Biology and Ecology of Hexacorallians in the San Juan Archipelago

Christopher D. Wells

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Reading Committee:

Kenneth P. Sebens, Chair

Megan N. Dethier

Jennifer L. Ruesink

Program Authorized to Offer Degree:

Biology Department

University of Washington

ABSTRACT

Biology and Ecology of Hexacorallians in the San Juan Archipelago

Christopher D. Wells

Chair of the Supervisory Committee:
Kenneth P. Sebens
Biology Department and School of Aquatic and Fishery Sciences

Hexacorallians are one of the most conspicuous and dominant suspension feeders in temperate and tropical environments. While temperate hexacorallians are particularly diverse in the northeast Pacific, little work has been done in examining their biology and ecology. Within this dissertation, I describe a novel method for marking soft-bodied invertebrates such as hexacorallians (Chapter 1), examine the prey selectivity of the competitively dominant anemone *Metridium farcimen* with DNA metabarcoding (Chapter 2), and explore the distribution of the most common hard-bottom hexacorallians in the San Juan Archipelago (Chapter 3).

In the first chapter, I found that both methylene blue and neutral red make excellent markers for long-term monitoring of *M. farcimen* with marked individuals identifiable for up to six weeks and seven months, respectively. I also found that fluorescein is lethal in small dosages to *M. farcimen* and should not be used as a marker. Neutral red could be used for long-term monitoring of growth and survival in the field, and in combination with methylene blue could be

used to mark individuals in distinguishable patterns for short-term studies such as examining predator-prey interactions, movement of individuals, and recruitment survival.

In the second chapter, I found that *M. farcimen* captures a wider variety of prey than has been previously described, likely all prey that are large enough to detect and that cannot escape. Additionally, comparisons between DNA metabarcoding and published results from traditional gut sampling techniques showed that many more taxa can be found by DNA metabarcoding. Terrestrial prey were surprisingly high in abundance within the diet of *M. farcimen*, likely due to the animals living on floating docks. These data highlight the need for consideration of space and time in a sampling regime and the usefulness of the metabarcoding method in identifying prey within the gut of planktivorous animals.

In the final chapter, I found that depth, light, flow, and substratum slope had significant impacts on the distribution of hard-bottom hexacorallians, whereas predation pressure and temperature had no detectable effect. Depth and light have a strong relationship with algal cover and most hexacorallians were conspicuously missing from high algal cover surfaces. Additionally, nearly every species increased in density with increased flow. These data call attention to the need for experimental studies examining the interactions between temperate hexacorallians and algae as well the effects of flow on distribution of anthozoans.

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DEDICATION

This work is dedicated to my grandfather, Earle (Pa) Ernest Houghton, for instilling within me a sense of discovery and interest in science and to Alexander (Zander) Charles Andrew Fodor for keeping me up all those late nights perfecting ice cream.

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CHAPTER I: INDIVIDUAL MARKING OF SOFT-BODIED SUBTIDAL INVERTEBRATES *IN SITU* – A NOVEL STAINING TECHNIQUE APPLIED TO THE GIANT PLUMOSE ANEMONE *METRIDIUM FARCIMEN* (TILESIUS, 1809)

ABSTRACT

The ability to recognize individuals and track growth over time is crucial to population dynamics research as well as studies of animal behavior. Invertebrates are particularly difficult to track as they often molt, have regenerative capabilities, or lack hard parts to attach markers. We tested, in laboratory and field studies, a new way of marking sea anemones (order Actiniaria) by injection of three vital stains (i.e., neutral red, methylene blue, and fluorescein). Neutral red and methylene blue did not affect growth or survival, but fluorescein was lethal at high concentrations. Marked individuals could be identified up to seven months after injection with neutral red, six weeks with methylene blue, and three days with low concentrations of fluorescein. Neutral red could be used for long-term monitoring of growth and survival in the field, and in combination with methylene blue could be used to mark individuals in distinguishable patterns for short-term studies such as examining predator-prey interactions, movement of individuals, and recruitment survival.

INTRODUCTION

The ability to recognize individuals and track growth over time is crucial to population dynamics research, animal behavior studies, as well as to parameterize bioenergetics models (Kitchell *et al.*, 1977; Caswell, 2001). Several methods for marking individuals have been used on marine invertebrates including the use of inserted tags, external tags or colors applied to hard parts, and staining techniques (e.g., Feder, 1955; Sebens, 1976; Kurth *et al.*, 2007; Hale *et al.*, 2012).

However, invertebrates often lack hard parts to attach markers, molt, or have regenerative capabilities and these methods frequently involve removing the animal from the field to mark them.

Only external staining techniques have been used to mark sea anemones (order Actiniaria) (Sebens, 1976). The method used by Sebens (1976, 1977, 1980, 1981b, 1982) requires the anemone to either be exposed during a low tide or taken out into the air to apply the stain. For subtidal anemone species this would involve undue stress during the removal process and then the difficult task of reattachment in the same location. Additionally, the impact of the staining process and the impact of the stain on growth and survival of the anemones has never been quantified.

The objective of this research was to develop a technique to mark subtidal anemones *in situ* while minimizing short- and long-term effects on growth and survival. This method was developed for use in studies of the population dynamics and a bioenergetics model for the giant plumose anemone *Metridium farcimen* (Tilesius, 1809). In both the laboratory and field, we experimentally tested a novel method of marking sea anemones through injection of three vital stains (neutral red, methylene blue, and fluorescein).

MATERIALS AND METHODS

Laboratory Experiment

Sixty-three individuals of *Metridium farcimen* (2.2-6.6 cm diameter, 3.7 cm mean) were collected off the pontoons of the Port of Friday Harbor marina, Friday Harbor, WA, USA (48.538°N, 123.015°W) and maintained in three sea tables (98 x 98 x 12 cm) at Friday Harbor Laboratories. Collections were limited to 63 individuals to limit impact on the wild population and so the sea anemones were not overcrowded in the laboratory tank. Collections were authorized by

the director of Friday Harbor Laboratories and by the port commissioners at the Port of Friday Harbor marina. This research did not involve any endangered or protected species. Anemones were maintained for two weeks before experimental treatments were applied to allow time for any pedal disk damage incurred during collection to heal. Seawater exchange was kept at 1.0 L/min during the healing period. This rate created a slow circular current, but was not fast enough to dislodge attached and attaching anemones. Anemones were fed 24-hr old *Artemia salina* (Linnaeus, 1758) nauplii daily.

One of three treatments (21 individuals per treatment) was randomly applied to each anemone through hypodermic injection with a 22-gauge, stainless steel needle. Anemones were injected with 1.0 mL of either 10% neutral red, 10% methylene blue, or with raw seawater alone as a control. All stains in this and the subsequent experiment were diluted in raw seawater. Both neutral red and methylene blue do not fully dissolve at this concentration in raw seawater and therefore some stain was injected in solid form. No work has been done to look at the effect of undissolved stain on animal tissues, although presumably solid stain would dissolve in the sea water in the coelenteron of the anemones and cause little damage. Anemones were injected about 0.5 cm above the pedal disk, directly into the coelenteron (i.e., gastric cavity). After anemones were injected, water exchange was increased to 1.5 L/min. A circulation pump (18.6 L/min) was added to each tank to increase the circular current on a six hour on, two hours off cycle.

Both neutral red and methylene blue are vital stains. Neutral red binds to lysosomes in live tissue, whereas methylene blue binds to DNA. Additionally methylene blue is used as an antimicrobial at very low concentrations and in a variety of fields of medicine (Wainwright and Crossley, 2002). Neutral red has been successfully applied to the outside of intertidal anemones

(Sebens, 1976), but its impact on growth and survival has yet to be quantified. Also, its efficacy to mark sea anemones when injected has not been tested.

Growth of anemones was monitored on a weekly basis for six weeks starting in July 2015 by measuring the major (i.e., maximum) and minor (i.e., perpendicular to the maximum) pedal disk diameters with digital calipers and calculating an average pedal disk diameter as described in Wells (2013). The effect of injecting neutral red and methylene blue on growth of *M. farcimen* was computed using a residual maximum likelihood linear mixed model in JMP 13 with days passed, tank, and individual anemone as random effects and solution injected as a fixed effect. Survival was checked daily.

Field Experiment

The previous experiment was repeated with small modifications to determine if the same growth and survival patterns seen in the lab would be observed in the field. On the underside of the 10 pontoons of the floating docks at Friday Harbor Laboratories, 50 specimens of *M. farcimen* (1.4-8.7 cm diameter, 3.1 cm average) were selected for one of five treatments (10 individuals per treatment with one individual on each pontoon). Number of anemones injected and subsequently measured was limited by dive-partner availability. Anemones were injected with 10% neutral red, 10% methylene blue, 10% fluorescein, or 0.25% fluorescein or raw seawater alone as a control during a SCUBA dive. Fluorescein is commonly used to observe water flow and fluoresces yellow-green. Anemones were injected with 2-6 mL of solution with larger anemones receiving more material. 16-gauge stainless steel needles were used to inject each anemone. Needle size was increased to reduce the chance of blockages in the needle from aggregates of undissolved stain, which can easily be corrected in a laboratory setting, but cannot while SCUBA diving. Anemones

were otherwise not disturbed. Neighboring non-experimental anemones were not removed; density of potential competitors was not controlled.

Photographs (12 megapixel stills) of anemones were taken (GoPro Hero4 Black Edition) weekly for the first six weeks and then every two to three weeks thereafter starting in July 2015. From these photographs, major and minor pedal disk diameters could be measured in the program ImageJ (National Institute of Health), which allowed growth rates to be calculated as described earlier. As anemones did not move extensively during the experiment, control anemones (i.e., seawater-injected anemones) could be tracked without external markings based on their position compared to marked anemones and their size. Effect of solution injected on growth rate was computed as in the laboratory experiment except with the addition of pontoon as a random effect. Survival was checked one day after the initial treatment and then every time as photographs were taken. Anemones were considered dead if there was major tissue necrosis typical of anemone death.

RESULTS

Neither methylene blue nor neutral red had significant effects on growth in both laboratory and field experiments ($p > 0.05$ for both, Figure 1), although sample size was small in the field experiment (N = 10 per treatment). In the laboratory experiment, mean diameter growth rates in methylene blue, neutral red, and sea water injected anemone were 0.10, 0.04, and 0.10 mm/day, respectively. In the field experiment, growth rates in methylene blue, neutral red, and sea water injected anemones were 0.0036, 0.0036, and 0.0042 mm/day, respectively, at 40 days. At 156 days, well beyond when methylene blue was visible, diameter growth rates for neutral red and sea water injected anemones were 0.020 and 0.0017 mm/day, respectively. Growth rates were one to two

orders of magnitude larger in the laboratory experiment, likely due to the abundant food available in the laboratory setting.

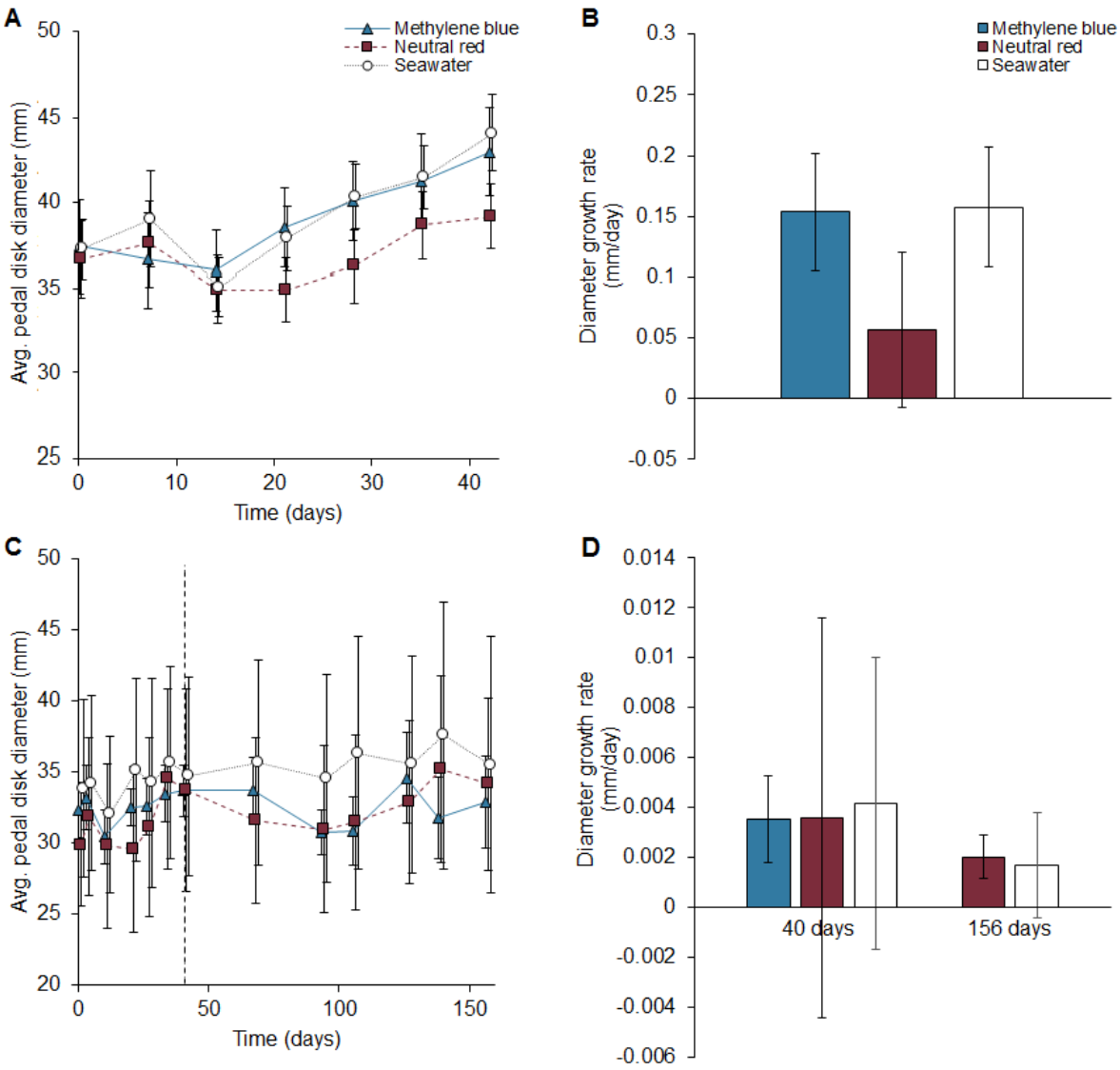

Figure 1. Growth of *Metridium farcimen* injected with 10% neutral red, 10% methylene blue, or seawater (control) in (A, B) laboratory and (C, D) field experiments. There was no significant difference between the marked anemone and control growth rates in either both laboratory or field experiments ($p > 0.05$, N = 21 and N = 10 respectively). Values are means ± standard error. The dashed line in panel C indicates the time at which methylene blue marked individuals lost sufficient color to be identified as marked. Neutral red individuals were still clearly visible at 156 days. Growth rates were compared at 40 and 156 days between all treatments that had visibly marked individuals.

One individual injected with neutral red and one control anemone were lost during the laboratory experiment, but were recovered in the drain trap. They had not died, but were damaged

so were excluded from the experiment. Fluorescein at high concentrations (10%) was lethal to *Metridium farcimen*; all anemones died within a week. Anemones marked with 0.25% fluorescein were visibly marked for a maximum of three days. Behavior was qualitatively similar to control anemones in 10% neutral red, 10% methylene blue, and 0.25% fluorescein (i.e., normal movement, feeding, and reaction times to physical stimuli). There could have been depressed growth within the first two weeks due to the injection process, but without a no-injection group this effect is not detectable.

Marked anemones were clearly stained within seconds, but the mark became brighter and dispersed throughout the animal over the course of an hour as free stain was absorbed from the coelenteron into the tissue. Injecting seawater did not change the color of *M. farcimen*. Methylene blue changed the color of the anemones to a brilliant blue (Figure 2B). The majority of the stain was observed bound to endodermal cells and additionally bound to cells surrounding the mouth and cinclides (i.e., blister-like openings to the coelenteron on the column) where anemones ejected coelenteric fluid during the injection process. Marked individuals were recognizable for six weeks, although acontia (i.e., defensive filaments in the coelenteron) kept a blue hue during the full length of the field experiment. With large anemones, acontia coloration was less apparent unless anemones were severely disturbed leading to acontia being extruded out the cinclides, a normal defensive reaction. Injecting anemones with neutral red changed their color to a deep red (Figure 2C). Neutral red became noticeably lighter in coloration over time, but marked individuals were recognizable until the conclusion of both experiments. Anemones were revisited two months after the conclusion of the field experiment (seven months post-injection) and marked anemones were still clearly stained. Staining patterns were similar to methylene blue. Neutral red has since been used in subsequent field experiments and neutral red marked anemones have been trackable for

over one year (Figure 3). Both 10% (before death) and 0.25% fluorescein injected anemones were a brilliant yellow post-injection with staining patterns similar to the other two stains (Figure 2D).

