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Nutritional drivers of adult locomotion and asexual reproduction in a symbiont-hosting sea anemone *Exaiptasia diaphana*

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Abstract

Some sedentary marine invertebrates have the potential to modify the environments they experience by moving, even as adults. Of particular interest are sea anemones, which, despite appearing immobile, can move throughout their lives. Individual locomotion may mitigate changes in environment conditions and therefore play an important role in the natural history of sea anemones, especially in naturally variable and/or stochastic environments. Sea anemones that associate with algal endosymbionts may respond to changes in nutrition, both autotrophic (from algae) or heterotrophic (from prey). Here, we describe the adult movement behaviors and asexual reproduction of the sea anemone *Exaiptasia diaphana* in response to changes in food availability and photosymbiont density. Anemones were collected from mangrove roots in the Florida Keys USA (24°49'21.91"N, 80°48'37.95"W) during January 2016 and exposed to a factorial experiment in which food availability and exposure to temperature shock were manipulated. Sea anemones exhibited a variety of responses, including (1) increased crawling along the substrate in response to starvation, (2) increased detachment from the substrate and reattachment in a new location in response to starvation, and (3) increased production of motile asexual clones in response to both starvation and temperature-induced changes in symbiont density. These responses are shaped not only by the direct consequences to the sea anemone, but also by the effects on the symbiotic algae, which exchange sugars, lipids, and oxygen for nutrients within the host. Observed patterns of movement and reproduction are likely advantageous for life in the dynamic mangrove root fouling communities where this anemone species occurs. The ability to disperse as an adult may give this otherwise sedentary invertebrate an advantage in naturally stochastic conditions or in rapidly changing environments.

Key words: *Aiptasia*; benthic; cnidarian; *Exaiptasia diaphana*; endosymbiosis; environmental change; food availability; mangrove roots; movement; Symbiodiniaceae; temperature

Introduction

An organism faced with changing environmental conditions must acclimate, move, or perish. In marine invertebrates, changes in physiology, body size, reproductive behavior, individual movement, and dispersal can all play a role in response to environmental change (Harley et al. 2006; Brooker et al. 2007; Ryan 2018). The capacity for, and adaptive value of, differing response strategies is strongly shaped by the natural history of organisms. For example, dispersal is expected to be solely accomplished by larval or juvenile forms in most sedentary and sessile marine species (Cowen and Sponaugle 2009). As such, investigating adult movement as a potential response mechanism is often overlooked in favor of studies of physiological acclimation to changing environments in nominally sessile organisms, even for species known to be mobile throughout their lives (e.g., mussels, snails, sea anemones). We have a limited understanding of the ecological role of adult movement in many species despite the potential for this behavior to reduce environmental variability, whether that variability is caused by stochasticity, seasonal fluctuation, or climate change. Understanding all of the ways organisms cope with environmental variation is key to making accurate ecological predictions under both ordinary and novel circumstances (Fox et al. 2019).

Here, we measure the ability of a nominally sessile sea anemone to engage in both short- and potentially long-distance movement in order to escape unfavorable conditions. Sea anemones can disperse as larvae, as new settlers, and as adults. The potential for adult movement is well accepted by those who keep sea anemones in the aquarium trade and evidenced by several studies. Adult movement occurs in response to changes in light (Pearse 1974), to the threat of

predators (Sund 1958; Edmunds et al. 1976), to agonistic behavior of conspecifics and other cnidarians (Sebens 1984; Chadwick 1987), or to ontogenetic changes as habitat requirements shift (Ottaway and Thomas 1971; Sebens 1981a). Individual sea anemones achieve directed movement through waves of muscular contractions that allow them to "crawl" along a surface (Parker 1916). The ability of sea anemones to travel short distances by crawling locomotion is well documented (McClendon 1906), but such behaviors are rarely integrated into eco-evolutionary hypotheses about the purpose or effects of movement (but see Fredericks 1976; Sebens 1981a; Chadwick 1987). Some anemones are also known to travel longer distances as adults. Distant dispersal may be achieved by detachment and drifting (Riemann-Zürneck 1998). We know that sea anemones can exhibit complex growth and reproductive responses to environmental fluctuations (Ryan 2018; Ryan and Miller 2019), but little is known about the potential roles of active movement or detachment in individual responses to changing environmental conditions, especially those that involve nutritional pathways.

Many tropical and temperate cnidarians, including sea anemones, host endosymbiotic dinoflagellate algae (Muller-Parker and Davy 2001). The responses of the holobiont – composed of a cnidarian host and its algal symbionts – to changing environmental conditions reflect both the host's and symbiont's environmental tolerances (Brown 1997). Algal symbionts can provide the animal with energy in the form of sugars and lipids in exchange for nitrogen produced by the animal (Smith et al. 1969; Yellowlees et al. 2008). In sea anemones, algal symbionts may also play a critical role in keeping animal tissues oxygenated when external conditions become hypoxic (Rands et al. 1992). The physical closeness of the two organisms can allow for rapid exchange of resources with minimal loss, a major advantage in resource-limited environments (Raven et al. 2009). The interaction between host and symbiont can also influence anemone

95 movement. Pearse (1974) found that *Anthopleura elegantissima* individuals display phototactic
96 movement only when symbionts are present. Individuals from high-light environments move
97 toward light, likely to increase photosynthetic products from symbionts. Fredericks (1976) found
98 that phototactic behavior in *A. elegantissima* is associated with oxygen enrichment by
99 photosynthesizing algal symbionts.

