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5 1 EFFECTS OF SPINE DAMAGE AND MICROHABITAT ON
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8 2 RESOURCE ALLOCATION OF THE PURPLE SEA URCHIN
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11 3 *Strongylocentrotus purpuratus* (Stimpson 1857)
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Abstract

Though phenotypically plastic responses in echinoids are well studied, the majority of the literature examines the effect of altered diet. Some species, however, such as the purple sea urchin, *Strongylocentrotus purpuratus*, occur over a large geographic range and thus experience a variety of substrate types and wave forces. To determine whether these factors impact resource allocation in adult intertidal *S. purpuratus*, a laboratory experiment was conducted using two types of microhabitat based on extremes inhabited by sea urchins in the field (flat substratum vs. concave pit), and two levels of spine damage to simulate wave exposure (monthly clipping vs. control). A suite of measurements were taken to determine the effects of these two treatments including: feeding and movement behavior, test morphometrics, demipyramid (jaw) allometry, spine growth cycles, and mass and stereological counts of gonad sections. When given the

opportunity, sea urchins remained in their pits for the duration of the experiment, which limited their ability to feed. This behavioral response caused a decrease in weight and volumetric growth, and an increase in jaw growth relative to test growth. Sea urchins in pits also grew more spheroid in shape (increased height:diameter ratio), while those on flat substrates decreased in height:diameter ratio. Further, both males and females in pits showed altered gametogenesis. While spine damage did not affect test growth, sea urchins with clipped spines showed decreased gonad index and altered production of mature oocytes. Altogether, these results are congruent with the life history strategy of long living species like *S.purpuratus*, in that it spreads reproductive efforts over multiple seasons, while diverting resources toward repairing damage, acquiring suitable refuge, and maximizing attachment area. Therefore, microhabitat and spine damage, by impacting movement, feeding, growth, and reproductive output, likely have important indirect impacts on the intertidal community by way of altered sea urchin grazing rates and susceptibility and value to predators.

Keywords: *Strongylocentrotus purpuratus* (Stimpson 1857), Phenotypic plasticity, Spine Damage, Microhabitat, Substrate complexity, Gonad Stereology

1. Introduction

Many echinoids occur in high energy and variable environments and show a high degree of phenotypic plasticity in these challenging habitats. Sea urchins exhibit morphological and behavioral plasticity in response to several different factors including, most notably, changes in food availability. The most commonly studied plastic response in sea urchins is that some

species can increase the size of their feeding apparatus relative to their body size when food resources are scarce, presumably to maximize feeding efficiency (Black et al., 1982; Ebert, 1980, 1996; Ebert and Russell, 1992; Edwards and Ebert, 1991; Levitan, 1991; Rogers-Bennett, 2003; Russell, 1987; Walker, 1981). In addition, both food quantity (Basch and Tegner, 2007; Ebert, 1968; Garrido and Barber, 2001; Meidel and Scheibling, 1999; Minor and Scheibling, 1997; Russell, 1998; Sellem and Guillou, 2007) and quality (Basch and Tegner, 2007; Kawakami et al., 1998; Lyons and Scheibling, 2007; Russell, 1998; Woods et al., 2007) affect the mass and reproductive stage of gonads. Such changes may not only affect a sea urchin's reproductive output, but also its survival and energetic value to predators as the gonads serve as the primary energy storage location (Bottger et al., 2006; King et al., 1994; Russell, 1998; Tomas et al., 2005; Walker, 1981).

