copepod nauplii and their > 2 µm prey at station SB (top), CB (middle), and NB (bottom). Linear fits at CB and NB are for all prey (autotrophs and heterotrophs) combined. R² values are 0.40 for both CB and NB, with p < 0.01 (CB) and P < 0.05 (NB). No significant trend was found at SB.

Fig. 18 Relationship between appendicularians and autotrophic picoeukaryotes (Peuks) at station SB during the 2001-02 sampling cycle. R² = 0.7, p < 0.0002 for curvilinear fit.

Fig. 19 Relationship between appendicularian abundance in southern Kaneohe Bay and freshwater runoff at the USGS Kaneohe Stream gaging station (R² = 0.53, p < 0.0001).
Fig. 20 Temporal variability in chaetognath (green), appendicularian (red), and medusa (blue) abundance at three stations in Kaneohe Bay during the 2001-02 sampling period. Lines are three point running means. Note the difference in scale for station NB.

Fig. 21 Relationship between chaetognaths and hydromedusae at three stations in Kaneohe Bay.

Fig. 22 Water temperature (A), solar radiation (B), and rainfall (C) in southern Kaneohe Bay during the 1998-99 (green) and 2001-02 (blue) sampling periods. Rainfall is reported from the USGS Luluku rain gage (top panel) located near the base of the Koolau mountain range in Kaneohe and the NOAA Coconut Island rain gage (bottom panel) located in southern Kaneohe Bay.

Fig. 23 Annual rainfall at Honolulu International Airport, located on the leeward coast of Oahu, and at the USGS Luluku rain gage, located near the windward base of the Koolau mountain range in Kaneohe. The red line illustrates the long-term median at each station.

Fig. 24 A) Chlorophyll a concentrations at station SB during the two study periods; B) Mean chlorophyll a concentrations at station SB during equivalent 8 month periods (August – March) in 1998-99 (black) and 2001-02 (green).
Fig. 25  Mean biomass of heterotrophic bacteria (Hbact), *Synechococcus* spp. (Syn), and autotrophic picoeukaryotes (Peuks) at station SB from July to March during the 1998-99 and 2001-02 sampling periods. Error bars are 95% confidence limits of the mean.

Fig. 26  Mean autotrophic eukaryote (Aeuk) abundance (cells ml$^{-1}$) and biomass (µg C l$^{-1}$) during the 1998-99 and 2001-02 sampling periods in southern Kaneohe Bay. Abundance is plotted on a log scale. Error bars are 95% confidence limits of the mean.

Fig. 27  Picoplankton abundance (cells ml$^{-1}$) in southern Kaneohe Bay during the 1998-99 (top) and 2001-02 (bottom) sampling periods. The picoplankton size fraction was divided into three groups: heterotrophic bacteria (Hbact), *Synechococcus* spp. (Syn), and autotrophic eukaryotes (Aeucks).

Fig. 28  Abundance of autotrophic (Aeucks) and heterotrophic (Heuks) nanoplankton in southern Kaneohe Bay during the 1998-99 (top) and 2001-02 (bottom) sampling periods. Nanoplankton are divided into four size categories: 2-5 µm (blue), 5-10 µm (green), 10-20 µm (gray), and >20 µm (red).

Fig. 29  Relationship between 10-20 µm (red) and >20 µm (black) autotrophic eukaryotes (Aeucks, cells ml$^{-1}$) in southern Kaneohe Bay and rainfall (mm) at the Coconut Island rain gage during the 1998-99 sampling period. $R^2 = 0.48$ and 0.57 for 10-20 µm and >20 µm Aeucks respectively, with $p < 0.001$ for both.
Fig. 30 Relative abundance of dominant copepod species in southern Kaneohe Bay during the 1998-99 sampling period.

Fig. 31 Relative abundances (ind l$^{-1}$) of copepods (top) and gelatinous zooplankton (bottom) in southern Kaneohe Bay from July to March during the 1998-99 and 2001-02 sampling periods. Error bars represent 95% confidence intervals of the mean.

Fig. 32 Mesozooplankton abundance (ind l$^{-1}$) in southern Kaneohe Bay during the 1998-99 (top) and 2001-02 (bottom) sampling periods. Both copepods (calanoids in gray and cyclopoids in red) and gelatinous zooplankton (chaetognaths in green, appendicularians in red, and medusae in blue) include both adults and juveniles.

Fig. 33 Relationship between appendicularians (ind l$^{-1}$) and total chlorophyll $a$ in southern Kaneohe Bay (station SB) during the 1998-99 sampling period. $R^2 = 0.51$, $p < 0.0001$. 

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Honolulu International Airport

Luluku

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CHAPTER 5

ZOOPLANKTON RESPONSE TO A MAJOR STORM RUNOFF EVENT

INTRODUCTION

Much of our view of the world ocean derives from haphazard sampling that is not designed to characterize short-term (days – weeks) changes in the marine environment. Nonetheless, natural perturbation events that alter water-column conditions on short time scales, such as sudden increases in nutrient availability from upwelling or terrestrial runoff, are ubiquitous throughout the world’s oceans. The episodic nature of perturbation events causes them to be chronically undersampled, and data on these processes are often serendipitous (e.g. Roman et al. 1995). However, perturbation responses can play a significant role in structuring plankton communities and dynamics. In particular, episodic nutrient inputs can shift the balance between growth and grazing in plankton communities, prompting a successional response in primary and secondary production (e.g. Margalef 1967). These biological responses have significant implications for regional processes such as fish production, as well as for processes of global concern, such as carbon cycling. The nature of the response likely varies with the magnitude of inputs and the extent of nutrient limitation in the water column, and the system in which it occurs. In particular, temperate and tropical systems are likely to respond very differently due to fundamental differences in environmental conditions, such as temperature and light availability (Saenger & Holmes 1992).
Temperate waters experience strong annual cycles in both light and temperature. New nutrients are added to surface waters in the winter by storm mixing, but light and temperature are both low, typically preventing significant phytoplankton response until spring. The zooplankton response is further delayed, as organisms ‘wake up’ and recover from the physiological stress of winter conditions, leading to significant decoupling of bloom primary and secondary production (e.g. Colebrook 1979).

In tropical systems, light is abundant and temperatures are high throughout the year. The dynamics of primary producers, herbivores and predators in the plankton community are not strongly decoupled on a seasonal basis, and temperature-dependent responses to change can be quite rapid. In particular, rapid zooplankton responses are facilitated by the year-round presence of an established and metabolically active community. The responses of tropical zooplankton communities at upwelling zones in the Pacific and Indian oceans (Timonin 1963, Vinogradov & Voronina 1963, Roman et al. 2002), and to the passage of instability waves (Roman et al. 1995) occur on the order of days to weeks, not months as is the case for classic temperate and boreal blooms.

This contrast between temperate and tropical responses might be even greater in coastal waters, which generally are subjected to larger natural nutrient perturbations than oceanic waters, particularly with regard to storm runoff. Runoff from adjacent terrestrial systems commonly contains elevated concentrations of dissolved nutrients, especially nitrogen, which often is a primary limiting nutrient in coastal systems, and silicon, which can limit diatom growth. However, despite their likely importance, few studies have investigated the impact of episodic nutrient inputs on tropical coastal plankton.
communities. The studies that have been conducted have generally focused on phytoplankton (e.g. Jokiel et al. 1993, Ringuet 2003); thus, the response of tropical zooplankton to dramatic changes in their prey concentrations remains largely unknown.

Long-term sampling in Kaneohe Bay, a subtropical embayment on the island of Oahu, Hawaii, has shown that the zooplankton community in these waters varies temporally, but generally stays within oligotrophic to mesotrophic “boundaries” with respect to abundance and biomass (Chapter 4; Scheinberg & Landry in prep). Bay waters are subject to frequent and substantial inputs of new nutrients via storm runoff from the adjacent watersheds (Hoover 2002), but the impacts are transient (Ringuet & Mackenzie submitted) and traditional long-term sampling methodologies are poorly suited to characterizing short-term changes in plankton community structure and composition in response to storm events. Kaneohe Bay was thus selected as the site in which to establish an intensive sampling program to investigate the response of an established and quasi-steady state tropical coastal plankton assemblage to nutrient enrichment by storm runoff.