100 Resources from algal symbionts are supplemented by the prey that anemones capture via
101 passive suspension feeding. The diets of sea anemones can be complex (Sebens 1981b) and
102 highly variable between sites or through time (Quesada et al. 2014). Chintiroglou and Koukouras
103 (1992) found that 99.15% of the coelenterons of *Anemonia viridis*, which lives symbiotically
104 with algae, were empty during winter sampling while only 85.64% were empty during summer
105 sampling, suggesting that sea anemone diets can be seasonal and highly variable. Anemones may
106 go long periods of time with no success in capturing prey. The availability of planktonic food
107 shapes competition among species (Svensson and Marshall 2015) and the distribution of species
108 in space (Lesser et al. 1994), but no previous study that we know of has addressed how variation
109 in diet could affect movement of sea anemones.

110 Many sea anemone species reproduce asexually, and movement can be an important
111 component to asexual reproduction. For example, *Exaiptasia diaphana* reproduces asexually by
112 pedal laceration. Small pieces of the pedal disc are detached from the adult and left behind, and
113 these tissue pieces develop into new adult anemones. Unlike other species that create large clonal
114 aggregations via asexual reproduction (e.g., *Anthopleura elegantissima*), *E. diaphana* adults
115 move away from their clonemates (S. Bedgood pers obs). Previous studies have revealed
116 nutritional drivers of asexual reproduction in *E. diaphana*. Hunter (1984) reported increased
117 pedal laceration when anemones were kept in the dark (no contribution from symbionts) but no

118 difference between feeding regimes, and Clayton and Lasker (1985) found that anemones
119 produced more pedal lacerates immediately after starvation. Clayton (1985) found an interaction
120 between feeding and symbiont state where anemones that were starved and possessed symbionts
121 produced the most pedal lacerates. Nutritional drivers may also impact asexual reproduction or
122 mitigate the risk of movement by leaving behind clones.

123 As heterotrophic and autotrophic pathways both contribute to nutrient acquisition in these
124 sea anemones, each pathway, as well as their potential interaction, on adult anemones has the
125 potential to influence processes at the individual, population, and community levels. Previous
126 work suggests that interactions between the host, its symbionts, and the environment likely
127 influence movement of symbiotic sea anemones (Pearse 1974; Fredericks 1976). In this study,
128 we explored the interactive effects of food limitation and symbiont density on the locomotion
129 behavior of the symbiont-hosting sea anemone, *Exaiptasia diaphana* (Rapp 1829; ICZN 2017)
130 from the Florida Keys, commonly referred to as *Aiptasia* and formerly known as *Aiptasia*
131 *pallida*. *E. diaphana* in this region are genetically distinct from other populations around the
132 world and may host dinoflagellate algal symbionts from three different genera (*Symbiodinium*,
133 *Breviolum*, and *Cladocopium*) in the family Symbiodiniaceae (Thornhill et al. 2013). By
134 manipulating the contribution of two potential sources of nutrition, we tested the hypotheses (1)
135 that anemones move more frequently in response to food-limited environments (i.e. anemones
136 actively forage), (2) that a reduction in heterotrophic nutrition provokes a similar movement
137 response as a reduction in autotrophic nutrition, and (3) that movement increases under dark
138 versus light conditions for individuals with symbionts as a response to the reduction in
139 photosynthate from algal symbionts. We also measured the influence of food limitation and

140 symbiont density on body size and clonal reproduction as these outcomes may reflect changes in
141 life history strategy in response to treatments alongside changes in movement.
142
143 **Methods**
144 *Species description*
145 The distribution of *E. diaphana* extends from subtropical to tropical shallow-water
146 marine habitats, where they attach to hard substrates (Thornhill et al. 2013). There is currently no
147 consensus on the local distribution of this species. The most common habitats where *E. diaphana*
148 are found in the Florida Keys include biofouling communities on docks or buoys, boulders in
149 shallow bays, and mangrove roots (S. Bedgood pers obs). We have observed that mangrove roots
150 have the highest density of anemones in this region. Invertebrates, including *E. diaphana*, attach
151 to the submerged portion of the roots. The availability of hard substrate in this environment is in
152 constant flux as new roots periodically enter the water and are then rapidly colonized by a
153 diverse assemblage of sponges, ascidians, algae, and anemones (Wulff 2004). Light in this
154 environment is highly variable due to shading by mangrove branches and macroalgae (S.
155 Bedgood unpubl data). Perhaps as a consequence of this dynamism, *E. diaphana* in the field have
156 variable symbiont densities and are usually found on the outer edges of mangrove root stands in
157 this region (S. Bedgood pers obs).
158
159 *Cold-shock efficacy experiment*
160 To manipulate symbiont density, we used a cold-shock protocol (Muscatine et al. 1991).
161 This is a unique approach to manipulating symbiont density as the majority of previous studies
162 have completely excluded symbionts as a control treatment. Here we include low and high

symbiont densities because aposymbiotic individuals are rare or absent in the field (S. Bedgood pers obs). To determine the efficacy of a cold-shock treatment in reducing symbiont density in anemones from this region, we collected 14 adult individuals of *E. diaphana* from mangrove roots on the west side of Otter Key in Sarasota, Florida, USA (GPS coordinates 27°18'50.09"N, 82°34'11.62"W) in December 2017. For the purpose of this study, we define "adult" anemones as individuals with a pedal disc area of 15 mm^2 or larger. Sea anemones were divided into either ambient or cold-shock treatments and were housed individually in 0.5-liter containers for two weeks. Ambient treatment anemones were maintained at room temperature (23°C) throughout the experiment, which was similar to field conditions. Cold-shock treatment anemones were also kept at 23°C except during a weekly cold shock of 4°C for four hours that was accomplished by placing aquaria in a temperature-controlled chamber. Water in the aquaria was ambient when placed in the chamber, and we completed water changes to all treatment groups after cold-shock anemones were removed from the chamber, rapidly increasing the temperature of cold-shock treatments to ambient temperature. We sacrificed and measured the symbiont density of all anemones after two weeks of treatment (total of two cold shocks).