Sea urchins also exhibit behavioral and morphological plasticity in response to risk of predation (Freeman, 2006; Kintzing and Butler, 2014; Matassa, 2010; Selden et al., 2009). In the presence of predators, predator cues, or crushed conspecifics, some sea urchins reduce their foraging (Freeman, 2006; Green, 2012; Matassa, 2010), or exhibit a flight response (Kintzing and Butler, 2014). This reduced foraging induced by risk of predation often translates to decreased grazing pressure for those species that sea urchins feed upon (Freeman and Hamer, 2009; Kintzing and Butler, 2014; Long and Hay, 2012). The effect of predator cues on sea urchin physiology, however, has only been examined in one study (Selden et al., 2009), in which predator cues induced thickening of skeletal material and a reduction in growth and gonad material in the green sea urchin *Strongylocentrotus droebachiensis*. Similar results have been found in other invertebrates (Rundle and Bronmark, 2001; Trussel et al., 2006), but no study has

examined the effects of predator cues on the physiology of other sea urchin species, and only one examines the combined effects of predator cues and refuge (Green, 2012).

The importance of substrate composition, texture, and rugosity on intertidal zonation and biogeography is well documented (Clemente and Hernandez, 2008; Holmes et al., 1997; McClanahan, 1994; Raimondi, 1988; Ricketts et al., 1988; Skinner and Coutinho, 2005). For sea urchins in particular, rough or rugose substrates are beneficial for both recruitment (Clemente et al., 2007) and adult attachment (Santos et al., 2005), while sandy bottoms reduce sea urchin movement and grazing (Clemente and Hernandez, 2008). It is thought that some sea urchins can impact their microhabitat by burrowing pits into suitably friable substrate, thus changing the surface area of intertidal habitats (Fewkes, 1890; Grupe, 2006; Hyman, 1955; Otter, 1932) and the diversity and abundance of species within their community (Davidson and Grupe, 2015; Schoppe and Weding, 1996). Hernandez and Russell (2010) showed that these pits impact sea urchin test shape, movement, feeding, and allometry. The fact that sea urchins tend to remain in pits (Grupe, 2006; Hernandez and Russell, 2010) suggests that in the face of frequent and changing wave stress, prime microhabitat is especially valuable.

Like substrate complexity, the role of wave shock in shaping the epibenthos is also well studied (Dayton, 1971; Dayton et al., 1984; Denny, 2006; Fowler-Walker et al., 2006; Madin et al., 2006; Paine and Levin, 1981; Robles et al., 2001; Rodil et al., 2007; Schoch et al., 2006; Tuya et al., 2007). Wave energy is complex and can be dramatically affected by fine scale topography (Denny et al., 2003; Gaylord, 2000; Guichard and Bourget, 1998; Helmuth and Denny, 2003), meaning that a single wave could affect organisms living within the same tidepool differently. Depending on their severity, waves can facilitate or inhibit spawning efforts and settlement (Denny et al., 2002; Denny and Shibata, 1989). Hydrodynamic stress can also render

movement difficult, resulting in reduced predation (Powers and Kittinger, 2002; Silva et al., 2010), and grazing in the intertidal (Kawamata, 1998; Konar, 2000; Konar and Estes, 2003; Lauzon-Guay and Scheibling, 2007a, 2007b; Underwood and Jernakoff, 1984). Further, because the species most often impacted by intertidal wave shock are primarily sessile, they tend to alter morphology to cope with the unpredictable environment (Ferrier and Carpenter, 2009; Fowler-Walker et al., 2006; Rogers-Bennett et al., 1995). Among these studies of wave action and invertebrates, the common theme is that while an organism's hydrodynamic shape is vital to survival, its attachment strength is even more important (Denny and Blanchette, 2000; Denny and Gaylord, 1996; Nicastro et al., 2010; Rilov et al., 2005; Santos and Flammang, 2008; Santos et al., 2005; Tuya et al., 2007) suggesting that dislodgement is one of the greatest risks in the intertidal. In sea urchins, spine morphology (Moureaux and Dubois, 2012; Tuya et al., 2007) and behavior (Kawamata, 1998; Lauzon-Guay and Scheibling, 2007a, 2007b; Nielsen, 2001) are also linked to wave shock. These plastic responses in sea urchins remain understudied with respect to changes in resource allocation and spine regeneration.