This study was conducted to determine the rates and magnitudes of response of tropical zooplankton to a nutrient perturbation event and to address the issues of bottom-up vs. top-down trophic interactions in coastal tropical waters. We expected that storm inputs of new nutrients would result in increased zooplankton abundance and biomass in this normally nutrient-limited system, and that this response would vary among groups of zooplankton due to differing reproductive and feeding strategies. Specifically, with instantaneous growth rates of ~ 2 d\(^{-1}\) at these temperatures (Hopcroft et al. 1998b), appendicularians were expected to respond rapidly and to exert significant grazing...
pressure on phytoplankton (Chapter 3, Scheinberg et al. in press). Copepod populations would likely increase more gradually than appendicularians due to their multi-step developmental process, with growth rates on the order of \( \sim 1 \text{ d}^{-1} \) (Huntley & Lopez 1992). Planktonic carnivores, such as chaetognaths, were also anticipated to respond rapidly to an increase in prey concentrations, leading to increased predation pressure and significant top-down control on community structure and abundance (cf. Szyper 1976, Kimmerer 1984).

**METHODS**

**Meteorology**

Rainfall and runoff into Kaneohe Bay were determined using data from the United States Geological Survey (USGS) Luluku rain gage, USGS Kaneohe stream gage, and the Hawaii Institute of Marine Biology (HIMB) weather station rain gage on Coconut Island in the southern sector of the bay (Figure 1). Solar radiation (\( \mu \text{m E m}^{-2} \text{ s}^{-1} \)) and wind speed (\( \text{km h}^{-1} \)) and direction (\( ^\circ \)) were also obtained from the HIMB weather station. Daily tidal range was obtained from the National Oceanographic and Atmospheric Administration (NOAA) tidal gage on Coconut Island.

**Field collection**

Intensive sampling was conducted following the first major storm event in a year of relatively normal rainfall. Several sites were established in southern Kaneohe Bay as part of an ongoing program monitoring bay water quality and response to runoff and other perturbations (Figure 1). Sites indicated in Figure 1 include those that directly pertain to
the present study (C Buoy and CRIMP), as well as those of previous monitoring programs in southern Kaneohe Bay used as a reference for baseline conditions (MP and SB; Kinzie III et al. 2001; Chapter 4, Scheinberg & Landry in prep). Daily sampling was conducted from the day of the storm, 29 November, through 20 December 2003, and continued on an approximately bi-weekly basis until March 2004. Zooplankton monitoring was restricted to station C Buoy, thus only data from this station will be presented here. Results from other studies on the bay-wide biogeochemical response to the storm will be presented elsewhere by Hoover et al. (in prep). Point sampling typically cannot distinguish explicitly between changes due to the temporal evolution of parameters and changes due to the advection of water past the sampling point. However, the residence time of water in southern Kaneohe Bay is long (~ 13 d, Bathen 1968) compared to the high growth rates (> 1 d⁻¹) of both autotrophic and heterotrophic populations at these temperatures (Newbury & Bartholomew 1976, Szyper 1976, Laws et al. 1984, Landry et al. 1984, Nielson & Kjørboe 1991, Hopcroft et al. 1998b). Thus, temporal changes are likely to be determined primarily by in situ processes rather than advection.

Water-column profiling was conducted using a YSI 6600 multi-parameter monitoring sonde. The sonde was configured to measure temperature, salinity, dissolved oxygen, pH, turbidity, chlorophyll-a fluorescence and dissolved oxygen every second as it was lowered through the water column from the surface to the bottom (0 – 15 m). Water samples were collected at the sea surface (~ 10 cm depth) and processed onshore for turbidity, salinity, dissolved nutrients (nitrate + nitrite, phosphate, silicate, ammonium,
total nitrogen, total phosphorus) and chlorophyll-a (details of methodology below). Net
tows (0.5-m diameter net, 64-μm Nitex mesh) were conducted beginning on 5 December
2003 to determine micro- and mesozooplankton abundance. Gentle vertical tows pulled
by hand minimized damage to fragile gelatinous animals, while the small mesh size
allowed for collection of nauplii, juvenile and adult copepods (Hopcroft et al. 1998a), all
post-hatching life history sizes of appendicularians (Fenaux 1998), as well as collection
of early stage chaetognaths (Baier & Purcell 1997). Net tow samples were preserved in
the field in 5% (final concentration) borax-buffered formalin.

**Nutrients**

Samples for analysis of dissolved nitrogen (NO₃⁻+NO₂⁻, NH₄⁺, total dissolved
nitrogen), soluble reactive phosphorus (SRP, historically reported as PO₄³⁻), total
dissolved phosphorus and silicate (Si(OH)₄) were filtered through acid-rinsed and
deionized H₂O-rinsed Whatman GF/C filters (nominal pore size of 1.2 μm). The filtrate
was frozen for later analysis using standard colorimetric methods (UNESCO 1994) on a
commercial autoanalyzer at the University of Hawaii.

**Phytoplankton abundance and pigment concentration**

Sample aliquots (20 – 100 mls) were filtered onto Whatman GF/C glass-fiber filters
for fluorometric analysis of chlorophyll a (Chl a). Filters were extracted in 5 ml of 90%
acetone in the dark at -20° C for 24 h, and Chl a was quantified using a Turner model
10AU fluorometer calibrated using HPLC measurements of pure pigment standards
(Holm-Hansen & Riemann 1978). Samples were also processed for pigment analysis by
high performance liquid chromatography (HPLC). Aliquots (0.3 - 1 liter) were filtered onto Whatman GF/C glass-fiber filters and frozen at -80 °C for later analysis. Prior to analysis, filters were placed in 5 ml of 100% acetone, ground using a hand-held glass/glass tissue homogenizer and extracted for 24 h at 0 °C in the dark. Pigment extracts were vortexed and centrifuged prior to analysis to remove cellular and filter debris. Samples (200 µl) of a mixture of 0.3 ml H₂O plus 3.0 ml extract were injected into a Varian 9012 HPLC system equipped with a Varian 9300 autosampler, a Timberline column heater (26 °C), and Spherisorb 5 µm ODS2 analytical (4.6 x 250 mm) column and corresponding guard cartridge. Pigments were detected with a ThermoSeparation UV2000 detector (λ = 436 nm). Pigment analyses were performed using the procedures described in Bidigare et al. (2004).

The relative abundance of net phytoplankton species (> 64 µm) was determined from preserved net tows. The large, often heterotrophic dinoflagellate, Protoperidinium spp., was present during this study and was included in the relative assessment of phytoplankton abundance as its trophic status was questionable. Phytoplankton species were identified according to Tomas (1997).

**Zooplankton biomass and abundance**

*Abundance.* Preserved net tow samples were washed through a 64-µm mesh sieve to remove preservative, diluted to 100 ml with 0.2-µm filtered seawater, and stained with Rose Bengal (in isopropyl alcohol, 0.05% final concentration) prior to analysis for abundance. A minimum of 100 individuals of each dominant family was counted using a
Leica Z9.5 stereomicroscope with an ocular micrometer. All dominant adults, juveniles and nauplii were classified to species and measured to the nearest 20 μm (n ≥ 50). Appendicularian trunk length was defined as the distance from the tip of the mouth to the posterior edge of the trunk. Chaetognaths were separated into three life stages based on gonad development (stage 1, 2 and 3) according to Casanova (1999). Stage 1, 2 and 3 chaetognaths typically are 3 ± 0.03, 9 ± 0.02 and 11 ± 0.03 mm in length in Kaneohe Bay (Chapter 4, Scheinberg & Landry in prep). Shrinkage of preserved gelatinous specimens was assumed to be 12% (Chapter 3, Scheinberg et al. in press). The dominant copepods, chaetognaths, appendicularians and cladocerans were identified to species according to Bückmann & Knapp (1975), Fenaux (1993), Bradford-Grieve et al. (1999), Casanova (1999) and Onbé (1999). Medusae were identified with the help of Bouillon (1999) and Wrobel & Mills (1998). All other organisms were sorted to the group level (polychaetes, bivalves, gastropods, crab larvae, decapod larvae, fish larvae, fish eggs).

**Biomass.** The biomass of appendicularians was estimated from the ash-free dry weight (W, μg) to trunk length (TL, μm) relationships of Hopcroft et al. (1998b) for *Oikopleura longicauda* (log W = 2.49 log TL − 5.97) and of Alldredge (1976) for *O. fusiformis* (log W = 4.21 log TL − 11.35), as reported in Hopcroft et al. (1998). Adult copepod biomass was estimated from the prosome length (L, mm) and ash-free dry weight (W, μg) relationships of Hopcroft et al. (1998) for *Oithona simplex* (log W = 3.47 log L − 8.76), *Oithona nana* (log W = 3.16 log L − 8.18), *Parvocalanus crassirostris* (In W = 3.25 In L − 19.65) and total nauplii (In W = 2.48 In L − 1570). The relationship of *P. crassirostris* was used in the absence of a length-weight relationship for *Bestiolina*
which likely yielded an underestimate of the biomass of this larger copepod species. The biomass of each chaetognath life stage was estimated from the length (L, mm) and weight (C, μg) relationship of Szyper (1976) for *Sagitta enflata* in Kaneohe Bay (*W = 0.0567 L^{2.83}*). Medusa biomass was estimated using the relationship of Bämmstedt et al. (1999) for bell diameter (L, mm) and dry weight (DW, μg) of *DW = 22.3L^{1.99}*. Biomass was converted to carbon (C) using a C:W ratio of 0.52 and a C:DW ratio of 0.45 (Alldredge, 1981).