We measured symbiont density by homogenizing whole sea anemones. The homogenate was diluted approximately 1:20 with deionized water, and cells were counted on a Brightline hemocytometer (Hausser Scientific, Horsham, Pennsylvania, USA). To standardize the symbiont density, we measured animal protein from the same diluted homogenate using the Lowry method for protein estimation (Lowry et al. 1951) with Bovine Serum Albumin as a standard. We report symbiont density in units of symbiont cells per μg protein (Muller-Parker 1984; Bergschneider and Muller-Parker 2008; Hiebert and Bingham 2012). Muscatine et al. (1991) reported a release

of 40-55% of algal symbionts from the sea anemones after this treatment. We achieved similar results (details reported below).

Nutrition and movement experiment

We collected 128 *E. diaphana* adult individuals from 26 mangrove roots along a 20-meter stretch of Zane Grey Creek in the Florida Keys, USA (GPS coordinates 24°49'21.91"N, 80°48'37.95"W) in January 2016. A maximum of five sea anemones were collected from each root to avoid over-representation of a single genet (clonal group) due to *E. diaphana*'s prolific asexual reproduction (Cary 1911; Bellis et al. 2018).

Sea anemones were transported to the laboratory where they were randomly distributed among 16 plastic 2 L aquaria, with eight sea anemones per aquarium. We chose these aquaria because they had a similar surface area to a small mangrove root and allowed for movement measurements across a flat clear surface. Air stones were used in each aquarium to provide water movement and oxygen, and six 27 watt 6500 K compact fluorescent lights were used to illuminate the aquaria from above on a 12/12-hour light cycle. Light intensity was no higher than 200 μmol m^{-2} s^{-1} and no lower than 150 μmol m^{-2} s^{-1}. These measurements were within the light intensities measured at the site during collections on a clear day at several roots (50 μmol m^{-2} s^{-1} to 500 μmol m^{-2} s^{-1}). The water of each aquarium was changed twice per week using 35 ppt salinity water prepared using Instant Ocean Sea Salt (Blacksburg, Virginia).

We conducted a fully factorial experiment manipulating food availability (starved, fed) and symbiont density (high density, low density; achieved via a weekly cold shock). Starved sea anemones were given no food after field collection, whereas fed sea anemones were given newly-hatched brine shrimp nauplii (*Artemia*, Brine Shrimp Direct, Ogden, Utah, USA) twice

weekly *ad libitum*, and Microvert Invertebrate Food (KENT Marine, Franklin, Wisconsin, USA) once weekly. Excess food was removed via water changes to all aquaria several hours after feeding. Symbiont density was manipulated as described above, using a weekly cold shock to create the low symbiont density treatment. Cold-shock treatments began immediately (first day of experiment) and continued to week three of the experiment for a total of three cold shocks applied to create and maintain the low symbiont density treatments.

To measure locomotion activity, we recorded movement every four minutes for all three weeks (see Supplemental Fig. 1), day and night, with a Canon Powershot S100 camera, operated with the automation software Canon Hackers Development Kit (CHDK). Photos were taken from below the aquaria, which were suspended from a structure one meter above the camera (see Supplemental Fig. 2). Photos were loaded into ImageJ (Schneider et al. 2012) as an image sequence for each 12-hour night or day period. We traced the path of each sea anemone that moved, tracking the center of the pedal disc (see Supplemental Fig. 2C). We also recorded the number of sea anemone movements, which we defined as locomotion across the substrate punctuated by no movement for 30 minutes or more on either end of the path. Because individual sea anemones were not easily identifiable across days, the total distance each anemone moved along the bottom of the aquarium during each 12-hour period was recorded and averaged for each aquarium. If anemones moved up the side of the aquarium, movement was not recorded and not included in the average, but this type of movement was rare (approximately one anemone per tank per week).

We recorded detachment and reattachment of anemones in this study, a behavior observed in other sea anemone species (see Riemann-Zürneck 1998) but previously undescribed in *E. diaphana*. We defined detachment and reattachment as occurring when the anemones

expanded, detached, and became neutrally buoyant. They then became caught in the flow, tumbled around the aquarium for several minutes to hours, and reattached in a new location. These events were recorded only if the detached anemone was observed to expand, detach, and tumble in the water, eventually reattaching in a new location.

Photographs of each sea anemone were taken weekly throughout the experiment and used to measure pedal disc area using ImageJ software (Schneider et al. 2012). We used pedal disc area to measure growth because it was the least invasive method and correlated closely with freeze dried mass (Supplemental Fig. 3).

Anemones produced clonal fragments via pedal laceration. The number of pedal lacerates produced in each treatment was recorded once at the end of the second week because counting lacerates was intensive and invasive. Pedal lacerates were defined as physically isolated pieces of anemone tissue that separated from the parent adult and remained attached to the substrate (Cary 1911). At the time data were recorded, pedal lacerates varied in size and stage of regeneration; some had fully formed tentacles, while others were newly separated pieces of tissue. No difference in the apparent stage of pedal lacerates was observed among treatments, so all stages were pooled for analysis. There was no way to determine which anemone produced which pedal lacerate, so clonal reproduction was averaged for each aquarium containing eight individual adults.