Wave shock can damage sea urchins directly by strong impact (Konar, 2000; Konar and Estes, 2003; Lauzon-Guay and Scheibling, 2007a, 2007b) or indirectly by suspended particle collision (Clemente and Hernandez, 2008). The calcite spines covering the test of the sea urchin serve as the primary interface with the environment and predators (Strathman, 1981), and aid in movement (Hyman, 1955), and therefore their repair is important. While the process of spine regeneration has been well established (Ebert, 1967, 1968, 1984, 1986, 1988; 1991), there is some debate as to whether this regeneration is independent of test and reproductive growth. Kawakami (1998) found that a poor quality diet can lead to spine shedding, suggesting a link between nutrition and spine maintenance. Others have found that damaged spines removed from

the test continue to grow in vitro for a short time, suggesting spine repair is independent of test and reproductive growth (Dubois and Ameye, 2001; Heatfield, 1970; Lewis et al., 1990). The only study to experimentally examine the impact of damaging spines found that spine repair resulted in increased calcification, suggesting a negative impact on test growth (Edwards and Ebert, 1991). Despite this finding, the study did not examine effects on reproductive tissue or behavior. It should also be noted that field observations spanning five decades from sites ranging from Alaska to Mexico indicate that level of exposure strongly influences spine abrasion (Ebert et al., 2013). Urchins in areas with greater exposure have spines that are blunted or completely removed, most likely from impacts of suspended material in crashing waves (Ebert, 1968; Ebert et al., 2013).

The purpose of this study was to assess the degree to which plastic physiological and behavioral responses affect the balance of energy and resources required for test and reproductive growth in sea urchins. The purple sea urchin, *Strongylocentrotus purpuratus* is an excellent model species for this experiment as it occupies a large geographic range which presents a variety of substrate types and hydrodynamic forces. This study focuses on smaller sea urchins as they grow faster and produce measureable growth responses more quickly to experimental treatments (Hernandez and Russell, 2010). Small *S. purpuratus* are preferred by some predators (Kenner, 1992; Matassa, 2010) and can reach reproductive maturity as small as 16 mm (Kenner and Lares, 1991).

This experiment used a two factorial (microhabitat and wave exposure) design to examine their influence on: 1) feeding and movement, 2) test growth and allometry, 3) spine regeneration, and 4) reproductive tissue quantity and quality. Each factor had two treatment levels: flat and pit for microhabitat, and high and low for wave exposure.

2. Methods

2.1 Experimental Design

Purple sea urchins were collected from the intertidal zone in Bean Hollow, CA, USA (37° 13'N 122° 24'W) on April 30, 2009, wrapped in wet kelp, and shipped to the lab (Villanova University, Villanova, PA, USA). Smaller sea urchins (≤ 20 mm diameter) are reliably found at Bean Hollow because of predictable recruitment (Ebert and Russell, 1988). These urchins were held in a recirculating seawater system and allowed three weeks to feed and acclimate. Throughout the experiment the temperature and salinity were kept between 12.5-13.5°C and 31-32 (measured daily). Weight, test height and diameter, and height and diameter of the canopy created by the spines were measured for each individual. Six urchins were randomly selected (diameter: $\bar{x}=21.66$ mm $\pm$ 4.18; height: $\bar{x}=10.36$ mm $\pm$ 2.57, weight: $\bar{x}=5.32$ g $\pm$ 1.95; these and all subsequent $\pm$ estimates are standard deviations) to obtain a pre-experimental average number of initial growth-repair cycles or spine rings. From these urchins, five spines were removed and kept for spine analysis (see below).