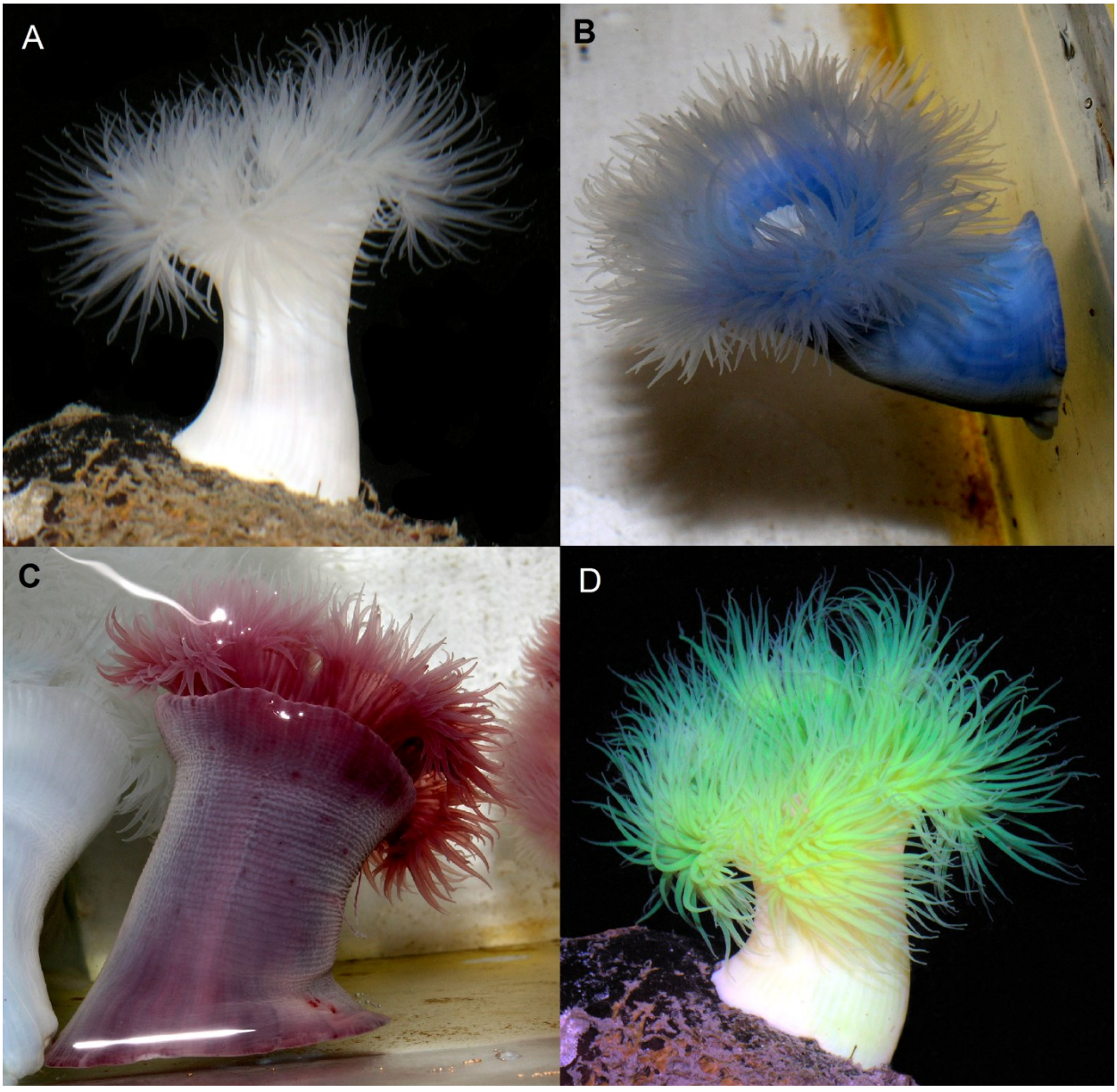

Figure 2. *Metridium farcimen* (A) before injection and injected with 1.0 mL of (B) 10% methylene blue, (C) 10% neutral red, and (D) 0.25% fluorescein. Endodermal tissue in the column and tentacles as well as cinclides are clearly marked in stained anemones.

Figure 3. Dr. Kenneth P. Sebens and Dr. Christopher D. Wells after diving at Long Island, WA, USA.

DISCUSSION

Both neutral red and methylene blue are effective stains for marking sea anemones in both a field and laboratory setting (Figure 2) with no detectable effect on growth rates in field and laboratory experiments (Figure 1). Neutral red makes an excellent long-term stain as anemones retain this stain for well over six months in this study (Figure 3), and we observed no significant impacts on growth or survival. Due to its shorter retention time (i.e., six weeks), methylene blue

can be used for short-term marking of anemones. Combining both neutral red and methylene blue in sea anemones would allow for recognizable patterns for identifying individual anemones (i.e., half blue-half red or purple), although interactive effects of using both dyes simultaneously were not quantified in this study and therefore some caution should be used. Short-term marking of anemones would allow for better tracking of movement and allow for high confidence in identifying individuals in predator-prey experiments with multiple predators or prey. The change in color of the anemones is not a concern for predator-prey studies because predators of anemones (e.g., nudibranchs and sea stars) do not generally rely on visual cues to find prey. Fluorescein is not recommended as a marker as anemones retained marks for minimal time (i.e., three days) at low concentrations and died at high concentrations. This finding was rather unexpected as fluorescein is often used with invertebrates to visualize water flow (e.g., Eerkes-Medrano *et al.*, 2015; Nishizaki and Carrington, 2015; Voltzow, 2015). Fluorescein could be used to mark an anemone if marking is only needed for a day (Figure 2).

This method may have utility in other types of sea anemone research in laboratory and field settings, such as labeling tissues for tracing lineages of tissues grafted between anemones (e.g., Kraus *et al.*, 2007). Labeling could also be used to track a genet as it asexually reproduces, an extremely common trait in sea anemones (reviewed in Shick, 1991), or to track tissues amputated to cause regeneration. This method could be expanded to include corals and other cnidarians, although it is unknown how these stains would interact with the calcified parts of scleractinians and octocorals. It would be particularly interesting to mark the scyphistomae of scyphozoans and examine how tissue is allocated to the budding ephyrae.

While qualitative observations of behavior were recorded during the lab experiment, effects on reproduction, feeding, or other activities were not quantified and it is possible that these

dyes could have other unanticipated effects on *M. farcimen*. This could be particularly important for future studies that include measures of fitness or other longer-term metrics. Similarly, dye would change an anemone's albedo, which would be especially important for those in the intertidal zone that are at risk of stress from heat or drying out. Additionally, many anemones associate with algal symbionts and how these stains affect the symbiosis as well as the growth and survival of the symbionts is unknown.

While methylene blue and neutral red make effective markers for sea anemones, the internal impact on other taxa is unknown. Neutral red had no effect on survival of termites when taken up in their water (Evans, 2000). Submersion of amphipods and gastropods in neutral red, which marked internal structures, did not reduce survival (Howard, 1985; Drolet and Barbeau, 2006). Both internal and external applications of methylene blue have few to no complications on targeted tissues in humans (Granick *et al.*, 1987; Varghese *et al.*, 2008). External marking with neutral red did have a negative impact on growth and survival in larval amphibians (Travis, 1981; Jung *et al.*, 2002) while external application of methylene blue reduced predator avoidance (Carlson and Langkilde, 2013). Exposure to methylene blue and neutral red, separately, affected development, pupation, and survival of female mosquito larvae, but not of males (Barbosa and Peters, 1970).

It is clear that methylene blue, neutral red, and fluorescein can have a diversity of effects on different taxa, ranging from long-term healthy staining to death. Caution should be used whenever applying a marking technique to a new taxon. Impacts of the staining process (e.g., injection, submersion, or taking stain up through food or water) and the stain itself on survival, growth, or any other parameters critical to the experiment must be quantified and addressed before proceeding with other experiments.

CHAPTER II: DNA METABARCODING PROVIDES INSIGHTS INTO THE DIVERSE DIET OF A DOMINANT SUSPENSION FEEDER, THE GIANT PLUMOSE ANEMONE *METRIDIUM FARCIMEN*

ABSTRACT

Benthic suspension feeders can have significant impacts on plankton communities by depleting plankton or by modifying composition of the plankton through prey selectivity. Quantifying diets of planktivorous animals can be difficult because plankton are frequently microscopic, may lack diagnostic characters as larvae, and are digested at variable rates. With the use of DNA metabarcoding, the identification of gut contents has become faster and more accurate, and the technique allows for higher taxonomic resolution while also identifying rare and highly degraded items that would otherwise not be observed. We used DNA metabarcoding to examine the diet of the giant plumose anemone *Metridium farcimen*, a large, abundant, competitively-dominant anemone on subtidal rock surfaces and floating docks in the northeast Pacific Ocean. Gut contents of 12 individuals were compared to 80 and 330 μm filtered plankton samples collected 1 hr previously at 0.02, 0.28 and 1.5 km away. The objectives of this study were both to determine if *M. farcimen* has a selective diet and to compare our findings with published, traditional gut content analyses. We found that *M. farcimen* captures a wider range of prey than previously suspected, likely all prey that it can detect and that cannot escape, and metabarcoding can find many more taxa than traditional sampling techniques. Gut contents (73.8 operational taxonomic units (OTUs)) were less diverse than 80 μm filtered plankton samples (91.3 OTUs), but more diverse than 330 μm filtered plankton samples (52.7 OTUs). The diet of the anemones was 52% arthropods with a surprisingly high insect input (10%), which was not present in the plankton. There were no overrepresented OTUs in the gut contents compared to the plankton but there were underrepresented OTUs (19 and 14 OTUs for 80 and 330 μm filtered plankton, respectively). This

study highlights the need for consideration of space and time in a sampling regime and the usefulness of the metabarcoding method in identifying prey within the gut of planktivorous animals.

INTRODUCTION

Benthic suspension feeders can have major impacts on the plankton communities they prey on (Sebens and Koehl, 1984; Young and Gotelli, 1988; Kimmerer *et al.*, 1994; Gili and Coma, 1998; Petersen, 2004; Whitten *et al.*, 2018). In coastal marine areas, dense populations of suspension feeders can often filter the immediately overlying water volume several times a day (Jørgensen, 1980; Davies *et al.*, 1989; Riisgård, 1991; Petersen and Riisgård, 1992; Vedel *et al.*, 1994; Petersen, 2004). Grazing experiments with polychaetes and ascidians have found that these suspension feeders may be able to deplete phytoplankton up to 30 cm away from the bottom in estuaries (Riisgård *et al.*, 1996a; Riisgård *et al.*, 1996b; Vedel, 1998).

Escape capabilities, morphological defenses, toxicity, and size vary greatly in the plankton and these factors can heavily influence capture probability of planktonic prey (Dodson, 1974; Browman *et al.*, 1989; Suchman and Sullivan, 1998; Viitasalo *et al.*, 1998; Engström *et al.*, 2001; Viitasalo *et al.*, 2001; Safi *et al.*, 2007). Many bivalves preferentially select plankton, with size, shape, and motility being more important factors than biomass (Cucci *et al.*, 1985; Shumway *et al.*, 1985; Defossez and Hawkins, 1997; Safi *et al.*, 2007). Prey selectivity can result from the inability of predators to capture certain prey, the preferential capture or consumption of palatable and energetically valuable species, or active rejection of prey. Studies on prey selectivity allow for better understanding of the effects of predators on their ecosystem, prey capturing mechanisms, and dietary niche partitioning (Suchman and Sullivan, 1998; Costello and Colin, 2002; Leray *et al.*, 2019).

Unfortunately, quantifying the diet of planktivorous animals can be difficult because plankton are microscopic, frequently lack diagnostic characters as larvae, and are digested quickly but at variable rates (Purcell, 1977; Sebens and Koehl, 1984; Zamer, 1986; Fancett, 1988; Larson, 1991). Because of this, marine plankton are difficult to identify in gut contents past the class or order level when using traditional visual identification techniques (e.g., Purcell, 1977; Sebens and Koehl, 1984; Fancett, 1988).

With the advent of high throughput sequencing and powerful molecular techniques such as DNA metabarcoding (Taberlet *et al.*, 2012), identification of specimens within community samples, such as in plankton and gut contents, can be rapid, accurate, and relatively cheap (Aylagas *et al.*, 2014; Brandon-Mong *et al.*, 2015; Nielsen *et al.*, 2018). DNA metabarcoding has been used to successfully identify taxa within gut contents of fishes (Leray *et al.*, 2013b; Leray *et al.*, 2015; Albaina *et al.*, 2016; Harms-Tuohy *et al.*, 2016), to evaluate biodiversity of insects (Yu *et al.*, 2012; Ji *et al.*, 2013; Brandon-Mong *et al.*, 2015), and to find rare taxa with environmental DNA (Evans *et al.*, 2016; Valentini *et al.*, 2016; Deiner *et al.*, 2017). Metabarcoding can be used to reanalyze diets of previously examined species to get higher taxonomic resolution while also identifying rare and highly degraded items that would otherwise not be observed (Nielsen *et al.*, 2018).

We used DNA metabarcoding to examine the diet of the giant plumose anemone *Metridium farcimen* (Cnidaria, Anthozoa, Actiniaria). *M. farcimen* is a large, abundant anemone on subtidal rock surfaces and floating docks in the northeast Pacific Ocean (Hand, 1955b; Ricketts *et al.*, 1968; Kozloff, 1973; Fautin *et al.*, 1989; Fautin and Hand, 2000) that feeds on small zooplankton (Koehl, 1977a; Purcell, 1977; Sebens, 1981a; Shick, 1991). *M. farcimen*, which can extend over a meter into the water column (Fautin *et al.*, 1989), is well-adapted for high-flow environments (Koehl,

1977a, b, c) and is a competitive dominant species on rocky subtidal ledge communities (Nelson and Craig, 2011). Gut contents of *M. farcimen* in the San Juan Archipelago, WA, USA were compared to concurrent, nearby plankton samples. The objectives of this study were both to determine if *M. farcimen* has a selective diet and to compare findings from traditional gut content analyses (i.e., suction and dissection) with metabarcoding analysis.

MATERIALS AND METHODS

Sample collection and gut content extraction

The available suspended food was quantified by sampling the plankton at three sites within the San Juan Archipelago: beside the Friday Harbor Laboratories (FHL), Friday Harbor, WA, USA floating dock (48.5452°N, 123.0124°W); 280 m southeast of the docks (48.5436°N, 123.0100°W); and in San Juan Channel (48.5490°N, 122.9924°W), 1.5 km northeast of the docks. Samples were taken during an ebb tide between 1:00 and 2:30 pm on August 4, 2016, a season when plankton is highly diverse. Two simultaneous plankton tows were performed at each site. Each pair of tows consisted of an 80 µm mesh size net to capture a broad range of plankton and a 330 µm mesh size net to capture large zooplankters. Both plankton nets were 50 cm wide, and approximately 98 m^3 (125 m long) of water was sampled. Samples were immediately preserved in 95% ethanol in the field and kept at -20 °C in the laboratory.

Sixteen *M. farcimen* were collected from the floating docks at FHL by hand one hour after the plankton tows. All collected anemones were within 20 m of each other. Anemones were kept in seawater in bags on ice until gut contents could be extracted (between 0.5 and 3 hr following collection). Material attached to the pedal disk (i.e., the bottom of the anemone attached to the substratum) was carefully removed and discarded. In the laboratory, anemones were bisected and

then small shallow longitudinal incisions were cut within the coelenteron, allowing efficient extraction of gut contents. Gut contents were extracted by massaging the coelenteron, rubbing the sharp end of a scalpel against the mesenterial filaments, and flushing the coelenteron with 45 µm filtered seawater. Material extracted from the gut consisted of partially digested food, copious amounts of mucus, mesenterial filaments, acontia, and some gonadal material in sexually mature individuals. Large food particles (e.g., hydromedusae) were cut up into small pieces to facilitate later grinding.