Darkness experiment

During the third week of the experiment, anemones were kept entirely in the dark for three days to determine if the absence of light affected movement. A black plastic tarp was draped over the entire system with only a small amount of light entering from below so that long

exposure times could capture images. Light intensity in all aquaria read as 0 μmol m^{-2} s^{-1}. Movement recorded over the three days leading up to the dark period and the three days during the dark period were compared to test the effect of dark exposure. We measured movement and detachment during this time as described above.

Statistical analyses

We conducted all analyses in R 3.4.2 (R Core Team 2017) using a variety of parametric and non-parametric tests. Data from the cold-shock efficacy experiment were replicated by individual anemone (n = 6 or 7), and data from the nutrition and movement experiment and the darkness experiment were pooled by aquarium with eight anemones in each aquarium and a total of four aquaria in each treatment (n = 4). To verify the assumption of normality, we used a Shapiro-Wilk test on each set of data. Movement data and detachment data were not normally distributed, whereas symbiont density, asexual reproduction, and anemone size data were normally distributed. We analyzed the data from the cold-shock efficacy experiment with a two-sample t-test. We used several approaches with the anemone movement experiment. Movement data were transformed ($\log_{10}[x+1]$) to achieve normality, and we analyzed movement and clonal reproduction data using a two-way ANOVA with feeding and symbiont density as main factors followed by Tukey HSD tests for multiple comparisons. Detachment data were non-normal regardless of transformations, so we analyzed them via a Kruskal-Wallis rank sum test and used Dunn's multiple comparisons test for post hoc evaluation of differences between treatments. We used a generalized linear mixed-effects model (GLMM) to analyze the size of sea anemones throughout the experiment, with tank identity as a random effect and feeding, temperature, and week as fixed effects. We analyzed differences in the darkness experiment by comparing

277 movement and detachment data between the three days of darkness and the previous three days
278 of light using a repeated-measures ANOVA.
279
280 **Results**
281 *Cold-shock efficacy experiment*
282 Sea anemones that were cold-shocked had a lower symbiont density than those that were
283 kept at an ambient temperature (two-sample t-test, $t(12) = 2.56$, $P = 0.030$; Fig. 1). Our treatment
284 reduced symbiont density to 47% of the symbiont density observed in the control on average,
285 which was within the range reported by Muscatine et al. (1991). Cold-shocked sea anemones
286 appeared lighter in color than ambient-temperature sea anemones throughout the experiment,
287 suggesting that symbionts remained at a low density in this treatment for the duration of the
288 experiment. Based on these results, hereafter we refer to the ambient treatments as “high
289 symbiont density” and the cold shocked treatments as “low symbiont density.”

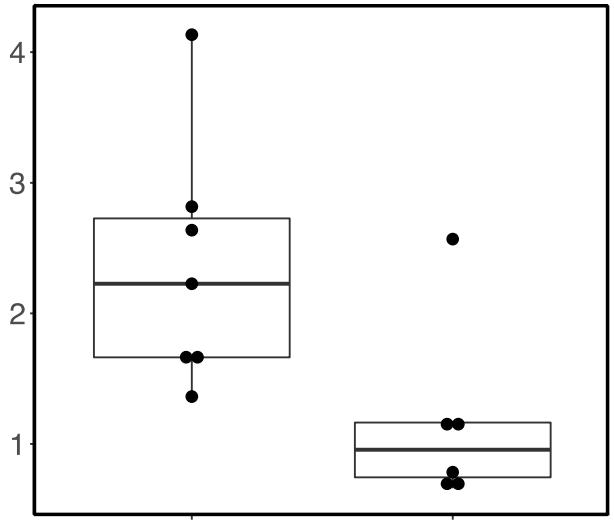

Figure 1. Boxplot of symbiont density of anemones after being kept at an ambient temperature (23 °C) or cold shocked for 4 hours (4 °C). Symbiont density was determined by counting cells and standardizing with animal protein concentration. Data were analyzed with a two-sample t-test. Lower and upper box boundaries 25th and 75th percentiles, respectively, line inside box median, lower and upper error lines smallest or largest data points within 1.5 times the interquartile range, respectively. Overlaid points are symbiont densities of individual anemones (cold shock n = 7, ambient n = 6).

Nutrition and movement experiment

Anemones in all treatments increased movement when first introduced to aquaria, so we allowed one week for acclimation to aquaria and treatments before recording movement rates (see Supplemental Fig. 4). Movement of anemones via crawling across the substrate was greater in starved treatments than in fed treatments (two-way ANOVA, $F(1,12) = 15.90$, $P = 0.002$), but there was no effect of symbiont density on movement (two-way ANOVA, $F(1,12) = 2.23$, $P = 0.162$; Fig 2A) or interaction between feeding and symbiont density (two-way ANOVA, $F(1,12) = 0.86$, $P = 0.373$). Fed anemones moved an average of 3.03 ± 1.46 ($\overline{X}$ ± SE, n = 8) mm per anemone per week, whereas starved anemones moved an average of 14.47 ± 3.50 (n = 8) mm per anemone per week, almost five times as much.

The path length of each anemone, measured by taking the length of a path defined by no movement at the start and end, and the number of anemone movements within a replicate (aquarium) both played a role in the average movement value (Fig. 2A). We analyzed path length and the number of anemone movements separately. Path distances of starved sea anemones were appreciably longer than those of fed anemones (two-way ANOVA, $F(1,12) = 5.96$, $P = 0.031$); fed anemones moved an average of 15.75 ± 6.32 mm per movement, and starved anemones moved an average of 39.46 ± 6.97 mm per movement (two-way ANOVA, $F(1,12) = 5.96$, $P = 0.031$). These path distances are larger than the movement rates because they exclude anemones that did not move. Starved sea anemones were also characterized by a greater number of movements than fed anemones (two-way ANOVA, $F(1,12) = 13.35$, $P = 0.003$). We recorded an average of 4.88 ± 1.16 movements per week in the fed treatments and an average of 16.25 ± 2.65 movements per week in the starved treatments. Both the path length and number of movements in each replicate aquarium demonstrated the same result; starved anemones moved more frequently and farther than fed anemones. Symbiont density did not affect any metric of movement (two-way ANOVA, path - $F(1,12) = 0.90$, $P = 0.362$, number - $F(1,12) = 0.002$, $P = 0.969$).