Sea urchins (n=44) with a test diameter range of 13-23 mm were selected and ranked by size into 11 replicates of 4 individuals. Each individual within a replicate was assigned to one of four treatment groups based on the combination of two fixed factors (spine damage and microhabitat) with two levels each (clipped vs. not clipped, and pit vs. flat, respectively): pit-clipped (PC), pit-no-clip (PNC), flat-clipped (FC), and flat-no-clip (FNC). This sorting method was used to reduce size variation among the four treatments (PC: $\bar{x}=18.10$ mm $\pm$ 2.15; PNC: $\bar{x}=18.18$ mm $\pm$ 2.33; FC: $\bar{x}=18.11$ mm $\pm$ 2.07; FNC: $\bar{x}=18.21$ mm $\pm$ 2.11). Once all individuals were assigned, sea urchins were soaked in the fluorochrome calcein for 24 hours (Haag et al.,

2013). Calcein binds to developing skeletal material, leaving a stain that indicates the size at time of tagging, and is the standard for measuring sea urchin growth (Ebert, 1980; Haag et al., 2013; Russell and Urbaniak, 2004).

Once all individuals were tagged, all spines of PC and FC sea urchins were clipped 3-4 mm above the milled ring, which serves as a muscle attachment point near the base of the spine. This clipping treatment has been previously used to simulate wave exposure (Ebert, 1968; Edwards and Ebert, 1991). The process of removing sea urchins from the sea table for measurement (morphometrics) and clipping was repeated approximately every four weeks from May 25th to November 5th, 2009. All sea urchins were handled similarly regardless of treatment group to remove the effects of being out of the sea table.

Sea urchins were housed in a PVC “suspension system” described in Hernandez and Russell (2010). In this system, each sea urchin is placed on a sandstone block (Fig. 1) which is suspended below the surface of the water in the sea table. Submersible pumps and air stones provided rigorous circulation and aeration around the replicates. The pit treatment blocks each had symmetrical concave cavities ~30 mm in diameter and ~14 mm deep pit drilled in their center (n=22) while the flat treatment blocks were not drilled (n=22, Fig 1). Each sandstone block was fenced with open-top, plastic mesh (10 mm diagonal openings) that extended above the waterline and corralled individuals on the surface of their blocks. Dry weights of the blocks were recorded as well as pit diameters and depths before and after the experiment to determine whether sea urchins had any effect on the blocks.

2.2 Feeding and Movement

Sea urchin feeding was compared using total weight of *M. pyrifera* fed to each sea urchin over the experimental period. Sea urchins were fed 2.5x2.5 cm pieces of kelp (*Macrocystis*

205 *pyrifer*) once a day. This kelp is the preferred food of *S. purpuratus* (Leighton, 1966). During
 206 each feeding, cages were assessed for remaining food and resupplied so that each sea urchin was
 207 left with three pieces of kelp until the next day. For example, if an individual was found to have
 208 no remaining food, three pieces of kelp were weighed and given to that individual. At the time of
 209 feeding, sea urchin position was recorded as a proxy for movement similar to Hernandez and
 210 Russell (2010) with each individual registered as in the same (0) or in a different (1) position
 211 than the previous day. Total 1's for each sea urchin were divided by the total number of days
 212 (145) to obtain a proportion of movement over the experimental period.

2.3 Morphometrics and Allometry

215 Approximately every four weeks, sea urchin height and diameter of both test and spine
 216 canopy were measured and test volume was later estimated using these data (Elliott et al., 2013).
 217 Sea urchins were allowed to drip dry on paper towels for approximately one minute before whole
 218 animal wet weights were recorded. At the conclusion of the experiment, sea urchins were
 219 dissected after final measurement. Dissections began with the extraction of the perivisceral fluid
 220 using a syringe via the peristomal membrane. The animals were then dissected into five body
 221 components: body wall (test, spines, and peristomal membrane), Aristotle's lantern, gut, gut
 222 content, and gonad. Immediately following dissection the wet weights of all body components
 223 were recorded. Body component indices were calculated as the percentage of the total wet
 224 weight (James and Heath, 2008; Russell, 1998). Once the wet weight of the gonad material was
 225 recorded, a 1-2 mm section of one gonadal lobe from each urchin was removed for histological
 226 analyses. These sections were immediately placed in a primary fixative solution for thin
 227 sectioning (3% glutaraldehyde made in a 0.2 M cacodylate buffer). Body wall and lantern