To remove excess mucus and enrich samples in prey DNA, gut contents were rinsed with 95% ethanol in a 45 µm mesh net. Ethanol rinses efficiently break up anemone mucus compared to seawater rinses. Material within the 45 µm mesh net was massaged to further break up large pieces. During this process there is a risk of losing partially digested items without exoskeletons. Contents were transferred into sterile sample tubes with 95% ethanol and kept at -20 °C overnight. As there were still large particles within the sample, samples were centrifuged at 2000 x g for eight minutes at 20°C, the supernatant was decanted, and the pellet was ground up within a mortar and pestle into a fine paste. The paste was placed back into another sterile tube with 95% ethanol, centrifuged at 2000 x g for eight minutes at 20°C, and the supernatant was decanted. The whole pellet of each sample was used for DNA extraction using the MoBIO PowerSoil® DNA Isolation Kit following manufacturer's instructions. Genomic DNA for both plankton and anemone samples were quantified with a Qubit fluorimeter and diluted to 10 ng/µL.

PCR and Library Preparation

We used a hierarchical tagging approach with a combination of randomly-assigned tailed PCR primers and single indexed Illumina Y-adapters to sequence all samples in a single Illumina MiSeq run. Three PCR replications were performed per sample. DNA amplification was

confirmed on 1.5% gel electrophoresis and then triplicates were pooled. DNA was purified using solid phase reversible immobilization beads to remove primers, primer dimers, salts and dNTP's. DNA samples from the 16 anemones and 6 plankton samples (3 distances, 2 size classes) were used to amplify a highly variable fragment (~313 bp) of the cytochrome c oxidase subunit (COI) region with the PCR primers mlCOIintF and jgHCO2198 (Geller *et al.*, 2013; Leray *et al.*, 2013b). Despite some amplification bias, this set of primers generates useful estimates of relative abundance (Leray and Knowlton, 2015). PCR reactions were performed in a total volume of 20.0 µL, containing 13.2 µL of nuclease free water, 2.0 µL of Clontech 10X Advantage 2 PCR buffer (Takara Bio Inc., Kusatsu, JP), 1.0 µL of mlCOIintF (10 µM), 1.0 µL of jgHCO2198 (10 µM), 1.4 µL of dNTP, 0.4 µL of Clontech 50X Advantage 2 (Takara Bio Inc., Kusatsu, JP), and 1.0 µL (10 ng) of DNA. The reactions were incubated in a Biometra T3 thermocycler (Analytik Jena, Jena, DE), starting with 5 min of denaturation at 95 °C, followed by 35 cycles of 30 s at 95 °C, 30 s at 48 °C, and 45 s at 72 °C, with a final extension of 72 °C. A negative PCR control and extraction control were performed to test whether the reagents were free of contaminants; both were negative for contamination. Purified PCR products were quantified using an Invitrogen Qubit™ fluorimeter and then diluted to 30 ng/µL. The PCR products of samples amplified with different tailed primers were pooled before library prep as detailed by Leray *et al.* (2013a) and Leray *et al.* (2016). Samples were prepared for sequencing with the Illumina TruSeq DNA PCR-free LT Library Prep Kit, which includes end-repair and dA-tailing chemistry, and then ligated with adapters.

Bioinformatics

Sequences were demultiplexed and Illumina adapters were trimmed using Flexbar (Roehr *et al.*, 2017). DADA2 (Callahan *et al.*, 2016; Callahan *et al.*, 2017) was then used to remove primers, discard low quality sequences and infer exact amplicon sequence variants (ASVs) using

the following parameters: maxN = 0, maxEE = c (2, 2), truncQ = 10, trimLeft = 26. DADA2 uses sequence quality scores and abundance information to generate an error model that best fit the data, and subsequently uses the error model to infer ASVs. ASVs, which can differ by as little as one nucleotide, were clustered into operational taxonomic units (OTUs) at a 97% identity threshold using VSEARCH (Rognes *et al.*, 2016). To further improve estimates of alpha and beta diversity, spurious OTUs were removed using the LULU algorithm (Frøslev *et al.*, 2017) (parameters: minimum ratio type = "min", minimum ratio = 1, minimum match = 84, minimum relative co-occurrence = 0.95). This tool, which uses sequence similarity and co-occurrence patterns, was shown to reduce taxonomic redundancy and improve similarity with the true taxonomic composition of test samples (Frøslev *et al.*, 2017). Taxonomic names were assigned to each remaining OTU using an iterative approach. First, BLASTn searches were used to compare one representative sequence of each OTU to a local database of DNA barcodes deposited in GenBank (ascension number PRJNA400342, 375 sequences from specimens belonging to 220 genera, Benson *et al.*, 2017). Many of these species were collected off the floats near the source of *M. farcimen*. An OTU was considered to match a local barcode when the level of sequence similarity was higher than 98%. 104 OTUs (24% of total OTUs) were identified from this dataset. Second, unidentified OTUs were assigned taxonomic information using the Bayesian Least Common Ancestor Taxonomic Classification method (BLCA) (Gao *et al.*, 2017) against a curated database of metazoan mitochondrial gene sequences (Midori-Unique v20180221 available at www.reference-midori.info, Machida *et al.*, 2017). Assignments with less than 50% confidence were not taken into account. Third, the numerous OTUs that remained unidentified using BLCA were compared to the whole NCBI NT database (May 2018) using BLAST searches (word size =

7; max e-value = 5e-13) and assigned the taxonomy of the lowest common ancestor of the first 100 hits.

Statistical Analyses

Samples with over 95% *M. farcimen* sequences and less than 7000 amplified sequences were dropped from the analysis. It was assumed that these individuals were either not feeding or prey sequences were hidden by the abundance of *M. farcimen* sequences leading to insufficient data to categorize diet; including these data could bias the results. After dropping these four of 16 samples, the remainder were rarified to the lowest number of sequences by dropping out sequences at random (7268 sequences). An unequal number of sequences can affect estimates of diversity due to the positive relationship between sample size and number of OTUs. This rarefied data set was used for all further analyses. All data were analyzed in R version 3.5.2 with the vegan 2.5-4 package (R Core Team, 2018; Oksanen *et al.*, 2019).

To illustrate the sequencing effort, rarefaction curves were built, one for each sampling effort (i.e., gut contents and the two plankton sizes). A plateauing curve indicates an exhaustive sampling effort.

There are five ways we defined community composition in this study: the number of OTUs or taxa in a sample or in a treatment (e.g., anemone guts), herein referred to as richness; the fraction an OTU or taxon was of the total sequences, herein referred to as abundance; the fraction of the total OTUs or taxa, herein referred to as diversity; the closeness of the abundances of the OTUs, herein referred to as the evenness; and the fraction of anemone guts containing a particular OTU or taxon, herein referred to as incidence. Mean evenness for each treatment was calculated using Pielou's evenness index (Pielou, 1966).

Matrices of community dissimilarity based on the Bray-Curtis index were created using both number of sequences and presence/absence of OTUs (i.e., the Sørensen index). Differences between diet composition of *M. farcimen* (n=12) and 80 (n=3) and 330 µm (n=3) filtered plankton communities were tested using permutational multivariate analyses of variance (PERMANOVA, Anderson, 2001) with 9999 permutations. Patterns of species composition were visualized in two-dimensional space using non-metric multidimensional scaling ordination plots (nMDS) with 9999 permutations. Similarity percentage analysis (SIMPER) were used to determine what OTUs were significantly contributing to the Bray-Curtis dissimilarities calculated between groups of samples (Clarke, 1993).

RESULTS

A total of 3,109,361 high quality metazoan sequences passed the quality controls with an average of 101,000 sequences per plankton sample and an average of 12,500 non-*Metridium* sequences for *Metridium farcimen* gut content samples. Four *M. farcimen* gut contents did not provide a sufficient number of sequences (< 7000) and were dropped from further analyses, leaving 12 samples. 107 OTUs (24% of all OTUs) were identified to species. 357 OTUs (82% of all OTUs) were identified to the phylum level. There was a total of 438 OTUs. Rarefaction curves for both plankton and *M. farcimen* plateaued, which indicates that few to no additional species were present but failed to appear in the barcoding effort (Figure 4).

M. farcimen gut contents were richer in total (356 OTUs) than either the 80 µm filtered plankton (160 OTUs) or the 330 µm filtered plankton (97 OTUs). Gut contents were less rich on average (73.8 OTUs) than the 80 µm filtered zooplankton samples (91.3 OTUs) and more diverse than the 330 µm filtered zooplankton samples (52.7 OTUs). The 80 and 330 µm filtered zooplankton samples were more even (0.64 and 0.68, respectively) than the gut contents (0.57).

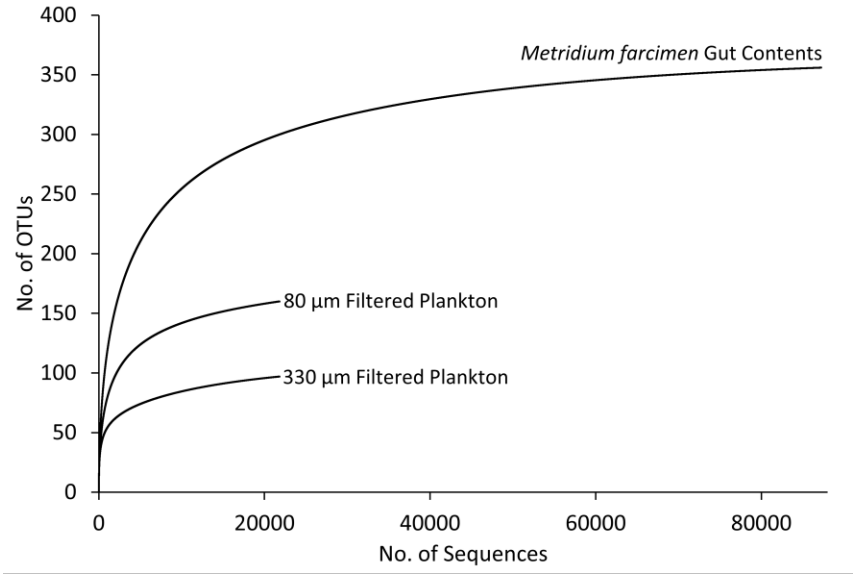

Figure 4. Rarefaction curves to evaluate the completeness of the sequencing effort at describing the diversity of dietary items in the gut contents of the giant plumose anemone *Metridium farcimen* and in nearby plankton samples using two different mesh sizes. All curves plateaued which indicates that this sampling was exhaustive.

Mean incidence was lowest in the gut contents (21%) and higher in the 80 and 330 µm filtered plankton (57% and 54%, respectively).

The gut contents of *M. farcimen* contained on average 14.8 classes belonging to 10.5 animal phyla (Table 1). *M. farcimen* diet contents were primarily made up of arthropods (52% of sequences), especially insects, crab larvae, barnacle larvae, and copepods (Table 2). Hexanauplia was the most diverse class for both the gut contents and the 80 µm and 330 µm filtered plankton samples (14.5, 9.3, and 15.7 OTUs, respectively) and had the highest proportion of sequences for all three samples (22%, 38%, and 33%, respectively, Table 1). The class present in the largest fraction of anemones was Hexanauplia (Table 3). Overall, metabarcoding of gut contents detected many more taxonomic groups than found by Purcell (1977), Sebens (1981a), or Sebens and Koehl (1984) for *Metridium* spp. through standard visual identification – on average, eight metazoan classes were identified in visual identification methods (Purcell, 1977; Sebens, 1981a; Sebens and Koehl, 1984) whereas 26 classes were found using metabarcoding (Table 4).

Table 1. Average proportion of sequences from plankton samples and the gut contents of the giant plumose anemone *Metridium farcimen* with average number of OTUs in parentheses. Proportions of sequences in *M. farcimen* guts most closely resembled the 80 µm filtered plankton except with elevated levels of the classes Malacostraca and Ostracoda.

Phylum	Class	*Metridium farcimen* Gut Contents	80 µm Filtered Plankton	330 µm Filtered Plankton
Annelida	Polychaeta	9% (10)	9% (11)	Present (1)
	Unidentified	Present (1.3)	Present (1.7)	
Arthropoda	Arachnida	Present (0.7)		Present (0.3)
	Branchiopoda	3% (1.7)	6% (2.7)	7% (2.7)
	Hexanauplia	22% (14.5)	38% (15.7)	33% (9.3)
	Insecta	10% (1.3)	Present (1)	Present (0.7)
	Malacostraca	8% (3.2)	Present (2)	5% (6)
	Ostracoda	5% (0.9)		Present (0.3)
	Unidentified	4% (5.2)	Present (3)	Present (1.7)
Bryozoa	Gymnolaemata	2% (4.1)	5% (7.3)	29% (8)
	Unidentified	Present (0.6)		
Chaetognatha	Sagittoidea	Present (0.2)	Present (2.7)	2% (4.7)
Chordata	Actinopteri	Present (0.3)	Present (0.3)	1% (2.3)
	Ascidiacea	Present (0.1)		
Cnidaria	Anthozoa	Present (0.2)		
	Hydrozoa	1% (2.7)	1% (4.7)	6% (6.7)
	Scyphozoa	Present (0.8)	Present (0.7)	5% (0.7)
	Unidentified	Present (0.1)		
Ctenophora	Tentaculata	1% (0.8)		Present (1.3)
Echinodermata	Echinoidea	Present (0.5)	Present (1.7)	Present (0.3)
	Holothuroidea	Present (0.5)	Present (0.7)	
	Ophiuroidea	Present (0.1)	Present (2.3)	
Mollusca	Bivalvia	1% (2.8)	3% (10)	Present (0.3)
	Gastropoda	1% (3.6)	Present (3.7)	Present (2.3)
	Polyplacophora	Present (0.2)	Present (0.3)	Present (0.3)
	Unidentified	Present (0.1)		
Nematoda	Chromadorea	Present (0.5)		
Nemertea	Anopla		Present (0.3)	
	Enopla	Present (0.3)	Present (0.3)	
Phoronida	N/A		Present (0.3)	
Platyhelminthes	Rhabditophora	Present (0.6)	Present (0.7)	
	Unidentified	Present (0.9)	Present (0.7)	
Porifera	Demospongiae	3% (1.3)	2% (1)	Present (0.7)
	Homoscleromorpha		Present (0.3)	
	Unidentified	Present (0.2)		
Rotifera	Monogononta	2% (2.3)	14% (4.7)	Present (0.7)
Unidentified Animal	N/A	26% (11.9)	19% (11.7)	11% (2.3)

Table 2. The 15 most abundant OTUs in the gut contents of the giant plumose anemone *Metridium farcimen* (68% of sequences). *M. farcimen* diet is highly diverse, but consists primarily of arthropods (e.g., insects, crab larvae, barnacle larvae, and copepods).

Lowest Taxonomic Group	Class	Avg. No. of Sequences
Lasius brevicornis	Insecta	703
Metazoa sp. 26	N/A	562
Metazoa sp. 1	N/A	524
Metacarcinus gracilis	Malacostraca	450
Balanus sp. 1	Hexanauplia	437
Metazoa sp. 2	N/A	356
Pseudocalanus newmani	Hexanauplia	322
Spionidae sp. 1	Polychaeta	285
Metazoa sp. 9	N/A	240
Sarsiellidae sp. 1	Ostracoda	216
Halichondria panicea	Demospongiae	193
Evadne nordmanni	Branchiopoda	185
Balanus nubilus	Hexanauplia	174
Laonice cirrata	Polychaeta	171
Arthropoda sp. 2	N/A	162

Table 3. OTUs contained in more than half of the gut contents of the giant plumose anemone *Metridium farcimen*.

Lowest Taxonomic Group	Class	No. of *Metridium* Containing
Gastropoda sp. 1	Gastropoda	9/12
Calanoida sp. 1	Hexanauplia	9/12
Campanulariidae sp. 1	Hydrozoa	9/12
Ploima sp. 1	Monogononta	9/12
Balanus glandula	Hexanauplia	8/12
Tisbe sp. 1	Hexanauplia	8/12
Metazoa sp. 2	N/A	8/12
Metazoa sp. 3	N/A	8/12
Macoma lipara	Bivalvia	7/12
Halichondria panicea	Demospongiae	7/12
Amonardia perturbata	Hexanauplia	7/12
Cyclocanna welshi	Hydrozoa	7/12
Metazoa sp. 5	N/A	7/12
Polychaeta sp. 1	Polychaeta	7/12
Polycladida sp. 1	Rhabditophora	7/12

Table 4. Percent of diet in all published *Metridium* species. Non-metazoan and unidentified categories (e.g., eggs and embryos) were excluded. Purcell (1977) and Sebens (1981a) used relative abundance, Sebens and Koehl (1984) used relative biomass, and we used relative number of sequences.