Because the behavior was rare, we pool and report detachment data collected over all three weeks during both the nutrition and movement experiment and the darkness experiment (Supplemental Fig. 1). Regardless of treatment, 85.83 ± 6.88 % ($\overline{X}$ ± SE, n = 6) of anemones detached during the night rather than day. Feeding treatment had a significant effect on detachment (Kruskal-Wallis test, $H1 = 8.24$, $P = 0.004$), but symbiont density did not (Kruskal-Wallis test, $H1 = 0.03$, $P = 0.868$). We were not able to test for an interaction between feeding and symbiont density because detachment data were not normally distributed. An average of

22.40 ± 5.39 % (n = 8) of anemones detached per aquarium per week in starved treatments, while an average of only 1.04 ± 0.68 % (n = 8) of anemones detached per aquarium per week in fed treatments (Fig 2B). A post hoc analysis revealed differences between the starved low symbiont treatment and fed high symbiont (Dunn's multiple comparisons test, $Z = -2.15$, $P = 0.015$) and fed low symbiont treatments ($Z = -2.93$, $P = 0.002$). However, the fed high symbiont treatment was not different from the starved high symbiont treatment ($Z = 1.015$, $P = 0.155$) suggesting that an interaction between feeding and symbiont density was likely.

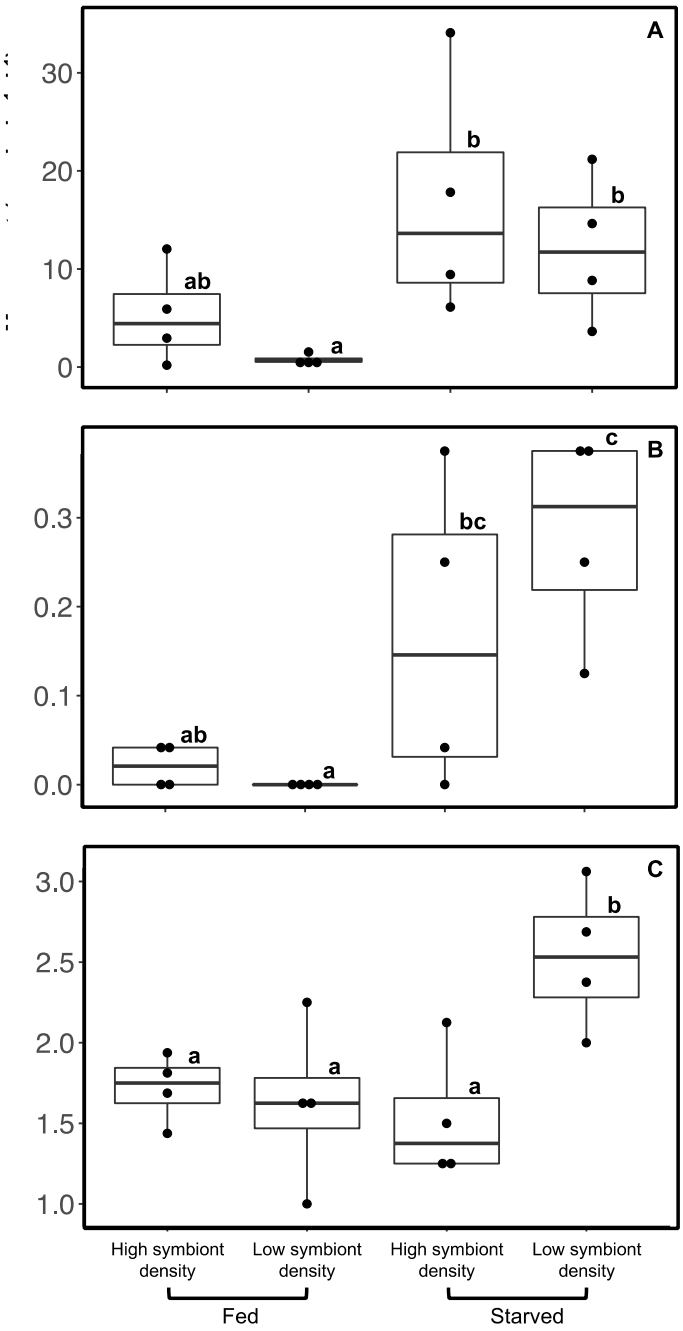

Figure 2. Boxplot of movement, detachment, and reproduction of *E. diaphana* in four treatments. The high symbiont density treatments were kept at an ambient temperature and the low symbiont density treatments were cold shocked weekly. (A) Average movement of each anemone analyzed with a two-way ANOVA and Tukey HSD. (B) The proportion of anemones that detached in each aquarium analyzed with a Kruskal Wallis test and Dunn's multiple comparisons test. (C) Average reproduction via asexual pedal laceration analyzed with a two-way ANOVA and Tukey HSD. Lowercase letters indicate significant differences among treatments. See Figure 1 description for boxplot explanation. Overlaid points are the means of each aquarium (n = 4 for each treatment).