components were then soaked for 24 hours in 5% sodium hypochlorite (chlorine bleach) to remove soft tissue, rinsed in tap water, and air dried for spine ring analysis. Aristotle's lantern demipyramids were examined under UV light for presence of calcein marks which indicate growth since the time of tagging (Johnson et al., 2013; Russell and Urbaniak, 2004).

2.4 Spine Regeneration

The five longest and most well-formed spines from each sea urchin (including the six pre-experimental individuals) were chosen for spine repair cycle analysis. This standard for selection of spines has been used previously (Selden et al., 2009), and limits choosing spines that may have been broken during handling or processing. Spine length, base diameter, and tip diameter were measured using an ocular micrometer on a light microscope. Spine cross sections were prepared according to methods adapted from Ebert (1967, 1968). Each spine was dipped in a solution of 22 parts terpinol, 1 part methyl salicylate and subsequently placed in a vacuum for 2 minutes. This process served to make repair cycles more distinct and easier to quantify by removing echinochrome pigment from the center of the spine.

Thin cross sections of the spines were obtained by mounting them upright on microscope slides with Crystal BondTM glue and grinding them down by hand with sandpaper (grit: 220 then 600). Once spines were about 1 mm in length, the glue was either re-melted or dissolved with acetone, and the spines were flipped upside down and re-mounted. The spine base and milled ring, which exhibit no spine rings, were then ground away. Finally, spines were flipped over once more and ground until cross sections were paper-thin (~100 microns). Each spine was examined under a light microscope and counted the number of complete dark and light cycles, beginning at the center and moving outwards (Fig 2).

2.5 Gonad histology

To examine potential effects of microhabitat and spine repair on gametogenic status, gonad sections from one lobe of each individual were prepared using fixation, rinsing, post-fixation, and dehydration methods according to Bottger et al. (2006). Tissues were then placed in a 1:1 mixture of propylene oxide and EMBED 812® (Electron Microscopy Sciences) embedding medium for an hour at room temperature, and then kept in a 1:2 mixture overnight. Tissues were then submerged in EMBED 812 for 30 minutes before being transferred to capsules and being cured in EMBED 812 for 24 hours at 60 C°.

Blocks were then sectioned using an RMC 6000 ultramicrotome with glass knives at ~500nm until 5 separate testes or ovaries were recovered from each sea urchin. These sections were stained with toluidine blue and viewed under an Olympus EH2 light microscope and photographed for stereological quantification using a Spot Insight QE camera. Using ImageJ software, a grid was placed over each picture (500 px²/sq, or 0.001 mm²/sq). The number of intersecting grid points that fell within the following objects was recorded: spermatogenic cells (SC) and new sperm in males (S), primary oocytes (PO) and mature ova (O) in females, and nutritive phagocytes (NP) and blank luminal space (L) for both sexes (Fig 3).

2.6 Analyses

A 2x2 factorial analysis of variance (ANOVA) was used to compare differences in response variables across the four treatment groups when the data met the parametric assumptions of normality and homogeneity of variance. The response variable data that met these assumptions were: sandstone block measurements (g and mm), feeding rates (number of

pieces of kelp remaining and weight of food delivered in grams), morphometrics (mm), and spine-ring counts. Logarithmic (body component indices) and arcsine transformations (movement data, gonad stereological proportions) were used to meet parametric assumptions.

The change in sandstone block weight (g) and pit size (diameter and depth, mm) was compared among experimental groups as well as against three control flat blocks and three control pit blocks using Kruskal-Wallis ANOVA and one-way ANOVA respectively. Student's T-tests were used to assess the change in H:D ratio of the sea urchin test and to compare sea urchin spine rings of experimental groups with pre-experimental controls.