Phylum	Class	Purcell 1977 – *M. senile*	Purcell 1977 – *M. farcimen*	Sebens 1981 – *M. farcimen*	Sebens and Koehl 1984 – *M. senile*	This Study – *M. farcimen*
Annelida	Polychaeta	15%	15%	1%		8%
Arthropoda	Arachnida				Present	Present
	Branchiopoda				Present	3%
	Hexanauplia	45%	57%	87%	14%	26%
	Insecta					10%
	Malacostraca	Present	Present	4%	65%	5%
	Ostracoda	Present			1%	7%
Bryozoa	Gymnolaemata	Present	1%	1%	Present	2%
Chaetognatha	Sagittoidea					Present
Chordata	Actinopteri					Present
	Ascidiacea				6%	Present
Cnidaria	Anthozoa					Present
	Hydrozoa				12%	1%
	Scyphozoa					Present
Ctenophora	Tentaculata					Present
Echinodermata	Asteroidea			1%		
	Echinoidea					Present
	Holothuroidea					Present
	Ophiuroidea					Present
Mollusca	Bivalvia	34%	30%	1%		2%
	Gastropoda	6%	2%	1%		1%
	Polyplacophora					Present
Nematoda	Chromadorea	Present	Present		Present	Present
Nemertea	Enopla					Present
Platyhelminthes	Rhabditophora					Present
Porifera	Demospongiae				Present	3%
Rotifera	Monogononta					3%

There was a significant difference between the communities in the plankton samples and the gut samples for both number of sequences (PERMANOVA, $F_{2, 15} = 1.93$, $R^2 = 0.20$, $p < 0.001$) and presence/absence (PERMANOVA, $F_{2, 15} = 2.44$, $R^2 = 0.25$, $p < 0.001$, Figure 5 and Figure 6). There were significant differences between the 80 µm filtered plankton sample and the gut contents for both number of sequences (PERMANOVA, $F_{1, 13} = 1.31$, $R^2 = 0.11$, $p = 0.011$) and presence/absence (PERMANOVA, $F_{2, 15} = 1.72$, $R^2 = 0.12$, $p = 0.027$). There were 19 and 14 OTUs that contributed significantly to the difference between gut content and plankton samples (80 µm and 330 µm, respectively) (SIMPER, $p < 0.05$, Table 5 and Table 6). All of the significantly

contributing OTUs were much more abundant in the plankton than in the gut contents. The corrugated clam *Humilaria kennerleyi*, the hydrozoan *Clytia languida*, the brittle star *Ophiopholis kennerlyi*, and the peanut worm *Phascolosoma agassizii* were all more than 25 times less abundant in the gut contents compared to the 80 µm filtered plankton. The speckled sanddab *Citharichthys stigmaeus*, the periwinkle *Littorina scutulata*, the bryozoan *Membranipora membranacea*, and the hydrozoan *Clytia hemisphaerica* were all over 70 times less abundant in the gut contents compared to the 330 µm filtered plankton.

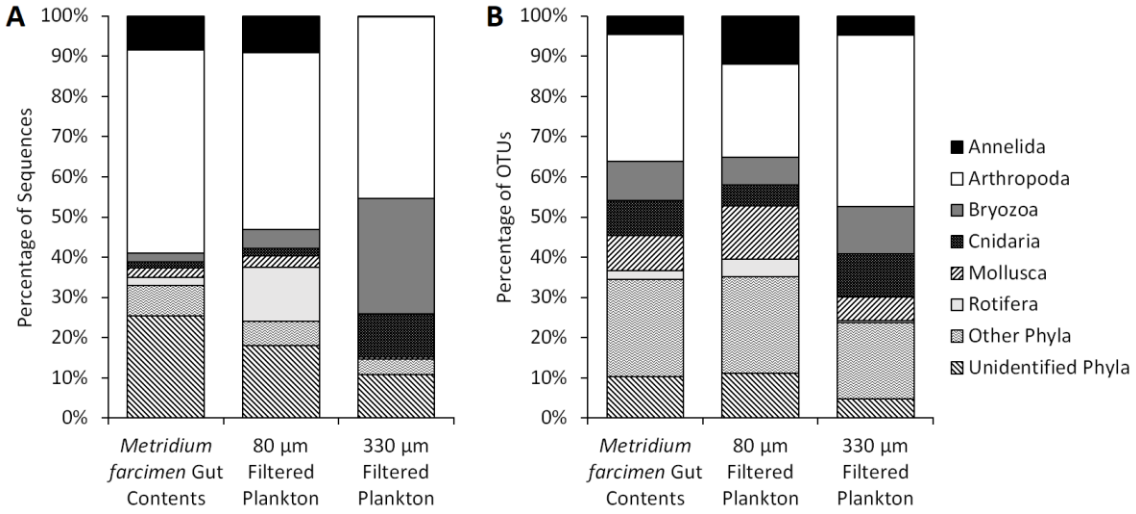

Figure 5. (A) Percentage of sequences and (B) OTUs of major phyla in the giant plumose anemone *Metridium farcimen* gut contents and nearby 80 and 330 µm filtered plankton samples. There were significant differences between the relative abundance of sequences of each community and presence/absence of OTUs (PERMANOVA, $p < 0.001$).

Amplicon sequence variants were clustered into OTUs if they were 97% similar. This can lead to genetically diverse species being clustered into more than one OTU. Cases arose in which sequences were classified to different OTUs, but were mapped to the same species. This may mean that there are some unknown cryptic species in the San Juan Archipelago or that this locus is more variable in these species, something this study cannot determine. Species with more than one OTU delineated were the syllid worm *Syllis elongata*, the sharp nose crab *Scyra acutifrons*, the

bryozoans *Celloporella hyalina* and *M. membranacea*, the sea gooseberry *Pleurobrachia bachei*, and the carinate dove shell *Alia carinata*.

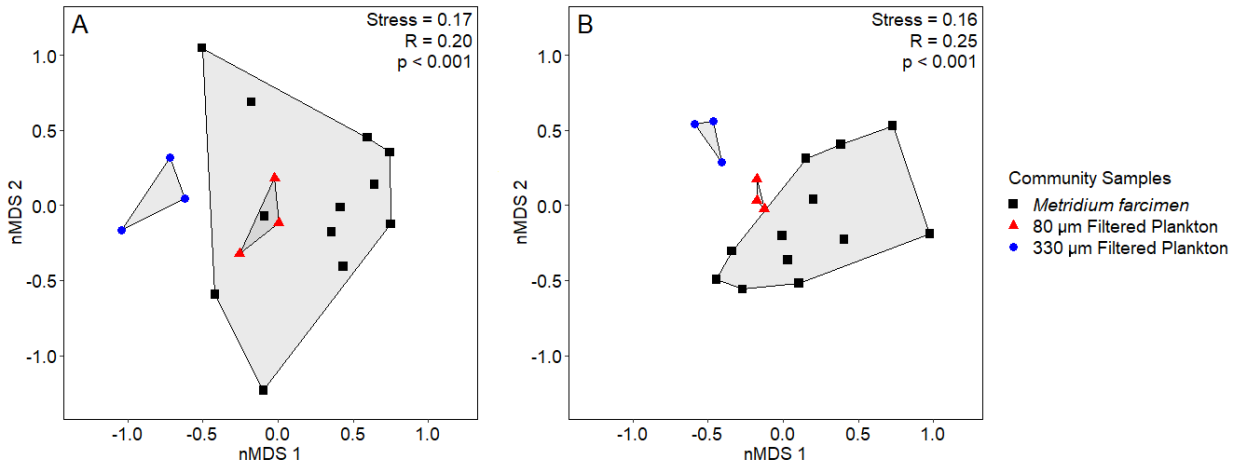

Figure 6. Non-metric multidimensional scaling ordination (nMDS) of (A) number of sequences and (B) presence/absence of OTUs for gut contents of the giant plumose anemone *Metridium farcimen* and nearby plankton samples. Each point represents one sample (plankton tow or gut content). Permutational analysis of variance (PERMANOVA) significance levels are indicated. There were significant differences between the diet of *M. farcimen* and nearby plankton samples.

DISCUSSION

The differences between the diet of *M. farcimen* and the plankton samples is surprising; the dispersion of the anemones' diet was found to be much larger than the breadth of plankton samples from either plankton sampling technique (Figure 6). This is probably because the gut contents are an integration of hours of anemone feeding, while plankton samples are a snapshot of the plankton at the time of the collection. It is well known that zooplankton are temporally variable, even at short timescales (Rodríguez *et al.*, 2000; Marques *et al.*, 2011). For example, to exclude the temporal variation component while additionally avoiding the issue of variable rates of digestion, Hansson (2006) released and recaptured lab-starved scyphomedusae and then examined

Table 5. OTUs that significantly contributed to the difference in composition between the gut contents of the giant plumose anemone *Metridium farcimen* and nearby 80 µm filtered plankton samples (SIMPER, $p < 0.05$). The fold decrease from plankton samples to gut contents is indicated.

Lowest Taxonomic Group	Class	Fold Decrease
Humilaria kennerleyi	Bivalvia	31
Leukoma staminea	Bivalvia	8
Nuttallia obscurata	Bivalvia	8
Cardiidae sp. 1	Bivalvia	7
Acartiidae sp. 1	Hexanauplia	121
Hexanauplia sp. 1	Hexanauplia	65
Calanus pacificus	Hexanauplia	5
Pseudocalanus newmani	Hexanauplia	2
Clytia languida	Hydrozoa	28
Ploima sp. 4	Monogononta	20
Ploima sp. 2	Monogononta	18
Ploima sp. 1	Monogononta	7
Metazoa sp. 28	N/A	36
Metazoa sp. 12	N/A	9
Ophiopholis kennerlyi	Ophiuroidea	32
Phascolosoma agassizii	Polychaeta	41
Terebellidae sp. 1	Polychaeta	12
Notomastus sp. 1	Polychaeta	9
Spionidae sp. 2	Polychaeta	5

Table 6. OTUs that significantly contributed to the difference in composition between the gut contents of the giant plumose anemone *Metridium farcimen* and nearby 330 µm filtered plankton samples (SIMPER, $p < 0.05$). The fold decrease from plankton samples to gut contents is indicated.

Lowest Taxonomic Group	Class	Fold Decrease
Citharichthys stigmaeus	Actinopteri	74
Littorina scutulata	Gastropoda	72
Membranipora membranacea OTU 3	Gymnolaemata	124
Membranipora membranacea OTU 2	Gymnolaemata	114
Membranipora membranacea OTU 5	Gymnolaemata	100
Membranipora membranacea OTU 4	Gymnolaemata	98
Acartia californiensis	Hexanauplia	10
Calanoida sp. 2	Hexanauplia	9
Diosaccus spinatus	Hexanauplia	6
Calanoida sp. 1	Hexanauplia	3
Clytia hemisphaerica	Hydrozoa	139
Obelia dichotoma	Hydrozoa	8
Arthropoda sp. 3	N/A	5
Pleurobrachia bachei OTU 1	Tentaculata	5

what they had consumed. This method could be adapted to work with anemones and other benthic suspension feeders by starving them in a lab, and, in the case of anemones, deploying them on panels in the field. Another potential reason for the high dispersion in the gut content samples is the lower evenness of the gut contents in combination with the lower mean richness. The 80 µm

filtered plankton samples were individually much richer than the anemone gut contents (91.3 and 73.8 OTUs, respectively), but the total richness was about half as much (160 and 356 OTUs, respectively). This indicates that each individual anemone had a vastly different diet, despite being within 20 m of each other, highlighting the small-scale spatial and temporal heterogeneity in zooplankton availability. Additionally, *M. farcimen* may be obtaining a portion of its diet from the benthic community.

Metridium farcimen had a selective diet, contrary to what was found by Purcell (1977). Sebens and Koehl (1984) found that *Metridium senile* had a selective diet, with positive selection for barnacle cyprids, ascidian larvae, and amphipods, and negative selection for eggs, copepods, and ostracods, compared to availability. In our study, *M. farcimen* did not seem to enrich its diet with more palatable or energetically valuable prey, but rather may have been unable to detect or capture some prey species. Success or failure in prey capture is likely more dependent on prey escape capabilities than predator preferences (Sebens *et al.*, 1996). No taxa were significantly higher in abundance in the gut contents, but several were significantly less abundant compared to the plankton samples. This result may seem surprising given the larger total richness in the diet of anemones, but individual gut content richness was lower than the 80 μm filtered plankton and mean incidence was half of both plankton samples. While it is unknown how these species avoided predation, Heidelberg *et al.* (1997) showed that some, but not all, zooplankters can detect passive suspension feeders in moving water and subsequently avoid predation. In addition, they showed that very small prey (e.g., nauplii) were less susceptible to predation. This may be because nematocyst discharge is affected by both chemical cues and mechanical stimulation (Thorington and Hessinger, 1988; Watson and Hessinger, 1988). Larger prey are more likely to impact tentacles at a higher force, increasing capture probability through mechanical or surface chemical detection.

The diet of *M. farcimen* was compositionally different from the diets found by both Purcell (1977) and Sebens (1981a). These discrepancies may be partially explained by the study locations. Purcell (1977) worked in Monterey, CA on *M. farcimen* associated with pilings at 8 m below the surface and Sebens (1981a) worked in Harper, WA, farther into the same estuary system as this study, but on subtidal piling surfaces 3 m below the surface. While Hexanauplia was the most diverse, rich, and abundant and had the highest incidence in the gut contents, likely because of its high diversity, richness, and abundance in the plankton (Table 1), we found a high abundance of insects and ostracods in the gut contents (Table 4). The most abundant insect prey (98% of insect sequences) was the yellow meadow ant *Lasius brevicornis*, which has mating flights in July to September (Pontin, 1963). It seems that *M. farcimen*, when associated with floating docks, may be getting a significant portion of their diet from episodic input from the nearby terrestrial environment. However, strong tidal currents and mixing could provide this resource to shallow subtidal populations on natural rock surfaces as well. This result highlights the need for further sampling across a broader temporal and spatial scale and across depth to better understand where *M. farcimen* and other benthic suspension feeders are deriving their energy. Other differences between our study and Purcell (1977) and Sebens (1981a), such as elevated levels of demosponge and rotifers, are likely because of the difficulty in identifying these groups using traditional gut content techniques. Both of these groups are small and would be difficult to distinguish from anemone tissue. Also, zooplankters in the gut of *M. farcimen* need not be digested at all. Purcell *et al.* (1991) found that ingested bivalve veligers were egested 7 h later, alive, by the medusae of the Atlantic sea nettle *Chrysaora quinquecirrha*, although the polyp stage of *C. quinquecirrha* was capable of digesting them.

It is clear that DNA metabarcoding is an efficient method for identifying even the partially digested gut contents of animals. To make the results ecologically relevant, significant DNA barcoding must precede metabarcoding studies, and this work can be exceedingly time consuming. The results of metabarcoding gut contents do not indicate what stage the prey species are at, while traditional techniques can. We recommend a paired sampling of traditional techniques to identify major patterns and metabarcoding to identify the microscopic and partially digested prey items for future intensive studies. We also recommend either using starved animals, or sampling the available plankton over a longer time period that would correspond with their prey retention time during digestion.

This work provides important insight into the diet of a competitively dominant sea anemone, which appears to capture a wider range of prey than previously suspected, seemingly limited to prey that are large enough to be detected but cannot escape. The gut contents of anemones are a composite of many hours of feeding and reflect the changing plankton community leading to a higher diversity than the surrounding plankton at any one time. The difference between individual anemones is much larger than the difference between plankton samples, despite the anemones being within 20 m of one another compared to the 1 km distance between plankton samples, likely because of small-scale temporal and spatial variability in the plankton communities and deriving part of their diet from demersal species. The surprising terrestrial input into the diet of *M. farcimen,* and the differences between this study and other published diet studies, highlight the need for consideration of space and time in a sampling regime and the usefulness of the metabarcoding method in identifying prey within the gut of planktivorous animals.

CHAPTER III: DEPTH, SLOPE, LIGHT, AND FLOW AFFECT THE DISTRIBUTION OF HARD-BOTTOM HEXACORALLIANS

ABSTRACT

The distribution of subtidal, passive suspension feeders could potentially depend on many abiotic and biotic factors such as light, flow, predation, and temperature. One of the most conspicuous and dominant groups of suspension feeders in temperate and tropical areas is hexacorallians. To date, there has not been a quantitative survey of the distribution of hard-bottom hexacorallians in a diverse temperate environment such as the San Juan Archipelago. We examined the effects of light, flow, depth, substratum slope, predation pressure, and temperature on the distribution and abundance of seven common, temperate, hard-bottom hexacorallians. We found that the most important factors affecting distribution were depth, light, and flow, while predation and temperature had little effect. Depth and light are strongly correlated, and both have a strong relationship with algal cover. Most hexacorallians were conspicuously missing from high algal cover surfaces. Additionally, nearly every species increased in density with increased flow. This study highlights the need for experimental studies examining the interactions between temperate hexacorallians and algae as well the effects of flow on distribution of anthozoans.