331

Anemones with low symbiont density produced more offspring via pedal laceration than those whose symbiont density was unmanipulated (two-way ANOVA, $F(1,12) = 4.82$, $P = 0.049$), but this effect was driven by an interaction with feeding (two-way ANOVA, $F(1,12) = 7.03$, $P = 0.021$; Fig 2C). The largest number of pedal lacerates was produced by the starved, low symbiont treatment, where each anemone produced an average of 2.53 ± 0.23 ($\bar{X} \pm$ SE, n = 4) pedal lacerates per week. In contrast, sea anemones in other treatments produced an average of 1.63 ± 0.11 pedal lacerates per week. We also observed that once tentacles were developed, lacerate-derived individuals moved just as far as large anemones, if not farther, and detached more frequently. However, because of their small size, movement and detachment were not always discernable in the time-lapse photo series, so these attributes were not recorded for pedal lacerates.

Anemone size changed throughout the experiment (Fig 3). A linear mixed effects model used to analyze anemone size during all three weeks of the experiment showed that feeding (GLMM, $X1 = 94.14$, $P<0.001$) and time (GLMM, $X3 = 47.41$, $P<0.001$) were the main factors influencing anemone size during the experiment. There was also an interaction between feeding and time (GLMM, $X3 = 29.60$, $P<0.001$). Fed anemones quickly increased in size between week 1 and week 2, whereas starved anemones remained the same size throughout the experiment.

Both feeding treatment (two-way ANOVA, $F(1,12) = 91.09$, $P< 0.001$) and symbiont density (two-way ANOVA, $F(1,12) = 4.93$, $P= 0.046$) affected final anemone size (Fig 3). There was a marginally significant interaction between feeding and symbiont density (two-way ANOVA, $F(1,12) = 4.61$, $P= 0.053$). Starved anemones remained the same size as they were when collected, whereas fed anemones almost doubled in size. Symbiont density did not

influence size when anemones were starved but contributed to significantly larger anemones when coupled with feeding.

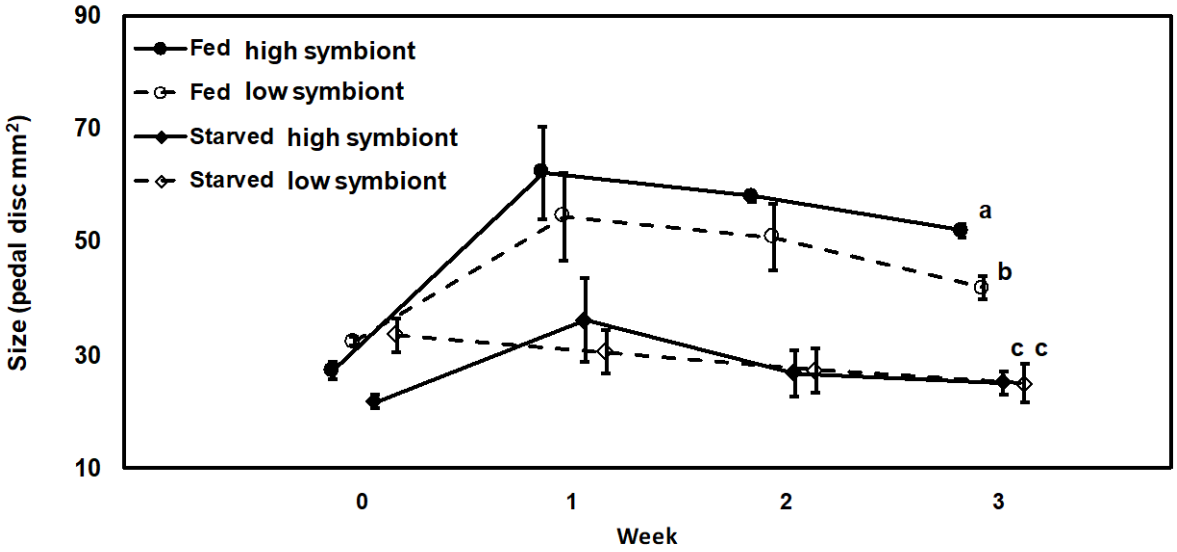

Figure 3. Mean (± SEM) sea anemone size for all four treatments measured at the end of each week analyzed with a GLMM and Tukey HSD. Points are offset horizontally to accommodate error bars. Lowercase letters indicate significant differences between treatments at end of week three. The sample size for each point is n = 4.

Dark vs. light experiment

Patterns of anemone movement did not change during the period of complete darkness. Starved anemones continued moving more than fed anemones, and the magnitude of movement was not significantly different from movement before the dark period (repeated measures ANOVA, $F(1,12) = 0.226$, $P = 0.643$).

Discussion

Despite being considered largely sedentary, *E. diaphana* has the ability to crawl or even detach from and reattach to the substrate. This behavior allows anemones to escape deleterious conditions and potentially choose habitat. Here, we hypothesized that nutrition, both from captured food and from photosymbionts, would affect the movement of anemones. We predicted that starvation and a low symbiont density would increase movement relative to anemones that were fed *ad libitum* and had a high symbiont density, respectively.

Movement and detachment in *E. diaphana* are driven by food availability. We found that starved sea anemones moved more frequently and farther than those that were fed. This response might be advantageous because small movements could alter the amount of food available to the individual. In mangrove root communities and other fouling communities in which *E. diaphana* is found, there is a high risk of smothering by macroalgae, other *E. diaphana*, sponges, and other suspension-feeding invertebrates (Ellison and Farnsworth 1992; Wulff 2004). Anemones may use the decrease in food availability that accompanies this smothering as a cue to move to a different location, escaping the low food environment. For example, *E. diaphana* individuals were regularly found attached to the outside of dense macroalgal growths, exposing their tentacles to higher water flow and potential zooplankton prey (S. Bedgood, *pes. obs.*).