**Statistics**

The significance of variability in the plankton community in Kaneohe Bay following the storm event was determined using paired and non-paired t-tests. Correlation analysis was used to determine relationships between variables and their significance established via regression analysis (Moore & McCabe 1993). Unless otherwise noted, errors are reported as 95% confidence limits of the mean.

**RESULTS**

**Meteorological & hydrological data**

Rainfall and streamflow data show that two major storm events resulted in elevated levels of freshwater runoff into southern Kaneohe Bay during the study period (Figure 2). The first and largest event occurred on 29 November, with a daily rainfall total of 26 cm at the Luluku rain gage and streamflow averaging 37 m³ s⁻¹. The flux of freshwater to the bay on this day was roughly 200-fold greater than baseline conditions. Rainfall and runoff declined gradually over the following 8 d until the second, less intense, storm...
occurred on 7 December. Rainfall and average streamflow during this storm were 13 cm and 19 m³ s⁻¹, respectively.

Wind speed decreased from 36 to 18 km h⁻¹ and reversed direction (NE to SW) in late November, as is typical of “Kona” storm conditions in the Hawaiian Islands. Wind speed generally remained low until strong trade wind conditions returned on 7 December (Figure 2). The tidal range during the 29 November storm was at a monthly low and remained relatively low until 4 December (Figure 2). Solar radiation decreased during the storm due to cloud cover, from a daily maximum of 980 to 335 µE m⁻² s⁻¹, and remained low for ~ 7 d (Figure 2). Surface water temperature varied little, with the exception of a drop in temperature of > 1 °C at the end of December (Figure 2). This decline in temperature was evident at both the surface and at depth and was not the result of freshwater runoff.

During the first storm event, low tidal range and wind speed resulted in an extended period of stratification comparable to the maximum stratification time measured following a storm event in this area (~ 8 d, Ringuet 2003; Figure 3). Relatively low and persistent trade winds beginning on 3 December led to a breakdown in water column stratification between 5 and 6 December. The second storm event resulted in a slight freshening of surface waters on December 7, but the water column was well mixed again within 24 h (Figure 3).
Nutrients and total chlorophyll a

Dissolved macronutrients (NO$_3^-$ + NO$_2^-$, PO$_4^{3-}$ and Si(OH)$_4$) increased significantly in the bay immediately following the first storm event on 29 November and were an order of magnitude higher than levels measured close to this station (Station MP) and in the center of the southern basin (Station SB) during bi-weekly monitoring from 1998 to 2001 (Figures 4a,b; Kinzie III et al. 2001). NO$_3^-$ + NO$_2^-$ levels peaked at 34 µM but declined to low levels in the days immediately following the storm. NH$_4^+$ was low initially but increased after the decline in NO$_3^-$ + NO$_2^-$ (Figure 4a). Both NH$_4^+$ and SRP levels remained well above baseline concentrations throughout the study period. Si(OH)$_4$ concentrations behaved similarly to NO$_3^-$ + NO$_2^-$, with high values immediately after the storm declining rapidly, then gradually dropping below baseline values (Figure 4b). The secondary storm runoff event on 7 December may have been responsible for a small increase in NO$_3^-$ + NO$_2^-$ and Si(OH)$_4$ on 11 December. SRP concentrations did not change following this storm.

HPLC and fluorometrically measured Chl a concentrations in surface samples were nearly identical (slope = 1.0, p < 0.001) and thus only HPLC-derived Chl a concentrations are reported here. Total Chl a peaked on 4 December, 3 days after the initial peak in macronutrients (Figure 4c). Chlorophyll-a levels remained elevated over baseline levels for ~ 3 d, coinciding with an increase in solar radiation and water-column stratification, then declined as stratification broke down on 5 December. Subsequently, Chl a concentrations increased gradually to a secondary peak of 3 µg l$^{-1}$ on 13-14
December, and varied around 2 µg l\(^{-1}\) for the remainder of the sampling period. Chl \(a\) returned to baseline concentration by the next sampling on 13 January 2004.

**Phytoplankton community composition**

Changes in phytoplankton accessory pigment concentrations over the sampling period are illustrated in Figure 5. Peridinin, the indicator pigment for autotrophic dinoflagellates, ranged from 0 – 87 ng l\(^{-1}\) and exhibited an initial 1-d peak on 2 December while remaining near or below detection thereafter. Prasinoxanthin increased rapidly on 3 December and peaked on 4 December at 586 ng l\(^{-1}\). The corresponding increases in other prasinophyte identifying pigments (violaxanthin, zeaxanthin and Chl \(b\)) indicates that these small, autotrophic flagellates bloomed directly following dinoflagellates. Zeaxanthin is also associated with cyanobacteria, and the increase in this accessory pigment between 3 and 6 December may indicate a parallel increase in cyanobacteria. Zeaxanthin is also found in submicron cyanobacteria, especially *Synechococcus* spp. (mean size = 0.9 µm in Kaneohe Bay), which are abundant in Kaneohe Bay (Chapter 3, Scheinberg et al. in press). However, the use of GF/C filters (nominal pore size 1.2 µm) biased these collections toward larger cells.

Fucoxanthin and diadinoxanthin concentrations indicate that diatoms also increased following the first storm (max = 837 and 75 ng l\(^{-1}\), respectively). While prasinoxanthin and fucoxanthin peaked on the same day, the peak shapes suggests that diatoms lagged prasinophytes by ~ 1 d. Fucoxanthin concentration reached its highest level during the second Chl \(a\) maximum on 15 December (1082 ng l\(^{-1}\)). Similar trends between total chlorophyll \(a\) and diatom pigments, and low concentrations of other phytoplankton
accessory pigments, suggest that diatoms dominated the phytoplankton community following the initial breakdown of water-column stratification on 6 December. This shift in community structure is illustrated in Figure 6. Minor increases also occurred for dinoflagellate and cyanobacteria accessory pigments during this time, following the trend for total chlorophyll $a$.

Divinyl Chl $a$ values were low relative to total Chl $a$, indicating that *Prochlorococcus* spp. likely did not play a significant role in the observed phytoplankton bloom. Alloxanthin (Allox) and 19'-butanoyloxyfucoxanthin (But) were also low, suggesting only a minimal contribution of cryptophytes and pelagophytes as well.

Net (> 64 µm) phytoplankton composition, from net tow samples collected daily after the breakdown in water-column stratification on 5 December, supported the observed succession from small phytoplankton to diatoms (Figure 7). Net phytoplankton were absent from the bay when first sampled on 5 December, with the exception of a sparse population of the autotrophic dinoflagellate, *Ceratium* spp. A diatom bloom was observed in net tows starting on 10 December, with a peak on 14 December, as also seen in fucoxanthin. During this time, the relative species composition of the diatom community shifted from a mixed assemblage of the pennate diatoms *Haslea* spp., *Pleurosigma* spp. and *Thalassionema nitzchoides*, and the dinoflagellate, *Ceratium* spp., to the centric chain diatoms *Guardia* spp. and *Chaetoceros* spp. The fucoxanthin peak on 14 December was almost entirely due to a bloom of *Chaetoceros* spp. The relative importance of *Chaetoceros* spp. decreased following this peak, but large diatoms were still abundant and responsible for a continuing gradual increase in the concentration of
fucoxanthin (and Chl a) through the end of the study period. *Protoperidinium* spp., a heterotrophic dinoflagellate similar taxonomically to *P. divergens* (Jeong et al. 1997), also exhibited a gradual increase in relative abundance following the centric diatom bloom towards the end of the study period. Accessory pigments and total Chl a returned to relatively typical concentrations by 13 January. Net phytoplankton were still present, but at much lower abundances relative to earlier samples. The assemblage was primarily composed of pennate diatoms, although dinoflagellates had increased in relative abundance.