INTRODUCTION

The distribution of passive suspension feeders in subtidal marine habitats depends on a range of abiotic factors such as light (Baynes, 1999; Irving and Connell, 2002; Miller and Etter, 2008), temperature (Hessler and Smithey, 1983; Jablonski *et al.*, 2000), flow (Wildish and Kristmanson, 1997; Smith, 2018), salinity (Milne, 2009), and sedimentation (Rogers, 1990; Irving and Connell, 2002). For example, shallow horizontal surfaces are often dominated by upright

algae, whereas vertical walls, which only receive a small fraction of the light received by horizontal surfaces (Brakel, 1979), are dominated by sessile invertebrates and crustose algae (Sebens, 1986b; Irving and Connell, 2002; Smith, 2018). This pattern is maintained by biotic interactions such as competition via overgrowth of invertebrates by algae (Young and Chia, 1984; Baynes, 1999), preferential larval settlement (Thorson, 1964; Saunders and Connell, 2001), and algal whiplash (Witman and Sebens, 1988) and abiotic factors such as ultraviolet light damage (Jokiel, 1980).

Along with light, water flow is important for passive suspension feeders, which depend on flow for delivery of food and oxygen, removal of waste and sediments, and larval transport (Yoshioka and Yoshioka, 1989; Patterson *et al.*, 1991; Sebens *et al.*, 1998; Palardy and Witman, 2014). Those that live within high flow environments have adaptations for dealing with drag and deformation of feeding appendages (Koehl, 1976, 1977c; Anthony, 1997). Individual organisms, colonies, and clonal aggregations have different optimal flow regimes and can significantly modify the flow, which can increase prey capture (Sebens and Johnson, 1991; Helmuth and Sebens, 1993; Sebens *et al.*, 1996; Sebens *et al.*, 1997). High densities of hexacorallians, for example, may benefit the component individuals by reducing high flow within the aggregation (Koehl, 1976, 1977a, d) and thus increase prey capture (Johnson and Sebens, 1993). This has been found for other suspension feeders such as bryozoans, octocorals, and polychaete worms where upstream individuals enhance prey capture of downstream individuals by inducing turbulence (Merz, 1984; Okamura, 1984, 1985; McFadden, 1986). In low flow environments, food can be depleted by upstream neighbors such as occurs in mussel beds (Okamura, 1986; Fréchette *et al.*, 1989). Prey can also benefit from particular flow regimes (Sebens and Koehl, 1984). Heidelberg *et al.* (1997) showed that increased flow allows for better detection of passive suspension feeders by some

copepods, but not by chaetognaths which were more likely to impact coral tentacles at higher flows.

Predation can also have major effects on the distribution of suspension feeders (Paine, 1969, 1974; Sebens, 1977; Young and Gotelli, 1988). For example, predation excludes the aggregating anemone *Anthopleura elegantissima* from the subtidal (Sebens, 1977; Annett and Pierotti, 1984) and highly modifies size structure of the plumose anemone *Metridium senile* (Harris, 1976, 1986). Temperature has also been shown to heavily influence benthic organisms (Naylor, 1965; Hiscock *et al.*, 2004; Vaquer-Sunyer and Duarte, 2011). Temperature can affect the distribution of organisms across depth gradients (Lloyd *et al.*, 2012), seasons (Coma *et al.*, 2000), and latitudes (Jablonski *et al.*, 2000). Additionally, heated effluent from natural (e.g., hydrothermal vents) and artificial sources (e.g., power plants) can exclude colder water species while giving a refuge for warmer water species (Naylor, 1965; Hessler and Smithey, 1983; Wells, 2013).

Hexacorallians (phylum Cnidaria, class Anthozoa) are prominent passive suspension feeders in most benthic ecosystems. Temperate zone hexacorallians, like their tropical relatives, can be the dominant space occupiers and some facilitate the recruitment and survival of species living in their understory (Taylor and Littler, 1982; Nelson and Craig, 2011). The northeast Pacific, including the San Juan Archipelago, WA, USA within the Salish Sea is one of the most diverse areas for temperate zone hexacorallians with at least 18 hard-bottom species in near-shore environments. The ecology of some of these species (e.g., *A. elegantissima* and *Metridium farcimen*) is relatively well-known (Koehl, 1977a; Purcell, 1977; Sebens, 1977; Francis, 1979; Harris and Howe, 1979; Bachman and Muller-Parker, 2007; Wells *et al.*, 2019) while others have

had little to no attention, despite their prevalence (e.g., *Epizoanthus scotinus* and *Cribrinopsis* spp.).

To date, no survey of temperate hard-bottom hexacorallians has been published from the San Juan Archipelago or other complex system spanning a wide range of environmental conditions. In this study, we looked at the effects of light, flow, depth, substratum slope, predation pressure, and temperature on the distribution and abundance of seven common hard-bottom hexacorallians. This was done through the use of phototransects from +4 to -38 m mean lower low water (MLLW) at 12 sites in the San Juan Archipelago, WA, USA. These patterns must be documented first before well-informed experiments can be designed to test the processes involved in determining distribution.

MATERIALS AND METHODS

Field Sampling

Ten field sites (Figure 7, Table 7) were chosen, employing several criteria, and using a bathymetric map. First, the sites had to reach a depth of -40 m mean lower low water (MLLW) within 300 m of the shore so divers could sample the whole site in one dive. Sites had to have a variety of horizontal, sloping, and vertical surfaces at all depths (e.g., not a vertical wall with no relief for 5+ meters) to allow for more balanced analyses. Additionally, the sites were chosen to span the range of flow regimes experienced within the San Juan Archipelago. Rosario Point did not reach -40 m MLLW, but was chosen because it had particularly low flow rates. Finally, sites were chosen so they would be widely distributed around the islands.

Between October 2016 and November 2018, hexacorallians were surveyed at each site from +4 to -38 m MLLW. At every 0.5 m depth, two photographs were taken. To ensure that

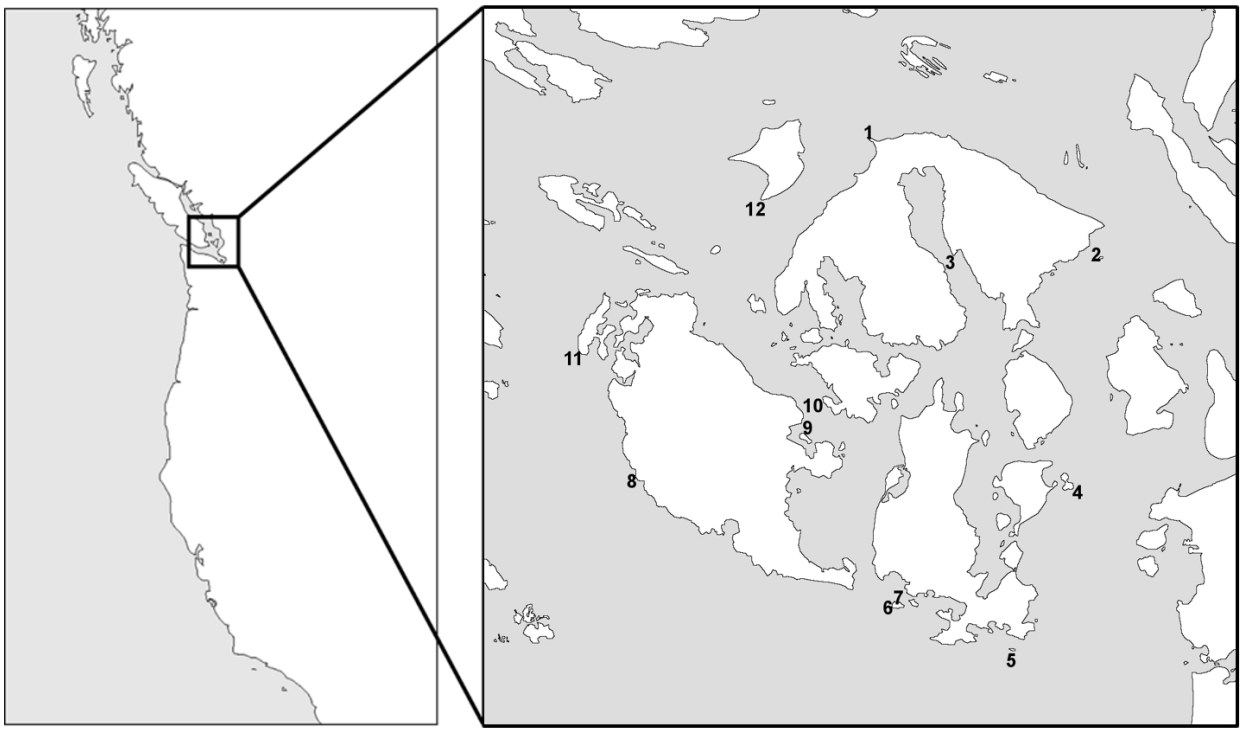

Figure 7. Field sites in the San Juan Archipelago, WA, USA. 1. Doughty Point, Orcas Island; 2. North Peapod; 3. Rosario Point, Orcas Island; 4. James Island; 5. Colville Island; 6. West Long Island; 7. Long Island; 8. Lime Kiln State Park, San Juan Island; 9. Cantilever Point, San Juan Island; 10. Shady Cove, San Juan Island; 11. Kellett Bluff, Henry Island; 12. Point Disney, Waldron Island.

Table 7. GPS coordinates and number of transects at each site.

Site	GPS Coordinates	Num. of Transects
Doughty Point	48.7127, -122.9524	2
North Peapod	48.6430, -122.7467	1
James Island	48.5087, -122.7694	1
Rosario Point	48.6434, -122.8746	2
Colville Island	48.4144, -122.8234	1
West Long Island	48.4421, -122.9313	2
Long Island	48.4436, -122.9240	1
Lime Kiln State Park	48.5158, -123.1528	1
Cantilever Point	48.5473, -123.0066	3
Shady Cove	48.5531, -123.0066	2
Kellett Bluff	48.5877, -123.2027	2
Disney Point	48.6759, -123.0447	2

photos were not biased towards particularly species-rich spots, divers ascended towards the shore from -38 m MLLW and took two adjacent photographs as soon as they reached the next 0.5 m increment. Photographs (12 megapixel stills) were taken with a GoPro Hero4 Black Edition (GoPro, San Mateo, CA, USA) attached to an aluminum 60 x 42 cm framer, 42 cm from the

substratum. The substratum was lit with two Light & Motion Sola 800 dive lights (Light & Motion, Marina, CA, USA) attached on either side of the camera. Depth, substratum slope, and substratum aspect were recorded in every photograph using a dive computer, inclinometer, and compass attached to the framer. The inclinometer could measure a maximum of 112°; none of the substrata exceeded the maximum. One to three transects of photographs were taken at each site (Table 7).

As part of a long-term monitoring program examining subtidal community dynamics (Smith, 2018), additional photographs were taken at two sites: Shady Cove, San Juan Island and Cantilever Point, San Juan Island. These photographs were incorporated into the current study for a total of 12 sites (Figure 7, Table 7). Horizontal transects were laid out at every 3 m depth interval from -3 to -27 m MLLW, and 10 randomly placed photographs were taken on each transect. Photographs (8 megapixel stills) were taken with an Olympus 8080 Wide Zoom digital camera (Olympus Corporation, Tokyo, JP) attached to a 35 x 25 cm aluminum framer with an Ikelite Substrobe DS160 strobe (Ikelite, Indianapolis, IN, USA) 34 cm from the substratum. Sampling was performed in October to January of 2007 through 2014. To supplement these horizontal transects, the larger framer was used between +4 and 0 m MLLW and between -28 and -38 m MLLW.

All photographs of substrata with a slope between 0 and 25° were considered horizontal surfaces, all those between 25 and 65° were considered sloping, and all those between 65 and 115° were considered vertical. For each photograph, the individuals of each species of hexacorallian were counted and the values within a slope category were pooled across each three-meter depth interval. For Shady Cove and Cantilever Point, transect data were also pooled across years. Site-specific density for each species at each combination of depth interval and slope category was found by dividing the pooled counts by the surface area sampled. While photographing, large

upright algae, especially kelps, were either held to the side when possible or physically detached at the holdfast so an accurate count of hexacorallians could be collected.

For each photograph location, a relative light index was calculated using the equation of McCune and Keon (2002) which estimates annual direct incident radiation. Annual direct incident radiation was used because our sampling was across the whole year, and these anthozoans species are perennial. The original equation incorporates latitude, substratum slope, and substratum aspect, but lacks a depth component, necessary for predicting light underwater. The modified equation, with the addition of a depth component, was as follows:

$$\mathrm{RLI} = \frac{-\mathrm{k\,D}\,(1.467 + 1.582\cos\mathrm{L}\cos\mathrm{S} - 1.5\cos\mathrm{A}\sin\mathrm{L}\sin\mathrm{S} - 0.262\sin\mathrm{L}\sin\mathrm{S} + 0.607\sin\mathrm{A}\sin\mathrm{S})}{\mathrm{M}}$$

RLI is relative light index and is unitless, k is the vertical extinction coefficient for the water across the year in m^{-1}, D is depth in meters, L is latitude in radians, S is substratum slope in radians, A is substratum aspect in radians, and M is the maximum attainable light and is unitless. For this equation, the highest light levels are on intertidal surfaces with a slope of 48 (0.50 radians) facing 152 or 208° (2.65 radians). Maximum attainable light (1.04) was calculated by solving for RLI using a depth of 0 m, the latitude of the most southern site (Colville Island, 0.85 radians), a slope of 0.80 radians, an aspect of 2.65 radians, and an M of 1. Dividing by the maximum attainable light makes the highest obtainable relative light 1.000 and other values are a proportion of the maximum value. The vertical extinction coefficient (0.294) was calculated using an RLI of 0.005, the minimum light level for upright algal survival (Markager and Sand-Jensen, 1992); a depth of 18 m, where the deepest upright algae were observed (Wells, personal observation), a latitude of 0.85 radians, a slope of 0.80 radians, an aspect of 2.65 radians, and an M of 1.04.

To examine the potential effect of predation pressure on distribution of hexacorallians, during all dives at each site, we recorded the number of the leather sea star *Dermasterias imbricata*,

a predator of hexacorallians (Mauzey *et al.*, 1968; Sebens, 1977; Annett and Pierotti, 1984; Bachman and Muller-Parker, 2007; Wells *et al.*, 2018). While there are other hexacorallian predators present within the system such as other sea stars, nudibranchs, and fish (Mauzey *et al.*, 1968; Harris, 1991; Crawford *et al.*, 2018), these species are not in high abundance and are relatively inconspicuous. Number of stars observed on each dive was divided by the dive time to get an average catch per unit effort (stars/hr) as a metric for potential predation pressure.

At each site, two 58 x 58 x 20 mm alabaster blocks were deployed on top of 225 x 112.5 x 75 mm clay bricks at -10 and -24 m MLLW for 15 to 19 days in August of 2018. Dissolution of alabaster blocks has been used in this location by Elahi *et al.* (2014) and Smith (2018), and has been an accurate and cost-effective surrogate for measuring mass transfer (Porter *et al.*, 2000). Both an InterOcean S4 electromagnetic current meter (InterOcean Systems LLC, San Diego, CA, USA) and a SonTek Hydra acoustic Doppler velocimeter (SonTek/Xylem Inc., San Diego, CA, USA) were deployed alongside alabaster blocks set out by Elahi *et al.* (2014) and Smith (2018) to correlate dissolution rate to flow speed under field conditions. That fitted line was then used to calculate average flow speeds at each of the sites. If the dissolution rate is calibrated in a flow regime similar to that at the field sites, then dissolution is a good predictor of mean flow speed (Porter *et al.*, 2000). The empirical equation solving for mean flow speed was:

$$\text{Mean Flow Speed} = \frac{\text{Dissolution Rate} - 0.0323}{0.00379}$$

Flow speed is measured in cm/s and dissolution rate is measured in grams of alabaster lost per day per cm^2 of alabaster initially exposed. If an alabaster block was fully dissolved at the end of the deployment, it was assumed that it took the whole time to dissolve, which gives a conservative estimate of mass transfer and therefore flow speed. Flow speed was averaged between the two blocks at each site to get a site-level flow speed.