How far can sea anemones move to escape deleterious environments? The fastest individuals measured travelled over 200 mm within 12 hours, which could allow them to move substantial distances over longer time frames. The physiological cost of movement is currently unknown, so movement over long distances without adequate food availability might be energetically limited. However, anemones in the starved treatments showed no decline in movement rate and in some cases increased their movement toward the end of our three-week experiment. Anemone size may also play a role in movement. Previous work on Fungiidae corals found that smaller individuals could move farther (Chadwick-Furman and Loya 1992), but the effect of size on movement in sea anemones is currently unknown. The average anemone size was the same across all aquaria at the beginning of the experiment, but it did diverge during the experiment (see Fig. 3). Size likely did not influence movement results as the effect of treatments on movement was apparent before size diverged.

Detachment of *E. diaphana* from the substrate, drifting, and eventual reattachment appeared purposeful, occurred mostly during the night, and were driven by starvation. This behavior was rare and would not have been detected without time-lapse photography. There is risk in this strategy, at least in mangrove root habitats, as the area immediately surrounding most mangrove roots is predominately soft sediment, unsuitable for attachment. The timing of detachment could increase the potential for successful dispersal as the risk of predation is likely lower during the night. Anemones may also compensate for the risk of detaching by leaving pedal lacerates behind in the original location. There is some evidence for this strategy as the treatment group with the highest asexual propagation rate also had the highest detachment rate. Given the intense competition for space on mangrove roots (Wulff 2017) and the regularity with which bare roots enter the water forming new patches, adult dispersal through drifting may be a reliable and important strategy for this species. Bellis et al. (2018) found genetically identical clones of *E. diaphana* on mangrove roots up to 50 meters apart. Detachment and reattachment to a new root could explain this pattern. Future field studies that address movement between roots are needed to link genetic structure to anemone movements in the field.

Medium- to long-distance post-larval dispersal by nominally sessile animals is not uncommon. Individual polyps of some corals including *Seriatopora hystrix* and *Pocillopora damicornis* have been observed to detach from the colony during stressful conditions and resettle in a new location (Sammarco 1982; Fordyce et al. 2017). At least two corallimorpharian species asexually produce buds that disperse in the water column to new locations (Chadwick-Furman and Spiegel 2000). Sea cucumbers can travel up to 90 km per day in a similar manner, expanding and increasing their water content by up to 700% (Hamel et al. 2019). The genetic structure of some *Metridium senile* (sea anemone) populations suggest that asexual clone dispersal is

414 common (Shick et al. 1979). *Diadumene lineata* is known to occasionally detach from the
415 substrate when exposed at low tide (Shick et al. 1979) or in fouled tanks (W. Ryan pers obs), just
416 as our *E. diaphana* detached when food was limiting. Riemann-Zürneck (1998) has even
417 suggested that free-living sea anemone individuals are common, frequently being found in
418 plankton trawls in an expanded buoyant state. Though many such observations are scattered
419 throughout the literature, no systematic effort to understand the role of adult dispersal in sea
420 anemones has yet been undertaken. Such behaviors may alter predictions about genetic
421 population structure or population growth rates and should be considered in future studies,
422 especially with regard to expected environmental change scenarios.
423 To investigate the effect of algal symbiont density on movement in sea anemones, we
424 experimentally manipulated symbiont density with weekly cold shocks. This method is
425 commonly used to produce low-symbiont-density or aposymbiotic anemones (Muscatine et al.
426 1991; Weis 1991; Gates et al. 1992). Exposure to water cold enough to induce bleaching does
427 occur in the Florida Keys, despite high average sea surface temperatures. Cold-water events
428 occur periodically in the Florida Keys, causing large die-offs of corals, gorgonians, macroalgae,
429 and sponges (Colella et al. 2012). The minimum water temperatures during January offshore of
430 Long Key (our collection site) reach 8.7 °C (Colella et al. 2012). However, within Zane Grey
431 Creek where our anemones were collected, water temperatures likely match air temperatures on
432 outgoing tides because a large shallow marsh empties through the creek (S. Bedgood pers obs).
433 Air temperatures periodically dip below 4 °C at Long Key, usually during January. Since 2000,
434 air temperatures during January have dropped to 4 °C three times at Long Key (NOAA National
435 Centers for Environmental Information, Asheville, North Carolina, USA). Although we could
436 not separate the physiological effects of symbiont density from those of the brief cold shock

itself, we interpret our results here in terms of symbiont density while acknowledging the potential effects of cold alone. Given the likely confounding of these two phenomena in field populations, we believe that our results reflect patterns relevant to the ecology of the animals in their natural setting.

The taxonomic identity of algal symbionts within anemones in this study is unknown, but sampling from adjacent sites (Crawl Key and Summerland Key) identified sea anemones with symbionts from three different genera including *Symbiodinium*, *Breviolum*, and *Cladocopium*. *Symbiodinium* was the most common genus while the other two genera were fairly rare and usually found in combination with *Sybiodinium* (Thornhill et al. 2013). Symbiodiniaceae genera and species are known to have different temperature-specific limitations to symbiosis (LaJeunesse et al. 2010; McGinty et al. 2012) and different carbon fixation rates (Rädecker et al. 2018). Our cold-shock treatment may have changed the composition of symbiont genera or species within the anemones. However, measuring this change goes beyond the scope of this study. Future work should address how environmental fluctuations affect symbiont composition within the host.