**Zooplankton abundance and community composition**

Zooplankton monitoring did not begin until 5 December, and thus this study only addresses changes in zooplankton abundance and community structure following the initial phytoplankton bloom and breakdown of stratification.

**Herbivores/Omnivores.** Omnivorous zooplankton (consumers of both autotrophic and heterotrophic prey) consisted primarily of the copepods *Oithona simplex, O. nana, Bestiolina similis* and *Parvocalanus crassirostris*, the appendicularians *Oikopleura longicauda* and *O. fusiformis*, the cladoceran *Penilia avirostris*, and larval bivalves and polychaetes. Adult copepods were most abundant immediately following the storm event, with the exception of *O. nana*, which varied little throughout the study (Figure 8). The smallest copepods, *O. simplex* (0.25 mm) and *P. crassirostris* (0.36 mm), were dominant, with populations peaking after the breakdown of stratification on 6 December, then declining gradually. The largest species, *B. similis* (0.5 mm), increased gradually to
a peak on 13 December. The ratio of copepod nauplii to adults oscillated but followed an increasing trend throughout the sampling period (Figure 9).

The maximum abundance of calanoid adults (*P. crassirostris* and *B. similis*) was significantly greater during the storm period than during long-term sampling in 1998 (p < 0.05, n = 25) and 2001 (p < 0.05, n = 30) (Chapter 4, Scheinberg & Landry in prep), and appeared to decrease following the storm in 2004 (p = 0.08, n = 4). In contrast, cyclopoid abundances (*O. simplex* and *O. nana*) were depressed during the storm period, with maximum abundances less than half those observed in 1998 (p < 0.05, Figure 10).

The dominant appendicularians in Kaneohe Bay, *Oikopleura longicauda* and *O. fusiformis*, increased rapidly following the break down in water-column stratification. *O. longicauda* increased from 0.4 to 2.5 ind l¹ in 1 d, while *O. fusiformis* increased from 0.1 to 0.6 ind l¹ during this same period. Due to their similar rates of increase, *O. longicauda* remained always more abundant than *O. fusiformis* (Figure 11a). Abundances of both species decreased rapidly following their initial 2-d bloom, but generally remained at or above their long-term means in Kaneohe Bay (Chapter 4, Scheinberg & Landry in prep) for the remainder of the sampling period (Figure 11b).

The dominant members of the meroplanktonic community, bivalve and polychaete larvae, also increased significantly in abundance relative to sample collections in 2001 (Figure 12). Bivalve abundance increased over an order of magnitude, from 0.02 to 0.6 ind l¹, between 8 and 10 December, and then decreased to more typical concentrations. Polychaetes increased on December 8 and subsequently peaked several times, reaching maximum abundances on 12 and 13 December over 2-fold higher than those recorded
during 2001 sampling. The cladoceran, *Penilia avirostris*, appeared for the first time in recent history in Kaneohe Bay, although their abundances were low and variable (max = 0.03 ind l\(^{-1}\), Figure 12). Other omnivores, such as gastropod larvae and larval crustaceans, comprised only a minor portion of the zooplankton community (mean abundances < 0.01 ind l\(^{-1}\)).

**Carnivores.** The dominant planktonic carnivores in the bay during the study period were the chaetognath, *Sagitta enflata*, the hydromedusae, *Obelia* spp. and an unidentified smaller hydromedusa. Although they also are planktonic predators, larval fish were not sampled quantitatively during this study and thus will not be discussed. A significant, gradual increase in total chaetognaths was evident over the 15-d sampling period (p < 0.05), but each growth stage responded differently (Figure 13). Small, stage 1 chaetognaths increased gradually and relatively consistently, while stage 3 adult chaetognaths also appeared to increase but fluctuated more. In contrast, less variability and no net change in abundance was observed for stage 2 chaetognaths. Hydromedusae also varied significantly following the storm event (Figure 14). The small, unidentified hydromedusa increased gradually over most of the 15-d sampling period (p < 0.05), and its abundance was correlated with that of stage 1 chaetognaths (R = 0.69, p < 0.01).

*Obelia* spp. peaked in abundance on 10 December at 0.02 ind l\(^{-1}\), directly following the peak in appendicularian abundance on 9 and 10 December. Both hydromedusae reached maximum abundances on the last day of the sampling period.

Unlike their prey, the mean abundances of adult chaetognaths and medusae were not significantly greater during the study period than in 1998, 2002 or in post-storm samples.
collected from 13 January to 9 March 2004. Their maximum abundances following the storm were well above mean historical levels, but were below maximum abundances recorded when the bay was receiving high nutrient input from sewage discharge (Peterson 1976, Szyper 1976).

**Zooplankton biomass**

Changes in the biomass of each zooplankton group over time are illustrated in Figure 15. Adult copepods generally were the most important group in terms of biomass, although appendicularians were most important during their bloom from 9-10 December. In general, omnivore biomass was highest early in the study period and decreased ($R^2 = 0.25, p < 0.001$), while predator biomass increased ($R^2 = 0.65, p < 0.001$) (Figure 16).

**DISCUSSION**

The aim of this study was to characterize the relative response times and successional pattern of zooplankton following a natural perturbation event in a coastal tropical system and to address issues of bottom-up vs. top-down control. The large magnitude of the 29 November 2003 storm, coupled with low wind speed and tidal exchange, resulted in optimal conditions for development of a phytoplankton bloom, and the subsequent opportunity to quantify the successional response of primary and secondary planktonic consumers in these waters. While an increase in abundance and biomass was observed in both phytoplankton and zooplankton populations in Kaneohe Bay, the magnitude and duration of the zooplankton response varied, most notably among omnivores that undergo direct development (egg to miniature adult), planktotrophic larvae (free-swimming and
feeding pelagic larvae of benthic species), copepods (indirect development) and gelatinous carnivores. Most striking was a shift in zooplankton community size structure, from small copepods to large, gelatinous zooplankton and larval meroplankton. The data indicate that both bottom-up controls (nutrient-enhanced growth of phytoplankton producing an increase in grazer biomass) and top-down controls (grazing on phytoplankton and predation on omnivorous zooplankton) influenced the structure and abundance of phytoplankton and zooplankton.

In general, successional patterns were similar to those observed at upwelling regions in the equatorial Pacific and Indian Oceans, where studies utilized distance from the upwelling as a proxy for time. Vinogradov & Voronina (1963) and Timonin (1969) observed an increase in zooplankton abundance and a shift in community structure, from herbivores and omnivores to predators, north and south of upwelling regions. Increases in zooplankton rate processes and biomass have also been observed with increasing distance from an upwelling zone (Roman et al. 2002).

**Phytoplankton succession**

Chlorophyll-a increased dramatically in a short-lived bloom immediately following the storm event, and then increased steadily for the remainder of the study period, interrupted only by a smaller, secondary bloom on 14-15 December. The maximum Chl a concentration in the initial bloom was over 6-fold higher than typical background in this area. The observed changes in phytoplankton community structure are consistent with the general observation that autotrophic flagellates and diatoms thrive under calm, high-nutrient, high-light conditions (Pinckney et al. 1999). Water-column stratification
during the 5 days immediately following the storm maintained high nutrient concentrations in a reduced-salinity surface layer, leading to a succession of phytoplankton groups, from autotrophic dinoflagellates to prasinophytes to diatoms. The early appearance of dinoflagellates is consistent with observations that these organisms are better adapted to utilize high nutrient concentrations under low turbulence conditions than other phytoplankton groups (Sellner et al. 2001). Given the high sensitivity of dinoflagellates to small-scale turbulence (Thomas & Gibson 1990, Berdalet 1992), the short duration of their bloom could have been the result of a moderate increase in wind speed on 2 December. Dinoflagellates did not contribute significantly to Chl a values after the initial bloom, although peridinin concentrations were measurable for much of the study period, and dinoflagellates were observed in net phytoplankton samples.

Prasinophytes and diatoms contributed roughly equally to total Chl a during the initial bloom based on Chl a:accessory pigment ratios determined in the subtropical coastal and central North Pacific (Letelier et al. 1993). Cyanobacteria also likely contributed to the initial bloom, but their contribution cannot be quantified directly from the zeaxanthin data due to the abundance of zeaxanthin-containing prasinophytes. Surprisingly, although diatoms responded to the initial nutrient input, they were responsible for less than half of total Chl a during the initial bloom. This contrasts with the majority of studies conducted in tropical and temperate waters (e.g. Malej et al. 1997, Landry et al. 2000), where diatoms dominate following a nutrient pulse.