HOBO Pendant Temperature/Light 64K data loggers (Onset Computer Corporation, Bourne, MA, USA) were deployed on the bricks to examine the relationship of temperature with hexacorallian distribution concurrently with the alabaster blocks. Before deployment, temperature loggers were deployed in a sea table (12.3 °C) and in a refrigerator held at 10 °C set to record temperature every minute for an hour. These measurements were used to generate a correction value for adjusting field measurements. In the field, loggers collected temperature data every minute for 13 days from August 20 to September 2 of 2018 starting and ending at 5:00 pm PST. Although these data are from a limited time period, late summer has the potential for the largest difference in temperatures between sites. Based on previous deployments at Cantilever Point, temperatures are essentially equal across depth for the coldest half of the year. The average temperature measured from the two data loggers was used as a site-level temperature measurement.

Focal Species

The effects of abiotic and biotic factors on the distribution of the seven most common and evenly distributed hard-bottom hexacorallians were examined in this study, excluding species that occurred at fewer than half the sites. These species are the aggregating anemone *Anthopleura elegantissima*, the orange cup coral *Balanophyllia elegans*, the crimson anemone *Cribrinopsis rubens*, the zoanthid *Epizoanthus scotinus*, an Isanthidae n. sp., the giant plumose anemone *Metridium farcimen*, and the painted anemone *Urticina grebelnyi*. *A. elegantissima* is a clonal, intertidal anemone which contains endosymbiotic zooxanthellae and zoochlorellae and occurs from southern Baja California to Alaska (Hand, 1955a; Muscatine, 1971; Francis, 1979; Littler *et al.*, 1983; McFadden *et al.*, 1997; Pearse and Francis, 2000). *B. elegans* is a temperate, aclonal, dendrophyllid coral that has been used extensively in reproductive studies for its limited dispersal;

it can only disperse meters in a generation because females produce crawl-away young (Gerrodette, 1981; Fadlallah and Pearse, 1982; Fadlallah, 1983; Hellberg, 1994, 1995; Bruno and Witman, 1996; Hellberg and Taylor, 2002). *C. rubens* is a brooding, aclonal, subtidal anemone (Siebert and Spaulding, 1976; Sanamyan *et al.*, 2019) with no previously-published ecological work. Stevens and Anderson (2000) describe an interaction between *Cribrinopsis fernaldi* and shrimps and this relationship has been observed in *C. rubens* as well (Wells, personal observation). *E. scotinus* is a colonial zoanthid occurring from southern California to Alaska (Wood, 1957; Lamb and Hanby, 2005). The ecology of this species is also poorly understood, but congeners form interesting epizootic relationships including living on urchin spines in the deep sea (Kise *et al.*, 2018) and creating pseudoshells for hermit crabs (Schejter and Mantelatto, 2011). *M. farcimen* is an aclonal, subtidal anemone on subtidal rock surfaces occurring from Kamchatka to southern California (Hand, 1955b; Ricketts *et al.*, 1968; Kozloff, 1973; Fautin *et al.*, 1989; Fautin and Hand, 2000) that feeds on small zooplankton (Koehl, 1977a; Purcell, 1977; Sebens, 1981a; Shick, 1991). *M. farcimen*, which can extend over a meter into the water column (Fautin *et al.*, 1989) is a competitively dominant species on rocky subtidal ledge communities (Nelson and Craig, 2011). *U. grebelnyi*, formerly considered *Urticina crassicornis*, is an aclonal anemone that occurs in both the subtidal and the intertidal (Hand, 1955a; Chia and Spaulding, 1972; Sebens and Laakso, 1977; Sanamyan and Sanamyan, 2006).

Statistical Analyses

All statistical analyses were performed in R version 3.5.2 with the packages boot 1.3-20, parallel 3.5.2, rcompanion 2.1.1, and vegan 2.5-4 (Canty and Ripley, 2017; R Core Team, 2018; Mangiafico, 2019; Oksanen *et al.*, 2019). Multiple permutational analyses of variance (PERMANOVA, Anderson, 2001) were run to determine the effects of depth, substratum slope,

flow speed, predation pressure, temperature, and relative light on the square root transformed densities of the seven focal species (3 m depth increments with one of 3 slope types at a site, n = 437) with site as a stratum (see Table 8 for a full list of models). Square root transformations were used to normalize the variance and are often used for count data where variance is proportional to the mean and from a Poisson distribution (Bartlett, 1936). Depth, substratum slope, and relative light were measured at the depth-slope increment-level, while flow speed, predation pressure and temperature were measured at the site-level. As there was a correlation between depth and light (Pearson's Correlation, r = -0.75, Pearson, 1896), flow speed and temperature (r = -0.66), flow speed and predation pressure (r = -0.55), and temperature and predation pressure (r = 0.65), these factors were never combined in any model. All interactive effects were included for each model. Akaike information criteria (AIC, Akaike, 1973) were used to choose the most appropriate model for each species, and were calculated as suggested in Anderson *et al.* (2008); the model with the lowest AIC was chosen to determine which factors impact distribution. Bootstrapped 95% confidence intervals (9999 permutations) were calculated with the transformed densities and then backtransformed for the figures. The main effects of depth and substratum slope were plotted for all modelled species. For all other main and interaction effects, only significant effects were plotted. Heat maps were produced for significant interactions to better visualize the interdependence of the main effects.

RESULTS

Site Data

Average alabaster dissolution rate ranged from 0.055 to 0.162 g/cm^2/day. Half of the sites had at least one block fully dissolve during the deployment. Average calculated flow speeds ranged

Table 8. Akaike information criteria (AIC) differences for all permutational analysis of variance models run for hexacorallian density. Lower values are better fitting models. Bold values within grey boxes are the best fitting models. D, depth; S, substratum slope; F, flow speed; P, predation pressure; T, temperature; and L, relative light index.

Density ~	*Anthopleura*	*Balanophyllia*	*Cribrinopsis*	*Epizoanthus*	Isanthidae	*Metridium*	*Urticina*
D*S*F	53	0	0	11	0	0	7
D*S*P	58	7	11	13	5	10	19
D*S*T	54	8	6	14	8	12	20
D*S	52	1	4	3	1	4	12
D	52	4	4	4	26	11	9
S	60	43	22	0	24	25	12
L*F	0	15	17	8	33	23	0
L*P	12	21	22	9	36	27	12
L*T	12	23	21	9	38	29	13
L	19	20	18	4	34	25	9

from 5.9 to 34.2 cm/s. Average temperature ranged from 11.3 to 12.4 °C with slightly warmer sites closer to the output of the Fraser River in the north or within a sound and slightly cooler sites closer to the entrance of the Strait of Juan de Fuca in the south. Catch per unit effort for the predatory sea star *Dermasterias imbricata* ranged from 0 to 26.8 stars/hr of diving (Table 9).

Table 9. Average dissolution rate, calculated flow rate, and temperature during the alabaster deployment and density of the predatory sea star *Dermasterias imbricata*. Asterisks next to dissolution rates indicates that the whole alabaster block dissolved before recovery and so these rates and the subsequent calculated flow speeds are conservative measurements.

Site	Avg. Dissolution Rate ($g/cm^2/day$)	Avg. Flow Speed (cm/s)	Avg. Temperature (°C)	Predation Pressure (stars/hr)
Doughty Point	0.151*	31.2	12.4	0.025
North Peapod	0.111	20.7	11.9	0.000
James Island	0.118	22.5	11.7	0.008
Rosario Point	0.055	5.9	12.5	26.786
Colville Island	0.146*	30.0	11.7	0.007
West Long Island	0.154*	32.1	11.8	2.410
Long Island	0.136*	27.4	11.5	0.690
Lime Kiln State Park	0.142*	29.1	11.3	8.028
Cantilever Point	0.075	11.3	11.9	1.659
Shady Cove	0.103	18.7	12.1	7.074
Kellett Bluff	0.162*	34.2	11.8	0.505
Disney Point	0.081	12.8	11.9	0.294

Hexacorallian Distribution

Aggregating anemone *Anthopleura elegantissima*

A. elegantissima was the only focal species restricted to the intertidal, with a depth range of +0.1 to +2.0 m MLLW (Figure 8) on surfaces with slopes from 0 to 100° (Figure 9). Maximum density was 1,580 polyps/m^2 with an average intertidal density of 58.4 polyps/m^2. During this study, 4,444 polyps were counted, but none were found at Cantilever Point, Point Doughty, Long Island, North Peapod, or Rosario Point. The model including light and flow speed had the best fit (Table 8). The distribution of the density of *A. elegantissima* had a significant interactive response to relative light level and flow speed, preferring well-lit, high-flow environments ($F_{1, 436} = 46.5$, $R^2 = 0.075$, $p = 0.001$, Figure 10).

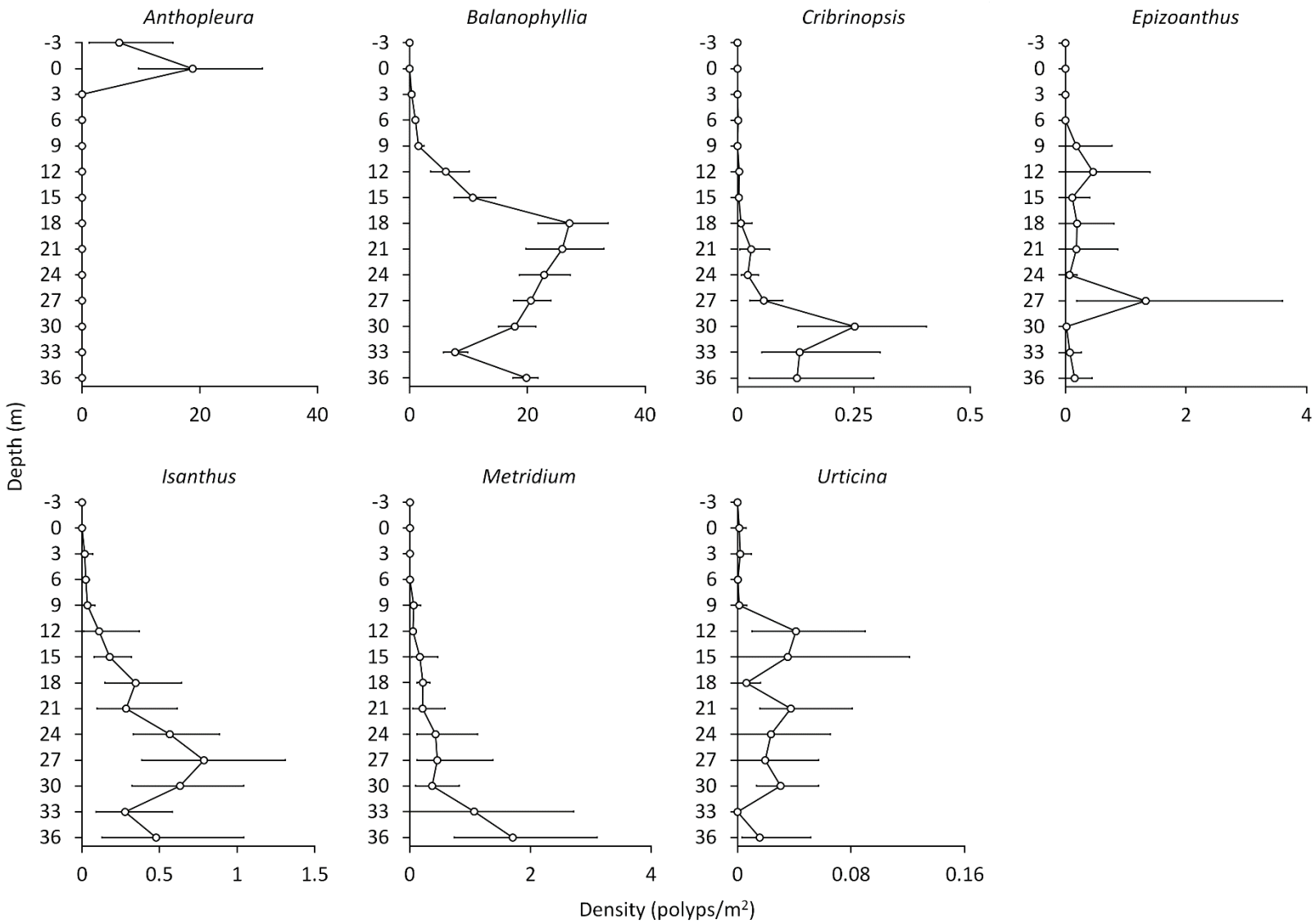

Figure 8. Depth distribution of abundant hard-bottomed hexacorallians in the San Juan Archipelago, WA, USA. Values are backtransformed means with backtransformed bootstrapped 95% confidence intervals.

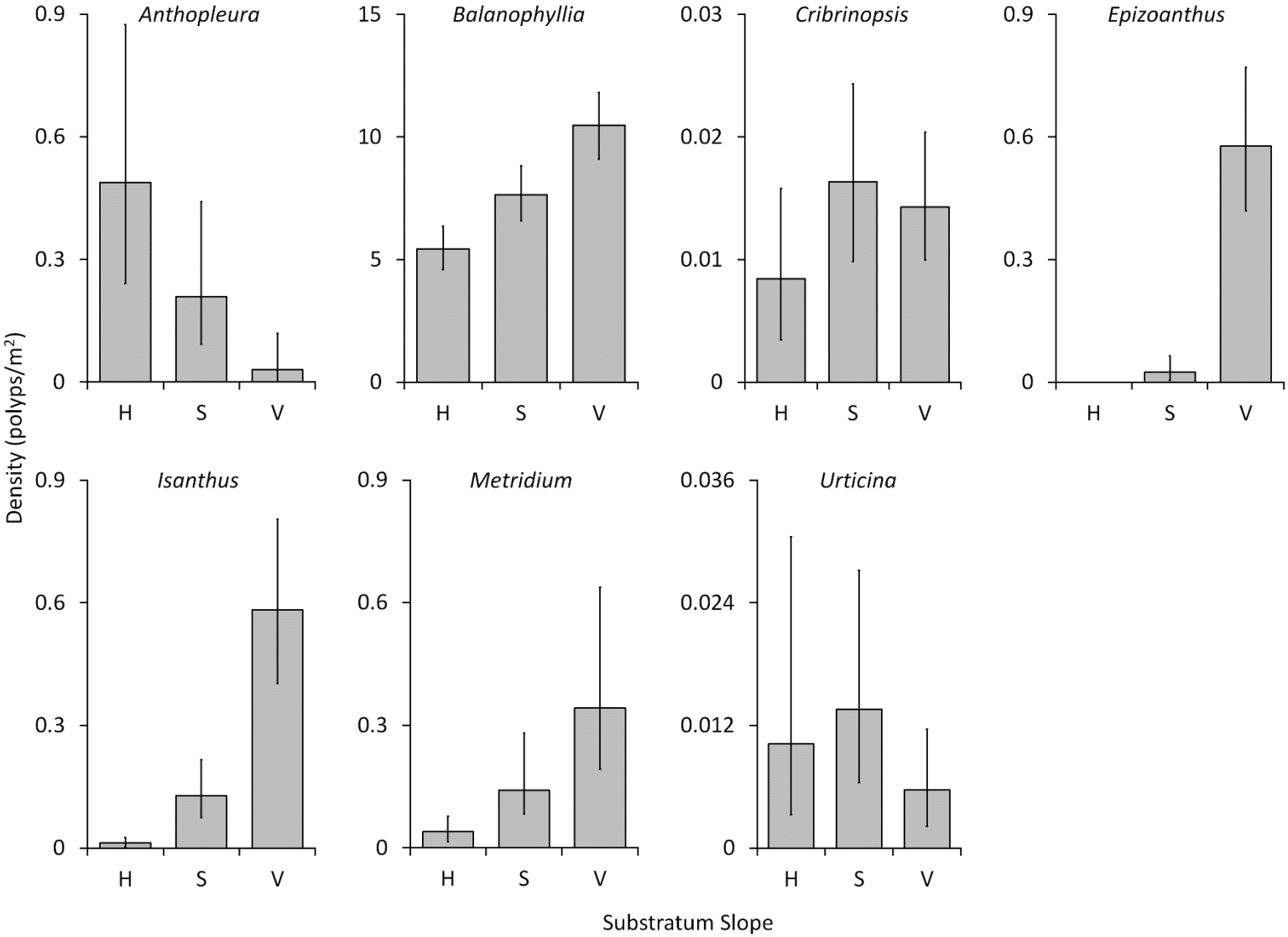

Figure 9. Mean density of abundant hard-bottomed hexacorallians on different slopes in the San Juan Archipelago, WA, USA. Values are backtransformed with backtransformed bootstrapped confidence intervals. Slope categories are abbreviated as follows: H, horizontal; S, sloping; and V, vertical. Values are backtransformed means with backtransformed bootstrapped 95% confidence intervals.