The symbiont density manipulation did not influence either crawling rate or the likelihood of detachment on the timescale of the experiment, even though symbiont density was reduced in the cold-shock treatments to half of the original density (Fig. 1). Likewise, the absence of adequate light for photosynthesis over a short period did not substantially influence the movement of sea anemones. The absence of a difference may indicate that the three-day duration of darkness was not sufficiently long to impact the dietary benefits from algal symbionts, that these sea anemones simply do not respond to the presence of light, or that we failed to capture an important variable as we were not able to measure directionality in the

response to light. Previous studies in *Anthopleura elegantissima* have found that anemones with and without algal symbionts show similar movement rates (Pearse 1974), but anemones hosting symbionts are positively attracted to light. Fredericks (1976) showed that this positive phototaxis occurred when the surrounding water was hypoxic or normoxic, but when oxygen was super saturated, anemones showed no attraction to light. Thus, phototactic behavior was hypothesized to increase oxygen availability by stimulating photosynthesizing symbionts (Pearse 1974; Fredericks 1976). It may be that anemones alter movement rate when food is scarce, but that symbiont density affects the direction of that movement with regard to light. Additional studies are needed to understand this interaction.

The symbiont density manipulation did influence body size and asexual reproduction. Sea anemones are able to grow and shrink rapidly in response to environmental conditions, including food and oxygen availability (Sebens 1981b; Chomsky et al. 2004; Ryan 2018; Ryan et al. 2019). Our initial predictions assumed that both prey capture and translocated products from symbionts were comparable nutritional pathways in *E. diaphana,* as they are in other sea anemones (Fitt and Pardy 1981). After two weeks of growth, fed anemones were larger; however, the presence of symbionts only increased body size in fed anemones. Starved anemones remained the same size regardless of symbiont status. This result contrasts with some previous studies. Clayton and Lasker (1985) found that the presence of symbionts only affected growth in *E. diaphana* when anemones were starved, and Leal et al. (2012) found that growth of *E. diaphana* is maximized when fed and kept in the dark (no symbiont contributions). However, both of these studies used treatments that completely eliminated symbiont contributions while our study likely reduced the contributions of symbiont in one treatment (low symbiont density). We acknowledge that our study is limited by having no treatment that completely excluded the contribution from

symbionts (aposymbiotic), but the low and high symbiont density manipulations that we include are realistic based on field observations. Future studies could include a variety of symbiont densities to resolve the effects of naturally varying symbiont densities and aposymbiotic controls.

Algal symbionts could influence anemone growth by two pathways: translocation of photosynthate or oxygen production. Without food, anemones have less nitrogen to translocate to their symbionts, potentially slowing the growth of those symbionts. Many cnidarians directly translocate nitrogen to their symbionts (Wang and Douglas 1998; Piniak et al. 2003). Symbionts within starved *E. diaphana* have a higher C:N ratio than those that were fed regularly (Cook et al. 1988), suggesting that symbionts are limited by anemone diet. However, Davy and Cook (2001) found that nutritional state did not affect the translocation of photosynthate from symbionts to host in *E. diaphana* even after 86 days of starvation. The reduction in nitrogen was mitigated by increased photosynthesis and increased carbon translocation per symbiont. Symbionts may be important as an internal source of oxygen in addition to nutritional enhancement, allowing the host anemone to grow larger without oxygen limitation. The interaction between nitrogen limitation and oxygen enrichment with the host warrants additional study, as even subtle differences in oxygen availability can also influence the size and performance of sea anemones (Ryan et al. 2019) and their metabolic rates (Szczebak et al. 2013).

The rate of asexual reproduction was highest in the least favorable condition (i.e., starvation with low symbiont density), which agrees with previous work (Clayton and Lasker 1985). This increase in asexual reproduction is likely limited by the duration of starvation as Clayton and Lasker (1985) found a higher rate of asexual reproduction in their starved group during the first four weeks of treatment, but their fed treatments produced more pedal lacerates

during the next four weeks of treatment, potentially as a result of increased size. Because pedal lacerates originate at the edge of the pedal disc, and larger anemones have a larger pedal disc circumference, size could play a role in asexual propagation (e.g., larger anemones produce more pedal lacerates). We found that size of anemones positively correlated with pedal laceration within the range of sizes included in the movement experiment (Supplemental Fig. 5). It is therefore likely that the larger anemones in the fed treatments (Fig. 2C) had a higher potential for offspring production. Despite being smaller, starved cold-shocked anemones produced more offspring than either of the fed treatments.

Asexual propagation can be an effective strategy to track phenotypic optima in a changing environment (Ryan 2018; Ryan et. al. 2019). Under deteriorating conditions, rapid reproduction may increase the chances of escape, as each clone can travel in a different direction. Small individuals formed from laceration may also be able to survive periods of metabolic stress better than larger parent clones, as they require fewer resources and have a higher surface area to volume ratio. In addition, there was some evidence in this study for pedal lacerates moving more rapidly along the substratum than adults (S. Bedgood pers obs), so this behavior may aid in escaping poor conditions, but no quantitative data were taken.

The locomotive capabilities of adult sea anemones may play a critical role in habitat choice throughout life, and so may influence all aspects of growth, survival, and reproduction. Thus, considering movement is critical to understanding the evolutionary ecology of these animals, including physiological measures, population structure, and species interactions. Furthermore, a species' ability to move directly affects how well it will be able to adapt to changing environmental conditions. Our study demonstrates the importance of considering multiple movement strategies, including both locomotion across substrates and more extreme –

529 and risky – dispersal events (i.e., detachment), and reinforces the importance of environmental
530 context in assessing the costs and benefits of symbiosis. It will become increasingly important to
531 consider all forms of movement and dispersal at multiple stages of an organism's life, even in
532 nominally sessile species such as sea anemones in order to predict species' distributions and
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534
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