Diatoms did, however, continue to increase following the initial bloom and were the dominant group by the end of the sampling period. The succession in > 64-μm diatom
species in net-collected samples appears to have been due, at least in part, to the concentration of silicate in the water column. A sharp decline in diatom abundance following a bloom of the highly-silicified centric diatoms, Chaetoceros spp. and Guardia spp., from 13 to 15 December coincided with a decrease in Si(OH)₄ below 2 µM, which is considered to be the threshold for silicate limitation of diatom production (Dortch & Whitledge 1992). Less silicified diatom species increased in relative abundance following this decline. In addition, heterotrophic Protoperidinium spp., some species of which are known to feed selectively on diatoms (Buskey 1997, Naustvoll 2000) and to respond positively to diatom blooms (Kjaeret et al. 2000), increased during this time. If the species collected was the heterotrophic Protoperidinium divergens, this dinoflagellate may also have played a role in the observed successional pattern.

The termination of the initial phytoplankton bloom was characterized by a precipitous 2-d decline in Chl a concentrations as the water-column stratification brokedown. Thus, dilution appears to have been the primary cause of the chlorophyll decline. However, the continued presence of high nutrient concentrations suggests that phytoplankton were still growing at high rates, despite their reduced concentrations. In fact, the consistently high concentrations of NH₄⁺ and SRP throughout the study period suggest that macronutrients were never limiting to phytoplankton growth. Comparison of surface and near-bottom samples from the CRIMP and surface data for C Buoy indicates that the elevated NH₄⁺ and SRP values after water-column mixing on 5 December may have had a benthic source (Hoover et al. unpub. data). Nutrient regeneration by zooplankton probably did not contribute greatly to the elevated NH₄⁺ and SRP concentrations since previous
studies in Kaneohe Bay suggest that the mesozooplankton contribution to nutrient fluxes should be only a small fraction of the observed total (Szyper et al. 1976).

Given the shallow depth of the bay and its proximity to land, it seems unlikely that trace nutrients, such as iron, were limiting during the sampling period. In addition, the continued increase in fucoxanthin concentrations and concomitant decrease in Si(OH)₄ suggests that diatoms were able to grow. Thus, the inability of phytoplankton to use the available NH₄⁺ and SRP in the latter part of the study period suggests top-down grazing control by omnivorous zooplankton.

**Omnivorous mesozooplankton response and their potential impact on phytoplankton**

The fluctuations in omnivorous zooplankton following the initial bloom indicate a close relationship between grazers and their phytoplankton prey, although the type of response appears to vary on a taxon-specific basis. Appendicularians exhibited the most dramatic shift following the phytoplankton bloom, with biomass increasing 6-fold in 1 d and abundances reaching values only rarely observed in Kaneohe Bay (Chapter 4, Scheinberg & Landry in prep). While the magnitude of the response was different between the two dominant species, both increased in abundance at similar rates (Figure 11).

In contrast to appendicularians, maximum total copepod abundances directly following the phytoplankton bloom were similar to those typically observed in these waters. The gradual increase in nauplii abundance over time suggests that, while copepods were able to take advantage of the new food source, the copepod reproductive
response was slow compared to that of appendicularians (Figure 9). Reproductive responses have been observed for copepods following phytoplankton blooms in both temperate and tropical systems (Kiørboe & Nielson 1990, Rollwagen Bollens & Landry 2000, Roman et al. 2002). Within the copepod assemblage, calanoids responded quickest to an increase in food availability. This phenomenon has been previously observed in the equatorial Pacific Ocean (Roman et al. 1995). Biomass-specific ingestion rates of calanoid and cyclopoid copepods in Kaneohe Bay are comparable (Calbet et al. 2000), however, the larger size of calanoid copepods (wider and in most cases longer) may have given them an advantage with respect to handling larger cells during bloom conditions. Even most small calanoid copepods can consume diatoms (e.g. Frost 1972, Checkley 1980), while the cyclopoids typically prefer smaller, more motile prey (Paffenhöfer 1993).

Among the omnivorous mesozooplankton, only the filter-feeding appendicularians had the capacity to graze rapidly enough to control primary production at phytoplankton growth rates observed in these waters under non-limiting conditions (typically ~ 1 d⁻¹, Laws & Allen 1996, Ringuet 2003). Combining abundance data and clearance rates of Oikopleura longicauda and O. fusiformis in Kaneohe Bay (0.8 and 0.9 l ind⁻¹ d⁻¹, respectively; Chapter 3, Scheinberg et al. in press) shows that appendicularians potentially cleared an average of 88% (50 - 260%) of the water column daily. Copepods, bivalve larvae, polychaete larvae and Penilia avirostris also are all capable of consuming micro- and nanoplankton prey (Bayne 1983, Turner et al. 1988, Martin et al. 1996, Turner et al. 1998, Calbet et al. 2000) and likely contributed to the maintenance of
relatively low phytoplankton standing stocks following the break down in water-column stratification. However, based on reported clearance rates and our abundance data, their estimated impact was well below that of appendicularians.

The pigment data suggest that prasinophytes (2 – 30 mm in size, Jeffrey & Veski 1997), cyanobacteria (1 – 20 μm; Chapter 4, Scheinberg & Landry in prep) and other, non-diatom prey were held close to baseline abundances by omnivore grazing throughout the latter portion of the study period. However, a gradual increase in total Chl a concentrations and the occurrence of a small bloom of > 64-μm diatoms (Dec. 14-15, Figures 5 & 7) provides evidence for a lack of grazer control on large diatoms. While minor stratification following the second storm may have contributed to the sharp increase in diatom standing stock on 14 and 15 December, this event is unlikely to have sustained by itself the continual rise in diatom standing stock through the end of the study.

An increase in mortality rate of omnivorous grazers due to predation may have led to the release of grazing pressure on large diatoms following the storm. Diatom abundance was lowest in the 7 days following the initial bloom (6 – 13 December), while omnivorous zooplankton were at their peak. Diatoms then increased as the biomass of omnivores decreased and carnivores increased (Figure 16). However, while predation may have released diatoms from grazing pressure, the above-average abundance of appendicularians was apparently still sufficient to maintain low levels of non-diatom prey during this time. Details of the relationships between carnivores and omnivores are addressed in the following section.
An explanation for the apparent release of diatoms from omnivore grazing pressure may be found in the size-selective grazing habits of appendicularians (Figure 17). Some appendicularians have been shown to process only relatively small prey due to size selection by the animal’s incurrent filter (Fernandez et al. in press). The 13-µm inlet filter of *O. fusiformis* (Alldredge 1977) would certainly have kept diatoms from entering their houses. However, the dominant appendicularian in Kaneohe Bay, *O. longicauda*, does not have inlet filters on its mucus houses and instead has two holes that could potentially allow in much larger cells. While it has been suggested that *O. longicauda* feed on larger particles than *O. fusiformis* (Chapter 3, Scheinberg et al. in press), these small appendicularians (trunk length 0.37 ± 0.03 mm) are unlikely consumers of the large, chain-forming diatoms that bloomed during this study, with individual cells typically ≥ 100 µm in length. A predation-driven decline in diatom-consuming calanoid copepods, coupled with the inability of appendicularians to feed on large diatoms may have enabled the continued growth of diatoms throughout the study.

Despite their apparent impact on phytoplankton production, omnivorous zooplankton were likely not food limited following the storm event. Assuming a typical range of gross growth efficiencies of 10 – 30% for omnivorous zooplankton (Omori & Ikeda 1984) and a carbon to Chl a ratio typical of Kaneohe Bay (113, Taguchi & Laws 1989), only at their least efficient did zooplankton require more than the available standing stock of phytoplankton biomass for growth and reproduction on any day of the sampling period. While this does not account for the loss of carbon due to appendicularian house
production, it suggests that phytoplankton production was more than sufficient to support zooplankton growth over the course of this study.

An additional and notable consequence of an appendicularian-dominated response to a phytoplankton bloom is the enhanced export of phytoplankton production to the benthos, through their mucus houses and fast-sinking fecal pellets. Appendicularians build and discard their mucus houses on the order of 6 – 24 times a day at these temperatures (> 26°C; Taguchi 1982, Sato et al. 2001) and their discarded houses are typically enriched 10-fold in particulate organic matter in Kaneohe Bay (Taguchi 1982). Based on measured sedimentation rates of discarded houses, Taguchi (1982) estimated that appendicularian houses in Kaneohe Bay can account for about 60% of the annual mean sedimentation of particulate organic carbon produced in the water column. In addition, fast sinking appendicularian fecal pellets represented ~ 13% of the net sedimentation of particulate organic carbon in these waters.