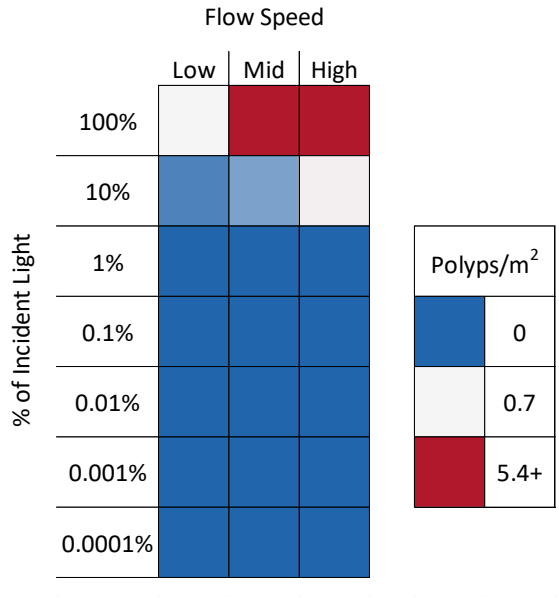

Figure 10. The interactive effect of light and flow speed for the aggregating anemone *Anthopleura elegantissima* in the San Juan Archipelago, WA, USA. *A. elegantissima* prefers well-lit vertical surfaces (PERMANOVA, $F_{1, 436}$ = 46.5, R^2 = 0.075, p = 0.001), which only occur in the intertidal. White is the 40 percentile and red is above the 80 percentile. During the analysis, light and flow speed were treated as a continuous variables. Here, flow categories are as follows: low, 0-11 cm/s; mid, 11-21 cm/s; and high, over 21 cm/s. Values are backtransformed means.

Orange cup coral *Balanophyllia elegans*

B. elegans was strictly subtidal with a depth range of -1.8 to -38.0 m MLLW (Figure 8) on all slopes (Figure 9). Maximum density was 262 polyps/m^2 with an average subtidal density of 26.6 polyps/m^2. During this study 26,418 polyps were counted; this species was the most abundant and evenly distributed hexacorallian. Polyps were found at every site except for Rosario Point, the lowest flow speed site (Table 9). The model including depth, substratum slope, and flow speed was the best fitting model (Table 8). *B. elegans* became more abundant with greater depth ($F_{1, 436}$ = 117.3, R^2 = 0.201, p = 0.001, Figure 8), steeper substratum slope ($F_{2, 436}$ = 7.60, R^2 = 0.026, p = 0.001, Figure 9), and higher flow speed ($F_{1, 436}$ = 20.60, R^2 = 0.035, p = 0.003, Figure 11). There were no interactive responses to depth, substratum slope, or flow speed.

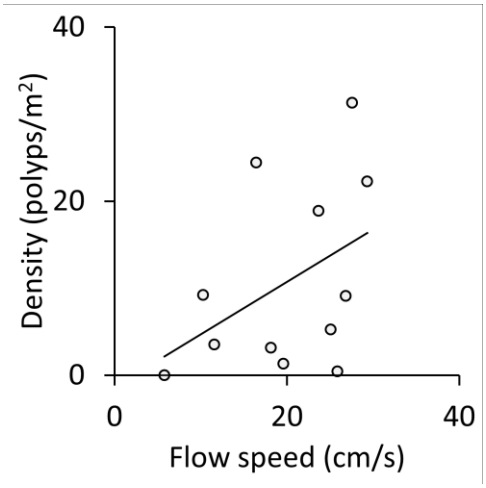

Figure 11. The effect of flow speed on density of the orange cup coral *Balanophyllia elegans* in the San Juan Archipelago, WA, USA. Sites with higher flow speeds have higher density of *B. elegans* (PERMANOVA, $F_{1, 436}$ = 20.60, R^2 = 0.035, p = 0.003). Values are backtransformed means.

Crimson anemone *Cribrinopsis rubens*

C. rubens was another strictly subtidal hexacorallian with a depth range of -6.8 to -37.7 m MLLW (Figure 8) on all slopes (Figure 9). Maximum density was 9.26 polyps/m^2 with an average subtidal density of 0.22 polyps/m^2. During this study, 188 polyps were counted, but none were found at Colville Island, Disney Point, Point Doughty, Long Island, or Rosario Point. The model including depth, substratum slope, and flow speed was the best fitting model (Table 8). The distribution of the density of *C. rubens* had a significant interactive response to depth and substratum slope ($F_{2, 436}$ = 3.75, R^2 = 0.015, p = 0.027), preferring deep, vertical surfaces, and an interactive response to depth and flow speed ($F_{1, 436}$ = 11.5, R^2 = 0.022, p = 0.001), preferring deep, high-flow environments (Figure 12).

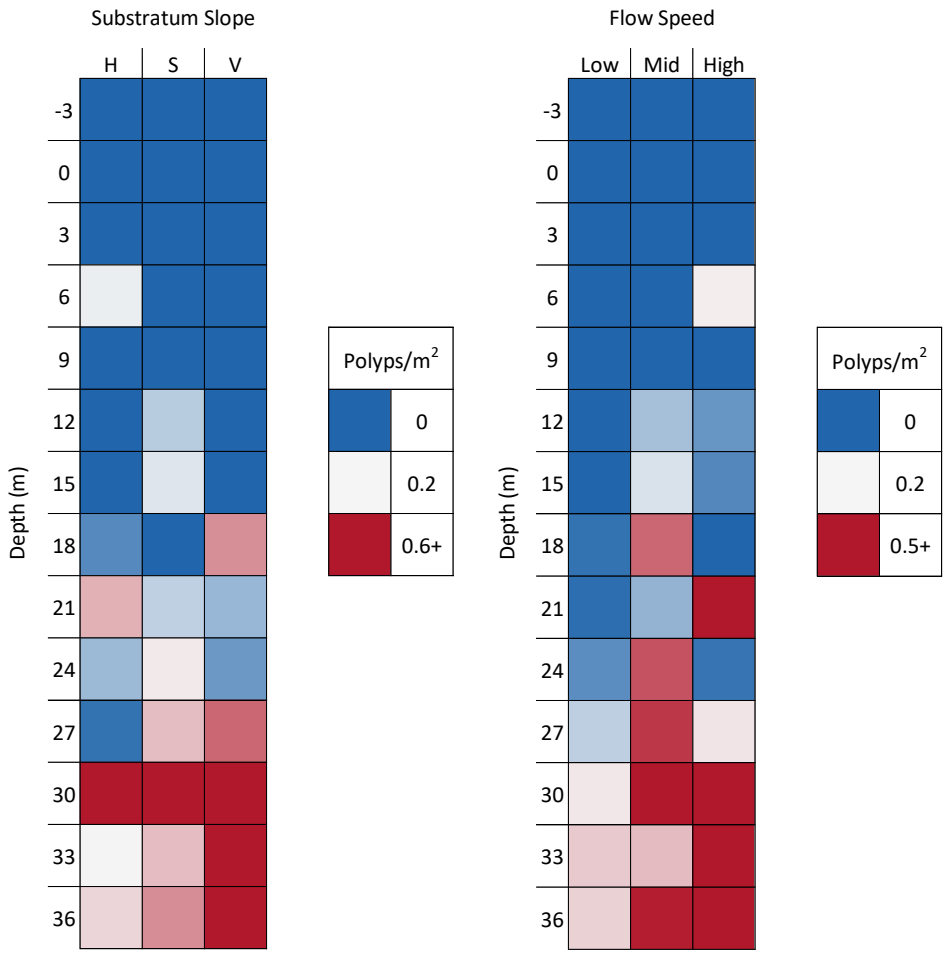

Figure 12. The interactive effect of depth and substratum slope and depth and flow speed for the crimson anemone *Cribrinopsis rubens* in the San Juan Archipelago, WA, USA. *C. rubens* prefers deep, vertical surfaces (PERMANOVA, $F_{2, 436} = 3.75$, $R^2 = 0.015$, $p = 0.027$) and deep, high flow environments (PERMANOVA, $F_{1, 436} = 11.5$, $R^2 = 0.022$, $p = 0.001$). White is the 40 percentile and red is above the 80 percentile. During the analysis, flow speed was treated as a continuous variable. Here, flow categories are as follows: low, 0-11 cm/s; mid, 11-21 cm/s; and high, over 21 cm/s. Slope categories are abbreviated as follows: H, horizontal; S, sloping; and V, vertical. Values are backtransformed means.

Yellow zoanthid *Epizoanthus scotinus*

The range of *E. scotinus* begins deeper than any other focal species. Depth range was from -9.0 to -38.0 m MLLW (Figure 8) on surfaces with slopes from 45 to 112° (Figure 9). Maximum density was 210 polyps/m^2 with an average subtidal density of 3.39 polyps/m^2. During this study, 3,944 polyps were counted, but none were found at Colville Island or Rosario Point. *E. scotinus* is the only hexacorallian for which the model only including substratum slope had the best fit (Table

8); individuals were absent on horizontal surfaces and relatively abundant on vertical surfaces ($F_{2,436} = 5.99$, $R^2 = 0.027$, p = 0.003, Figure 9).

Isanthidae n. sp.

The undescribed Isanthidae n. sp. was strictly subtidal with a depth range of -2.2 to -38.0 m MLLW (Figure 8) on all slopes (Figure 9). This species was found primarily on vertical surfaces and overhangs, with its pedal disk tucked into crevices or between other animals. Maximum density was 31.7 polyps/m^2 with an average subtidal density of 1.50 polyps/m^2. During this study, 2,211 individuals were counted. None were found at Colville Island or Rosario Point. The model including depth, substratum slope, and flow speed was the best fitting model (Table 8). Isanthidae n. sp. prefers high-flow environments ($F_{1, 436} = 13.8$, $R^2 = 0.023$, $p = 0.001$, Figure 13) and had a significant interactive response to depth and substratum slope preferring deep, vertical surfaces ($F_{2, 436} = 9.75$, $R^2 = 0.034$, $p = 0.001$, Figure 13).

Giant plumose anemone *Metridium farcimen*

M. farcimen was exclusively subtidal, with a depth range of -5.7 to -38.0 m MLLW (Figure 8) occupying all slopes (Figure 9). Maximum density was 64.3 polyps/m^2 with an average subtidal density of 1.90 polyps/m^2. During this study, 2,581 polyps were counted. None were found at Colville Island, Long Island, or Rosario Point. The model including depth, substratum slope, and flow speed was the best fitting model (Table 8). The distribution of the density of *M. farcimen* had a significant interactive response to depth and substratum slope ($F_{2, 436} = 4.43$, $R^2 = 0.017$, $p = 0.014$) being absent in the shallows, present on primarily vertical surfaces at mid depths, and abundant on all deeper surfaces (Figure 14). Additionally, there was an interactive response to depth and flow speed ($F_{1, 436} = 9.02$, $R^2 = 0.017$, $p = 0.005$); at mid depths *M. farcimen* is most

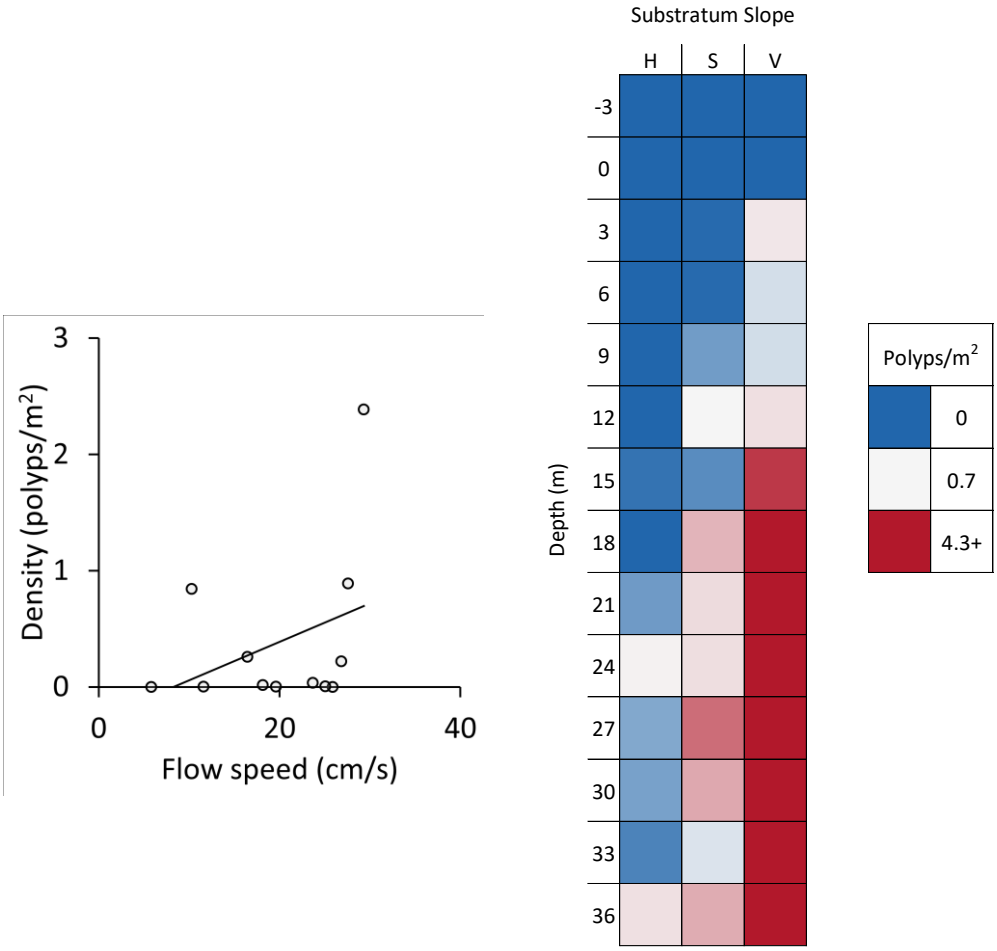

Figure 13. The effect of flow speed and the interactive effect of depth and substratum slope for an undescribed Isanthidae n. sp. in the San Juan Archipelago, WA, USA. Isanthidae n. sp. prefers high flow environments (PERMANOVA, $F_{1, 436} = 13.8$, $R^2 = 0.023$, $p = 0.001$) and deep, vertical surfaces (PERMANOVA, $F_{2, 436} = 9.75$, $R^2 = 0.034$, $p = 0.001$). White is the 40 percentile and red is above the 80 percentile. Slope categories are abbreviated as follows: H, horizontal; S, sloping; and V, vertical. Values are backtransformed means.

abundant at high-flow sites, and as depth increases, occurs over a wide range of flow speeds (Figure 14).