**Predation on omnivorous mesozooplankton**

Predation by carnivorous zooplankton following the storm event appears to have been a strong factor regulating omnivorous zooplankton abundance. All of the dominant gelatinous predators exhibited temporal variability suggestive of predator/prey relationships with the two dominant omnivores, appendicularians and copepods. A gradual decrease in juvenile and adult copepod abundance corresponded significantly to an increase in the abundances of *Sagitta enflata* (Figure 18a; $R^2 = 0.66$ & 0.18, respectively; $p < 0.001$) and total medusae (Figure 18b; $R^2 = 0.46$ & 0.31, respectively; $p$
< 0.01). *Sagitta enflata* in Kaneohe Bay are known to feed on the dominant species of copepods observed during this study and could have consumed up to 160% of the copepod population per day as their abundances increased over the sampling period (Kimmerer 1984). While the predation rates of medusae on juvenile and adult copepods are unknown for this system, the temporal patterns in their abundances suggest that copepods were a food source for these predators as well.

The potential grazing impact of appendicularians was likely reduced during this study due to predation by gelatinous zooplankton. Following their initial 2 d bloom, appendicularian abundances were held below 1 l⁻¹ despite a food supply sufficient to support maximum growth rates at these temperatures (2.0 ± 0.6 d⁻¹, Hopcroft et al. 1998b; as estimated in Chapter 3, Scheinberg et al. in press). Appendicularians are the preferred prey of *S. enflata* in Kaneohe Bay (Kimmerer 1984); and based on the mean ingestion rate of *S. enflata* on appendicularians (Kimmerer 1984) these predators could have consumed > 200% of the appendicularian standing stock daily during the post-storm period. The medusae, *Obelia* spp., also followed appendicularian abundances closely, increasing from 0.005 to 0.02 l⁻¹ directly following the initial peak in appendicularian abundance (Figure 19). Although their feeding rates are unknown, gut contents from both *Obelia* spp. and small hydromedusae collected in net tows indicate that both hydromedusae were feeding on appendicularians. Predator control of appendicularians by medusae and chaetognaths has also been implied from long-term abundance patterns in Kaneohe Bay (Chapter 4, Scheinberg & Landry in prep).
The potential impacts of chaetognaths on their prey vary with chaetognath life stage. Total chaetognaths, numerically dominated by juveniles, exhibited a significant, negative relationship with adult copepod abundances (particularly calanoids) over the sampling period (Figure 18a), while adult, stage 3 chaetognaths exhibited an inverse and lagged relationship with appendicularians (Figure 19). However, while fluctuations in the abundances of stage 3 chaetognaths suggest that they may have been tightly coupled to their preferred prey, they may also have been selectively preyed upon by other carnivores, such as larval or adult fish, due to their larger size.

CONCLUSIONS

A major storm runoff event in southern Kaneohe Bay resulted in a significant increase in both phytoplankton and zooplankton biomass in this tropical coastal embayment. Zooplankton community size-structure varied positively with nutrient loading, with larger components of the community, most notably gelatinous zooplankton, increasing while abundances of smaller zooplankton, such as copepods, remained at more typical levels. Appendicularian abundances were sufficiently high to clear a large fraction of the water column daily. As a consequence, the highest grazing impact of zooplankton following the initial phytoplankton bloom was likely on non-diatom phytoplankton, due to appendicularian size-selective filtration. However, predation pressure on omnivores by gelatinous predators reduced grazing pressure, allowing phytoplankton populations to increase gradually, especially diatoms, which were probably grazed less efficiently than smaller cells. Variations in phytoplankton and zooplankton community structure following this storm event suggest particularly tight
coupling between small cells, such as cyanobacteria and prasinophytes, omnivorous grazers and their predators.

Perturbations are common events in plankton community dynamics, yet their impact in oceanic systems may go unrecognized due to the short time scales over which they occur relative to typical sampling regimes. This is particularly true in tropical systems, where the response time of the zooplankton community is much shorter than in temperate waters. Through frequent sampling in a quasi-steady state tropical coastal ecosystem, we determined that nutrient-rich runoff can have significant impacts on the population dynamics of zooplankton in tropical coastal waters, via pulsed food influences on the growth and reproduction of both omnivorous and carnivorous organisms and that major planktonic responses can occur on a scale of 1-3 days. In general, tropical zooplankton may be more tightly coupled to meteorological and physical forcing events than their temperate counterparts.
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FIGURE LEGENDS

Fig. 1 Map of southern Kaneohe Bay, Oahu, Hawaii. Samples for zooplankton analyses were collected at Station C Buoy. Stations SB and MP were the sites of two long-term monitoring programs in Kaneohe Bay (Kinzie III et al. 2001; Chapter 4, Scheinberg & Landry in prep) that were used for comparison between storm values and baseline conditions throughout this study.

Fig. 2 Meteorological and hydrological conditions in southern Kaneohe Bay prior to and following the storm event on November 29, 2003. Daily rainfall (cm) and streamflow (m$^3$ s$^{-1}$) were determined using data from the United States Geological Survey (USGS) Luluku rain gage, USGS Kaneohe stream gage, and the Hawaii Institute of Marine Biology (HIMB) weather station rain gage on Coconut Island in the southern sector of the bay (top panel). Wind speed (km h$^{-1}$) and direction (°, second panel), solar radiation (μE m$^{-2}$ s$^{-1}$) and water temperature (°C, fourth panel) were also obtained from the HIMB weather station. Daily tidal range (m) was determined from the National Oceanographic and Atmospheric administration (NOAA) tidal gage also located on Coconut Island (third panel).

Fig. 3 Salinity profiles (in ppt) from Station C Buoy following the storm event on November 29, 2003. Measurements were taken from the surface to the bottom (0 – 15 m).
Fig. 4 Daily nutrient concentrations (μM, Figures 4 A and B) and total chlorophyll a (μg 1⁻¹, Figure 4 C) at Station C Buoy in southern Kaneohe Bay (left panels). Arrows and the dotted line indicate storms and the breakdown in water column stratification, respectively. Right panels show boxplots of biweekly data obtained from 10/98 – 7/01 for the plotted parameters as reported by Kinzie III et al. (2001) at a station close to the mouth of Kaneohe stream (MP) and in the middle of the southern basin (SB) (Figure 1). Data are plotted on the same vertical scales to illustrate the relative change in concentrations during the storm event. The “box” in box plots defines the 25th and 75th percentiles and the colored and black lines within the box are the mean and median, respectively. Lines extending from the ends of the box reach the 5th and 95th percentiles of the distribution of values observed during the sampling period.

Fig. 5 Phytoplankton pigment concentrations (ng 1⁻¹) during the storm event at Station C Buoy in southern Kaneohe Bay. Data from January 13, 2004 also are presented to illustrate the return to more typical conditions. The dotted line indicates a shift from stratification to a well-mixed water column between December 5 and 6.

Fig. 6 Phytoplankton pigment concentrations normalized to total chlorophyll a (ng/ng) prior to and following the storm event on November 29, 2003 at Station C Buoy. Data were not available for December 7.
Fig. 7 Relative percentage of net-collected phytoplankton species (> 64 μm) following the storm event. Samples were collected from Station C Buoy beginning on December 5, 2003. The large dinoflagellate, *Protoperidinium* sp., was common in net-collected samples and is thus included here, although it was most taxonomically similar to the heterotrophic species, *P. divergens*.

Fig. 8 Abundance of adult copepods (ind l⁻¹) at Station C Buoy. *Oithona simplex*, *O. nana*, *Bestiolina similis* and *Parvocalanus crassirostris* were the dominant copepods in Kaneohe Bay during this study.

Fig. 9 Temporal variability in the abundances of adult and naupliar copepods (ind l⁻¹) at Station C Buoy and the ratio of copepod nauplii to adults (ind/ind) over the sampling period.

Fig. 10 A comparison of the abundances of adult copepods (ind l⁻¹) in southern Kaneohe Bay during two long-term sampling efforts (1998, n = 25 and 2001, n = 30), the storm (Storm, n = 16) and following the storm from January – March, 2004 (Post-storm, n = 4). Box plots of *Oithona simplex* (OS), *O. nana* (ON), *Bestiolina similis* (BS) and *Parvocalanus crassirostris* (PC) represent the 25th and 75th percentiles, while lines extending from the ends of the box reach the 5th and 95th percentiles of the distribution. Thick and thin black lines within the box represent the mean and median, respectively. Circles above and below the boxes are the maximum and minimum values observed.
Fig. 11 Abundances of total (adult + juvenile) appendicularians, *Oikopleura longicauda* (OL) and *O. fusiformis* (OF), during the study period (Figure 11 A) and box plots of their relative abundances in southern Kaneohe Bay as recorded by previous long-term monitoring programs in 1998 (n = 25) and 2001 (n = 30), during sampling immediately after the storm (n = 16) and later post-storm sampling (n = 4) (Figure 11 B). Lines extending from the ends of each box reach the 5th and 95th percentiles of the distribution and circles above and below the boxes are the maximum and minimum values observed. Thick and thin black lines within the box represent the mean and median, respectively.

Fig. 12 Abundances (ind l⁻¹) of bivalve larvae (top), polychaete larvae (middle) and the cladoceran, *Penilia avirostris*, (bottom) in left panels. The break down in water column stratification is indicated with a dotted line. Right panels for each plot illustrate the abundance of these organisms in southern Kaneohe Bay during a long-term sampling effort in 2001 (n = 30), the storm event (Storm, n = 16) and following the storm from January – March, 2004 (Post-storm, n = 4). Boxes are the 25th and 75th percentiles and lines extending from the ends of each box reach the 5th and 95th percentiles of the distribution. Thin black lines within the box and circles above and below represent median and maximum and minimum values, respectively.
Fig. 13 Abundances (ind l⁻¹) of the chaetognath, *Sagitta enflata*, in southern Kaneohe Bay at Station C Buoy during the sampling period. Growth stages 1, 2 and 3 were defined according to Casanova (1991).

Fig. 14 Abundances (ind l⁻¹) of the hydromedusa, *Obelia* spp., and a smaller, unidentified hydromedusa at Station C Buoy during the sampling period.

Fig. 15 Estimated zooplankton biomass (µg l⁻¹) at Station C Buoy during the sampling period. The x-axis break separates the storm sampling period (December 5 – 20, 2003) and the post-storm sampling period (January 13 – March 9, 2004). Post-storm values represent the mean estimated biomass over this period. Biomass was estimated using the length-weight relationships reported in Hopcroft et al. (1998a,b), Szyper (1976) and Båmstedt et al. (1999).

Fig. 16 Relationship between phytoplankton, omnivorous zooplankton and carnivorous zooplankton biomass (µg l⁻¹) at Station C Buoy during the sampling period. The x-axis break separates the storm (December 5 – 20, 2003) and post-storm (January 13 – March 9, 2004) sampling periods. Post-storm values represent a mean estimated biomass for 2004. Omnivore biomass decreased significantly following the breakdown in water column stratification (R² = 0.25, p < 0.001), while carnivore biomass increased (R² = 0.65, p < 0.001).
Fig. 17 Appendicularian abundance (ind l\(^{-1}\)), prasinoxanthin and fucoxanthin concentrations (ng l\(^{-1}\)) at Station C Buoy during the sampling period. Pigment data were collected starting on 11/30, zooplankton sampling began on 12/15.

Fig. 18 Relationships between adult copepods (blue) and copepodites (red) with chaetognaths (top panels, A) and hydromedusa (bottom panels, B) over the zooplankton sampling period at Station C Buoy. Correlation analyses show significant relationships for all four parameters (p < 0.01) with an R of 0.81 and 0.76 for adult copepods and an R of 0.42 and 0.56 for copepodites with chaetognaths and hydromedusae, respectively.

Fig. 19 Temporal variability in appendicularian and hydromedusa abundances (top panel) and appendicularian and stage 3 chaetognaths abundances (bottom panel) at Station C Buoy.
Fig. 3
Fig. 4
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CHAPTER 6

CONCLUSIONS:

THE ROLE OF APPENDICULARIANS IN A SUBTROPICAL FOOD WEB

INTRODUCTION

The research presented in this dissertation is broadly focused on the ecology of appendicularians in the subtropical coastal ecosystem of Kaneohe Bay. The overarching hypothesis was that the ability of appendicularians to feed directly on bacteria-sized primary producers would provide a shorter and more direct route to higher order consumers and thus significantly increase the efficiency of energy transfer through the food web. Over the course of this study, it also became evident that appendicularians play a number of significant roles in the plankton assemblage of Kaneohe Bay -- as grazers, competitors and prey -- and that their importance varies substantially in time and space. The following discussion is a synthesis of key elements of my dissertation as they relate to three areas: 1) the regulation of appendicularians by food and predators in Kaneohe Bay (i.e., bottom-up and top-down controls), 2) the importance of appendicularians relative to other competing grazers, and 3) the relative importance of appendicularians and copepods in energy transfer to higher trophic levels. Finally, I discuss questions raised by this study and suggest possible avenues for future research.
Bottom-up vs. top-down control of appendicularians in Kaneohe Bay

Appendicularian abundances varied widely over the course of this study, ranging from 0 – 4 ind l⁻¹, with substantial variability over timescales from days to years. Both bottom-up and top down controls appear to be important. The boom/bust dynamics that characterize appendicularian populations suggest that these controls vary in their relative importance over short timescales.

The most direct evidence for bottom-up control is the rapid and dramatic increase in appendicularian abundance following the large storm runoff event discussed in Chapter 5. Both the increase and subsequent decline followed similarly dramatic changes in prey concentrations, suggesting tight coupling between appendicularians and their prey. Increases in appendicularian abundances following storm runoff events appear to be common in Oahu coastal waters (E. Parnell pers. comm., R. Scheinberg pers. obs.), and abundances in long-term monitoring datasets were positively correlated with runoff. Thus, the availability of sufficient food resources may be a relatively common constraint on population growth rates of appendicularians. Other data are more equivocal, however. For example, long-term monitoring data do show a general increase in appendicularian populations in 2001-02, but prey populations did not decline until several months after the initial increase in appendicularian abundance (Chapter 4). In addition, comparison of measured prey concentrations to those required to support maximum growth rates suggest that appendicularians may normally not be food limited (Chapter 2 and 3). Thus, while it seems likely that food pulses stimulate bursts in appendicularian abundance, other factors, such as predation, may be equally or more important as controls over the longer
The perturbations themselves may have some as yet unrecognized direct effect on predators (e.g., runoff effects on water clarity, particle load or turbulence) that creates an opportunity for population blooms of their appendicularian prey.

Appendicularians have previously been shown to be the preferred prey of chaetognaths in Kaneohe Bay (Kimmerer 1984). One new finding of the present study, from seasonal variability in the relative abundances of appendicularians and hydromedusae and the shift in gelatinous zooplankton community structure from 1998 to 2002, is that hydromedusae may be equally or more important in regulating appendicularian abundances in this system (Chapter 4). In the 2001-02 monitoring period, appendicularians only appeared in the bay after a significant decrease in hydromedusae, and they were consistently higher in abundance in 1998-99, when hydromedusae were virtually absent from the bay.

Appendicularians also appeared to be strongly regulated by predators following the storm event discussed in Chapter 5. Following their initial bloom, appendicularian abundances were held below 1 l⁻¹ despite a food supply that appeared more than sufficient to support maximum growth rates (Chapter 3). Both chaetognaths and medusae increased in abundance during this period. Ingestion rates determined by Kimmerer (1984), albeit at higher appendicularian abundances, indicate that chaetognaths could potentially have consumed >200% of appendicularian standing stocks daily following the initial bloom, and the medusa Obelia spp. closely followed appendicularian abundances, increasing from 0.005 to 0.02 l⁻¹ directly following the initial appendicularian bloom. Although Obelia spp. feeding rates on appendicularians have not
previously been measured, gut contents show that both *Obelia* spp. and an unidentified small hydromedusae were predators on appendicularians in Kaneohe Bay.

Predation pressure may also provide an impact on the relative abundances of the two appendicularian species in Kaneohe Bay. Although the generally lower abundances of *O. fusiformis* compared to *O. longicauda* were hypothesized in Chapter 3 to be due primarily to their reduced ability to take advantage of sudden increases in prey, *O. fusiformis* numbers increased at the same rate as *O. longicauda* following the storm event in Chapter 5. In this case, it seems likely that selective predation by visual predators, such as larval fish, could be important. Since larval fish feed on appendicularians while they are in their houses, the larger houses of *O. fusiformis* would likely be more visible, and thus targeted more frequently, than those of *O. longicauda*. Gut contents indicate that appendicularians can make up a substantial proportion of the diets of the abundant larval and juvenile anchovies (Nehu) in Kaneohe Bay (T. Clarke, pers. comm.).