Mottled anemone *Urticina grebelnyi*

U. grebelnyi was the only modelled species that occurred in both the intertidal and subtidal. It had a depth range of 0.0 m to -38.0 m MLLW (Figure 8) on surfaces with slopes from 4 to 112° (Figure 9). Maximum density was 4.76 polyps/m^2 with an average density of 0.115 polyps/m^2 across all depths. Intertidal density was 0.013 polyps/m^2 and subtidal density was 0.133 polyps/

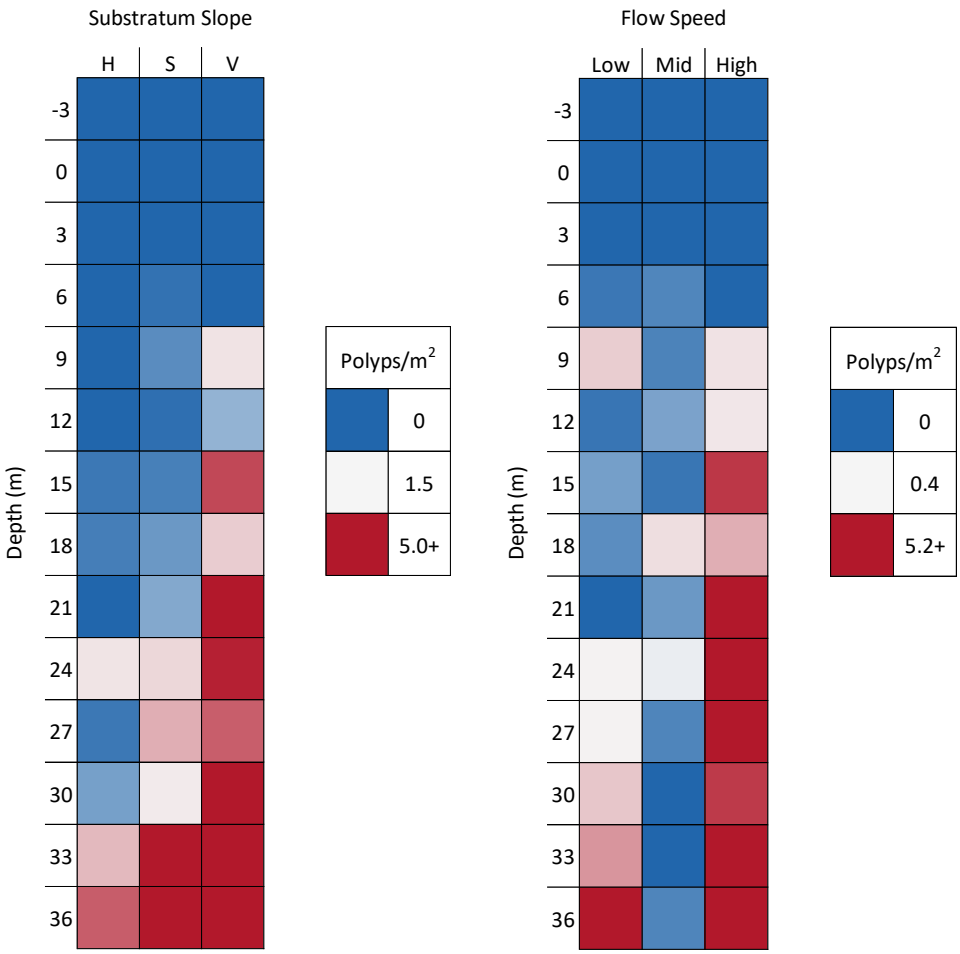

Figure 14. The interactive effect of depth and substratum slope and depth and flow speed for the giant plumose anemone *Metridium farcimen* in the San Juan Archipelago, WA, USA. *M. farcimen* is not present in the shallows, at mid depths is primarily on vertical walls, and in the deep is on all surfaces (PERMANOVA, $F_{1, 436} = 13.8$, $R^2 = 0.023$, $p = 0.001$). At mid depths *M. farcimen* is present at high flow sites and as depth increases, prefers a wide range of flow speeds (PERMANOVA, $F_{2, 436} = 9.75$, $R^2 = 0.034$, $p = 0.001$). White is the 40 percentile and red is above the 80 percentile. During the analysis, flow speed was treated as a continuous variable. Here, flow categories are as follows: low, 0-11 cm/s; mid, 11-21 cm/s; and high, over 21 cm/s. Slope categories are abbreviated as follows: H, horizontal; S, sloping; and V, vertical. Values are backtransformed means.

m^2. During this study, 135 polyps were counted with none found at Long Island, Rosario Point, or Shady Cove. The model including light and flow speed was the best fitting model (Table 8). There was a significant response to light level, with most animals found at lower light levels ($F_{1, 436} = 6.24$, $R^2 = 0.013$, $p = 0.004$, Figure 15). There was no response to flow nor an interactive response to light and flow speed. Generally, this anemone was found tucked between larger boulders.

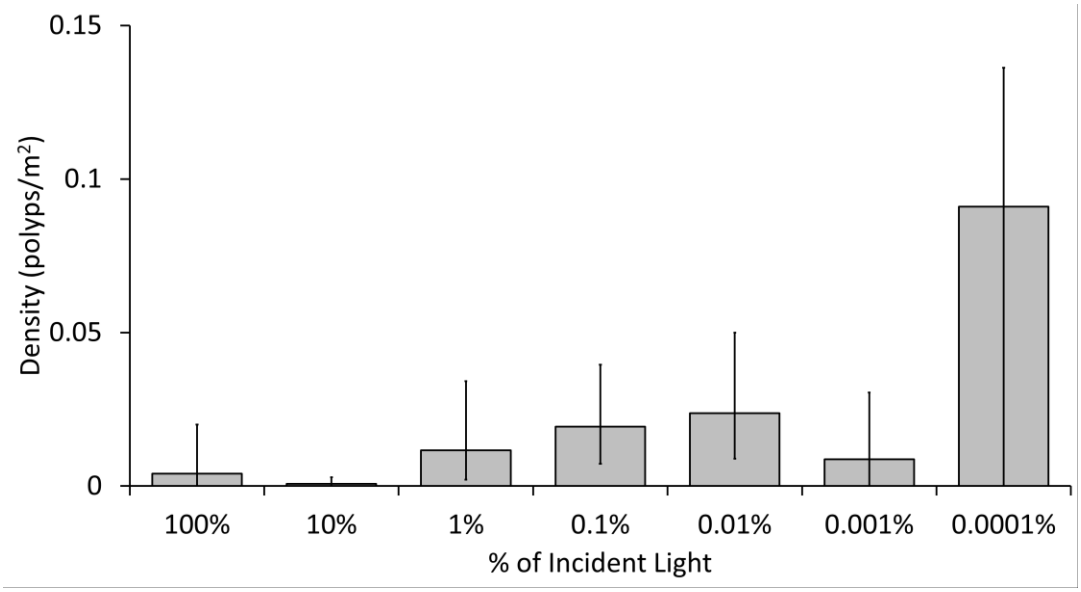

Figure 15. The effect of light on density of the mottled anemone *Urticina grebelnyi* in the San Juan Archipelago, WA, USA. *U. grebelnyi* prefers low light levels (PERMANOVA, $F_{1, 436} = 6.24$, $R^2 = 0.013$, $p = 0.004$). During the analysis, light was treated as a continuous variable. Values are backtransformed means with backtransformed bootstrapped confidence intervals.

Other non-modelled hexacorallians

Six other hexacorallians were captured in the photographs during the surveys, but were not present at enough sites (i.e., half or more) to create distribution models. These were the strawberry anemone *Corynactis californica*, Lisbeth's brooding anemone *Epiactis lisbethae*, the plumose anemone *Metridium senile*, the swimming anemones *Stomphia didemon* and *Stomphia coccinea*, and the stubby rose anemone *Urticina clandestina*. An additional two hexacorallian species were not captured in the photographs, but were observed at the research sites during dives – the white-spotted rose anemone *Cribrinopsis albopunctata* and the fish-eating anemone *Urticina piscivora*.

C. californica was present at only one site, West Long Island, as a single clonal mat from -17.7 to -20.8 m MLLW on a sloping surface. At this site there are multiple mats of several different color morphs of this species. We counted 23,739 *C. californica* in the photographs, with densities reaching a maximum of 4,532 polyps/m^2. This species has been observed at several other sites in the archipelago that were not part of this survey (Sebens, personal communication).

E. lisbethae was present at half the sites and was generally in low abundance; West Long Island was the only site where more than 10 were photographed. *E. lisbethae* was the only epiphytic species, found on stipes of the stalked kelp *Pterygophora californica* and the bullwhip kelp *Nereocystis luetkeana,* although most individuals were epilithic or epizootic. We recorded 1,479 *E. lisbethae* in photographs, with densities reaching up to 82.5 polyps/m^2.

M. senile was also present at only half the sites, but was even less abundant than *E. lisbethae*. Compared to its congener *M. farcimen*, *M. senile* has a wider depth range and can be found in the intertidal, from +0.5 to -38.0 m MLLW. During this study, 330 *M. senile* were photographed with densities reaching up to 51.6 polyps/m^2.

Only two *S. didemon* were found, one at -14.8 and one at -21.5 m MLLW, both on horizontal surfaces at Point Disney, Waldron Island. One *S. coccinea* was found at -37.4 m MLLW on a horizontal surface at North Peapod. One *U. clandestina* was found at -6.6 m MLLW and it was found between cobbles in gravel at West Long Island. *C. albopunctata* and *U. piscivora*, were both at Lime Kiln State Park, San Juan Island, but were not captured in the photographs.

DISCUSSION

These data show the distribution patterns and analysis of species-specific relationships with light, flow, depth, substratum slope, predation pressure, and temperature. The distribution of all but one focal species (*Epizoanthus scotinus*) of the seven taxa examined showed significant relationships with either depth or light. The effect of depth is a composite of many potential factors, such as light, temperature, salinity, oxygen, and plankton, although light is also affected by water clarity, substratum slope, and aspect. In this study, the same extinction coefficient was used for water clarity at all sites (0.294). The most conspicuous pattern was a highly reduced density of hexacorallians between 0 and -12 m MLLW. This depth corresponds with an elevated level of

large upright algae (> 40% cover), especially on vertical and sloping surfaces (Smith, 2018). The absence of hexacorallians in the presence of algae along a depth gradient has also been documented by Logan *et al.* (1984) and Witman and Sebens (1988); competition occurs between algae and hexacorallians, especially in the tropics (e.g., Gardner *et al.*, 2003; Edmunds, 2013; Bruno *et al.*, 2014). In the temperate environment, hexacorallians can be competitive dominants, but only where algae are absent or in low abundance (Dayton, 1975; Taylor and Littler, 1982; Witman and Sebens, 1988; Nelson and Craig, 2011). Witman and Sebens (1988) postulated that anemones were absent from surfaces near kelps due to the sweeping motion of the algae. This algal whiplash hypothesis seems likely, as negative effects also occur to other benthic suspension feeders such as barnacles (Hatton, 1938; Menge, 1976; Leonard, 1999) and can be a major component of interalgal competition (Dayton, 1975; Sousa, 1979; Kiirikki, 1996; Hughes, 2010). This shallow zone also experiences higher temperatures and lower salinities in pulses during the warmer months of the year, which could affect distribution and abundance (Murray *et al.*, 2015; Lowe *et al.*, 2016).

Two of the focal species, *Anthopleura elegantissima* and *Urticina grebelnyi*, were not distributed by depth *per se*, but rather directly by light. Of the two species, light was most important to structuring the population of *A. elegantissima* (Figure 10), an intertidal anemone with endosymbiotic microalgae. This anemone obtains anywhere from 13 to 45% of their carbon and 44 to 61% of their lipids from their endosymbionts (Fitt and Pardy, 1981; Fitt *et al.*, 1982; Shick and Dykens, 1984). Light was also important in structuring the populations of *U. grebelnyi*, a macrophagous predator without photosynthetic endosymbionts. They were found on horizontal and sloping surfaces more than vertical surfaces (Figure 9) and were more abundant on darker surfaces (Figure 15). This distribution likely allows them to avoid space competition with algae while still being able to prey on the associated macroinvertebrate community. Duggins *et al.*

(2016) found elevated levels of gastropods, polychaetes, crabs, and copepods associated with the kelp community. Currently, the diet of *U. grebelnyi* is unknown, but a similar species from the western Atlantic, *Urticina crassicornis*, feeds on jelly plankton, echinoderms, and large crustaceans (Wells, personal observation). *Balanophyllia elegans* also increases its density around the same depth range (Figure 8). The diet of *B. elegans* is also unknown, but adults readily feed on freshly hatched brine shrimp nauplii in lab. *B. elegans* are most abundant just as upright algae are disappearing (18-21 m) and they may be feeding on the elevated levels of copepods.

All but two focal species (*E. scotinus* and *U. grebelnyi*) were significantly impacted by water movement. Our data suggest that high flow, at least at the site-level, is beneficial for most species. Low-flow sites may never allow hexacorallians to experience their ideal flow regimes, while living at a high-flow site should allow for exposure to a range of flow throughout the tidal cycle. Flow can be a limiting factor in prey capture; limited flow can lead to upstream depletion of prey (Okamura, 1986; Fréchette *et al.*, 1989), but may also reduce the ability for planktonic prey to detect hexacorallians (Heidelberg *et al.*, 1997). Additionally, hexacorallians rely heavily on the flow regimes they occupy to disturb the boundary layer and increase diffusion of gasses (Patterson and Sebens, 1989; Patterson *et al.*, 1991). While being exposed to excessive drag may be a risk to some invertebrates, anthozoans can contract, avoiding the drag forces when flow is too high (Patterson, 1980; Sebens, 1984). Hexacorallians can also reduce form drag by bending over to become more parallel with the current (Koehl, 1977a). Within high flow environments, there should also be a larger variety of flow microhabitats, ranging from extremely low flows to near mainstream flows. Flow microhabitats of particular organisms can be exceedingly different from site-level flow regimes (Koehl, 1984). Unfortunately, with site-level flow measurements, we cannot say if these focal species are choosing particular flow microhabitats.

An effect of substratum slope was pronounced for most focal species. All, except for *B. elegans*, exhibited an interaction between slope and depth. The general pattern was an absence of hexacorallians between 0 and -12 m MLLW, an increase in abundance on vertical surfaces between -12 m and -24 m MLLW, and then an expansion to all slopes beyond -24 m MLLW. This pattern of surfaces becoming less distinguishable with depth has been described by Witman and Sebens (1988) and Smith (2018). Two species, *E. scotinus* and the Isanthidae n. sp., strongly preferred vertical surfaces at all depths (Figure 9 and 13). *E. scotinus* is seemingly an overhang specialist, being most abundant on walls with undercuts. When disturbed, they retract for an extended period of time, indicating that this species may be particularly sensitive to predation, although its consumers are currently unknown. The most conspicuous hexacorallian predator, *Dermasterias imbricata*, was never observed on overhangs and, during surveys, did not hold on well to the substratum, potentially due to the low amount of podia per biomass. It may be that *D. imbricata* is excluded from these slope refuges. In a laboratory setting, *E. scotinus* develops a heavy load of upright diatoms and filamentous red algae on its column if exposed to prolonged sunlight and seems sensitive to sedimentation (Wells, personal observation), which could be additional reasons for their specialized distribution.

Densities of the predatory sea star *D. imbricata* at the site-level do not seem to structure density of hexacorallians in the San Juan Archipelago. The inclusion of predation pressure reduced the performance of every model (Table 8). This result was surprising as previous experiments have found that predation and grazing can have significant impacts on subtidal benthic communities (Sebens, 1986a; Sieben *et al.*, 2011). *D. imbricata* can consume most of these species, but a size refuge exists for most large subtidal hexacorallians (Annett and Pierotti, 1984; Harris, 1991; Bachman and Muller-Parker, 2007; Wells *et al.*, 2018). For example, Wells *et al.* (2018) found

that the largest *D. imbricata* (~37 cm) could only eat a *Metridium farcimen* with a diameter of 14 cm; the maximum diameter of *M. farcimen* is about 20 cm. *D. imbricata* can structure *A. elegantissima* populations, excluding them from the subtidal (Sebens, 1977). The predation effects of *D. imbricata* are clearly more nuanced than site-level densities. The impacts of this predator on hexacorallians would be particularly interesting in regards to their effect on size-structure of competitively dominant space holders like *M. farcimen*.

Site-level temperature had little effect on the distribution of hexacorallians. The incorporation of temperature in the models always reduced model performance, similar to predator density (Table 8). Water in the San Juan Archipelago is well-mixed due to the large tidal exchanges, although small differences at each site did occur (Table 9). Warmer sites (+0.5 °C from the site averages) were closer to the output of the Fraser River in the north or were within a sound (i.e., Point Doughty and Rosario Point) and cooler sites (-0.4 °C from the site averages) were closer to the entrance of the Strait of Juan de Fuca in the south (i.e., Lime Kiln and Long Island). Smith (2018) found that temperature slowly decreased with depth during summer, but few patterns were found during other seasons. While there is not an abrupt thermocline at any point of the year (Smith, 2018), temperature could still have an effect on hexacorallian distribution; our data did not suggest this is a significant factor.

The quantified factors in total explained a small proportion of the total variation of the distribution of any focal species (R^2 = 0.01 - 0.08). These low values are not surprising given the type of data (counts) and the abundance of zeros in the data (up to 95.9% for *A. elegantissima*). Marine communities are extremely heterogeneous at the scale of meters (e.g., Fraschetti *et al.*, 2005; Smale *et al.*, 2010) due to phenomena such as competition (Connell, 1961; Paine, 1966;

Dayton, 1971; Paine, 1974; Lubchenco and Menge, 1978), predation (Paine, 1969; Connell, 1970; Dayton, 1975), and propagule supply (Berlow, 1997; Sams and Keough, 2012).

The ecology of several species (e.g., *C. rubens* and *E. scotinus*) are particularly poorly understood; further studies on their distribution patterns would be helpful in understanding overall subtidal community structure. In contrast, it is known that *A. elegantissima* and *M. farcimen* are competitive dominants in their respective habitats (Taylor and Littler, 1982; Nelson and Craig, 2011); for these species, next steps might be testing how they determine where to settle to reduce competition and predation as recruits. Distinguishing between processes affecting each species would require targeted experiments (e.g., translocations). This work gives important insight into the factors impacting distribution in temperate hexacorallians. While predation and temperature seem to be less important, depth, light, flow, and substratum slope are clearly important factors and should be examined further.

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