**Relative importance of appendicularians as grazers of microbial production**

The primary competitors of appendicularians for microbial prey in Kaneohe Bay apparently are protozoans and copepods. Grazing by protozoans, specifically 2-5 μm heterotrophic nanoflagellates (HNAN), can lead to picophytoplankton mortality rates comparable to mean rates resulting from appendicularian grazing. Combined, appendicularians and protists could have consumed an average of ~ 100% of picoplankton production daily in Kaneohe Bay during the long-term monitoring periods, indicating that grazing by these organisms alone could be responsible for the relatively
constancy of picoplankton abundances. The fact that picoplankton standing stocks were higher when appendicularian abundance was unusually low during the 2001-02 sampling period is consistent with a partial release from grazing pressure.

While the grazing impacts of protists and appendicularians appear on average to be similar, appendicularian abundances are more variable than those of protists, so that their grazing impact likely varies more with time. In particular, data suggest that on long time scales the relative grazing impact of appendicularians in Kaneohe Bay is higher during periods of normal rainfall and runoff than during low rainfall/runoff periods. On short time scales, the relative impact of appendicularian grazing could be especially high following storm events. When appendicularian abundances are extremely low during drought conditions, the control of picoplankton populations more clearly shifts to protists. Grazing impacts also may vary with picoplankton prey type since appendicularians do not digest cyanobacteria. The relative abundance of protists and appendicularians thus may affect the structure as well as the abundance of the picoplankton community.

Despite their relatively high and constant abundances in Kaneohe Bay, copepods appear not to be major grazing competitors for either appendicularians or protists. Adult copepods are much less efficient than appendicularians at consuming small-sized plankton (e.g. mean ~ 6% of picoplankton production daily vs ~50% for appendicularians). Grazing by juvenile copepods and nauplii would increase the total copepod contribution on both the nano- and picoplankton size ranges, but probably by only a factor of 2 – 3 overall based on typical abundances.
The relative importance of copepods and appendicularians in trophic transfer

The efficiency of trophic transfer depends heavily on the number of steps in a grazing chain. In Kaneohe Bay, appendicularians and copepods have the potential to enhance transfer efficiency by feeding directly on cells very much smaller than themselves. Appendicularians exhibit extraordinarily high grazing rates on microbial prey, but their episodic abundances suggest that these filter-feeders can only occasionally serve as a major intermediary between microbial production and higher trophic levels, and then only over time scales of days to weeks. Nevertheless, calculations based on the appendicularian grazing rates from Chapter 3, the appendicularian, copepod and chaetognath abundances from Chapter 4, and the copepod grazing estimates from Calbet et al. (2000) suggest that even intermittent outbursts of appendicularians lead to their dominance of trophic transfer.

To illustrate this point, comparable appendicularian- and copepod-mediated food chains were constructed using the chaetognath, Sagitta enflata, as the top predator. The appendicularian-mediated food chain has three levels, with energy flowing from picoplankton to appendicularians to chaetognaths. The copepod-mediated chain includes a fourth link from picoplankton to nanoplankton (specifically, 2-5 μm protists). Estimated mean grazing rates for 2–5 μm heterotrophic flagellates (Chapter 4, Landry et al. 2000), adult copepod grazing rates (on 2-5 μm cells, Chapter 4; Calbet et al. 2000), mean appendicularian clearance rates (0.8 l⁻¹ ind⁻¹ d⁻¹, Chapter 3) and mean chaetognath feeding rates on copepods and appendicularians (1.5 copepods chaetognath⁻¹ d⁻¹; 3.5 appendicularians chaetognath⁻¹ d⁻¹; Kimmerer 1984), were combined with mean prey
abundance data (Chapter 4) to determine the integrated efficiency of these two alternate energy pathways. For these scenarios, approximately 0.6% of picoplankton production is transferred through copepods to chaetognaths, versus 10% for the appendicularian-mediated chain. The appendicularian-mediated transfer thus is 17 times more efficient, equivalent to 1-2 steps in a typical multi-level chain (Ryther 1969). If, on average, appendicularians and nanoflagellates are similarly effective at grazing picoplankton in Kaneohe Bay, the combination of efficient grazing by appendicularians and selective predation by chaetognaths on appendicularians would result in chaetognaths acquiring 17 times more energy flow from microbes through the appendicularian grazing chain than via copepods.

These estimates are based on mean abundances over the sampling period, but appendicularian abundances in particular will change significantly when appendicularian populations bloom (and bust). As a result, the copepod-mediated chain will be relatively more important when appendicularian populations are low, though still likely less important than the appendicularian chain, while the appendicularian-mediated chain will be overwhelmingly dominant when appendicularians bloom. The appendicularian blooms following episodic inputs of new nutrients may provide a means of efficiently transferring these nutrients to storage (as biomass) in higher trophic levels. The result could be greater biomass at the latter levels than would otherwise exist.
Directions for future research

While the research reported in this dissertation demonstrates that appendicularians play important roles in tropical coastal food webs and energy and material fluxes, more research is needed in a variety of areas. I will comment on just three of these: 1) What is the contribution of appendicularians to particle fluxes via house and fecal pellet production; 2) To what degree does differential digestion of prey ultimately affect plankton community structure and energy and material fluxes; 3) How is predation on appendicularians actually accomplished?

Appendicularian houses and fecal pellets contribute significantly to the flux of organic matter out of the temperate surface ocean (Alldredge 1976a, Hansen et al. 1996), but their contribution to fluxes in tropical waters is not well known. Throughout this dissertation, estimates were made on the possible contributions of appendicularians to particulate carbon and nitrogen flux via their discarded houses and densely packed fecal pellets compared to copepods in Kaneohe Bay. In order to determine appendicularian contributions to carbon flux, data are needed on their house production rates, the carbon content of discarded houses and fecal pellets, and the sedimentation rate of houses and fecal pellets. In the literature, house production rates for O. longicauda in Kaneohe Bay range from 5 – 24 houses d\(^{-1}\) at ambient temperatures (Taguchi 1982, Sato et al. 2001). House carbon content values also range widely. Both will likely vary depending on a variety of biological and environmental factors, such as plankton community composition and water-column turbidity.
Using a range of values, the mean abundances of appendicularians could contribute anywhere from 0.4 to 144 \( \mu g \) C to particulate carbon flux daily (Alldredge 1976b, Taguchi 1982, Sato et al. 2001). After a storm event, their contributions should be higher due to elevated abundances and higher rates of house-turnover due to clogging by bloom phytoplankton.

Cyanobacteria are abundant throughout the tropical ocean, and while the appendicularian, *Megalocercus huxleyi*, does not digest *Synechococcus* spp., the inability of other species to digest these and other cyanobacterial prey has not been definitively established. The apparent inability of appendicularians to digest cyanobacterial prey may have significant implications for material and energy fluxes and for plankton community structure and dynamics. Between 30 and 50% of autotrophic biomass in Kaneohe Bay during the long-term monitoring periods was cyanobacteria, with *Synechococcus* spp. alone representing roughly 25%. This dominance could, in fact, reflect the relative invulnerability of cyanobacteria to one of the system’s dominant grazers. In addition to *Synechococcus* spp., the cyanobacterial community in Kaneohe Bay includes abundant *Crocosphaera* spp., which are significantly larger than *Synechococcus* spp. These larger cells should be more efficiently grazed than *Synechococcus* spp. and thus could potentially serve as a significant food source for appendicularians if they can be digested. Conversely, if these cells are grazed but not digested, they could represent a significant flux out of the water column as still-viable cells in sinking houses and fecal pellets. These cells then either could be consumed by benthic deposit feeders or possibly resuspended back into the water column. Thus, when appendicularians are abundant, they could
potentially shift the system toward cyanobacterial dominance and/or increased food supply to the benthos.

One surprising result of this dissertation was the close inverse relationship between hydromedusae and appendicularians. Hydromedusae predation on appendicularians has not previously been observed in Kaneohe Bay, but it may have played a substantial role in controlling appendicularian abundances during the study period. Important details of the feeding behaviors of chaetognaths on appendicularians are also poorly documented and could have important consequences with respect to predation pressure. In particular, if chaetognaths feed on appendicularians only when they are outside of their houses, appendicularians would be vulnerable to chaetognath predation for only $\sim 5 - 24$ min d$^{-1}$ (based on house production time = 1 min, Chapter 2), whereas if they feed on appendicularians while they are in their houses, they could play a much more significant role in structuring appendicularian populations. If the latter is correct, then *O. longicauda* would hypothetically be a more easily targeted prey for chaetognath predation than *O. fusiformis* because it is a comparably sized organism in a much smaller mucus house. Such a scenario would, for instance, lead to a size-selective predatory impact from chaetognaths that is exactly opposite to what would be expected from visually feeding predators (fish).
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