An ANCOVA was used to analyze sea urchin jaw/test allometry, comparing the volumetric growth across treatment groups with jaw growth as the covariate.

Finally, sex of sea urchins could not be determined until histological preparation. Two of the groups were limited to four males (PC, FNC), four male and four female sea urchins from each treatment group were selected for sex-based ANOVA comparisons. Selection of these individuals was based on the original 11 size groups to minimize variation in test diameter.

3. Results

3.1 Feeding, Movement, and Microhabitat Erosion

Microhabitat was the only factor significantly affecting feeding and movement (Table 1). Sea urchins in pits ingested less total food and moved less frequently than sea urchins on flat blocks (Fig 4). Further, neither factor produced significant changes in the weight of the sandstone blocks ($F_{5, 42}=1.5891$, $p=0.1841$). For pitted blocks, change in pit diameter (mm) and depth (mm) did not significantly differ among treatments nor against controls (diameter: F_4 ,

$_{20}=1.4159$, $p=0.2650$; depth: $F_{4, 20}=0.375$, $p=0.8237$). Therefore blocks were not eroded by grinding of spines or teeth.

3.2 Morphometrics, body component indices, and allometry

Only microhabitat exhibited significant effects on the dimensions of the sea urchin test, with urchins on flat blocks growing faster in diameter, weight, and volume than those in pits (Figs 5, 6). Though microhabitat had no significant effect on test height, these changes still resulted in a change in overall shape, as evidenced by a significant change in the H:D ratio (Table 1). When compared against zero (t-test), sea urchins in pits had significantly positive changes in H:D ratios ($t=4.4960$, $p>t=0.0001$) while those on flat blocks were significantly negative ($t=-3.6483$, $p<t=0.0008$), meaning that sea urchins in pits grew with a more spheroid shape while sea urchins on flat blocks grew in a flatter profile (Fig 6).

The change in diameter of the sea urchin spine canopy (mm) was the only morphometric dimension affected by spine clipping rather than microhabitat (Table 1). By the end of the experiment, the overall growth in diameter of the spine canopy was greater in not clipped urchins than in those that were clipped.

Gonad and Aristotle's lantern were the only body component indices that exhibited significant differences (Table 1). Aristotle's lantern index was greater in the pit microhabitat (Fig 7). Conversely, gonad index was negatively affected by clipping rather than microhabitat type (Fig 7).

Sea urchins in pits exhibited larger jaw growth with respect to test volume growth (Table1), while spine clipping had no effect on jaw growth. Therefore, the ANCOVA analyzing the jaw/test allometry was simplified to compare volumetric growth of all pit sea urchins versus all flat sea urchins, with jaw growth as a covariate (Fig 8). The significant interaction term

between jaw growth and treatment rejects homogeneity of slopes ($F_{3, 38}=8.8929$, $p=0.0050$), meaning sea urchins in pits had greater growth of Aristotle's lanterns relative to body size than sea urchins on flat microhabitats.

3.3 Spines

Average spine repair cycles per sea urchin confirms that cycles form in response to breakage (Ebert, 1967; Edwards and Ebert, 1991). There was a significant effect of spine clipping (Table 1), with clipped sea urchins showing more repair cycles than unclipped (Fig 9). Microhabitat type did not affect formation of spine rings, indicating that repair cycles are not added in response to spine tip abrasion. These results were further supported by the finding that spine base diameter was significantly wider in clipping treatments than in not clipped treatments (Table 1). The t-test comparing spine ring counts of non-clipped groups versus pre-experimental urchins showed no significant difference ($t=0.52$, $p>|t|=0.61$), indicating no effect of monthly handling or living within the "suspension system".

3.4 Gonad Stereology

Stereological examination of gonad histology revealed that treatments had different effects among gonad components and between sexes. In males, microhabitat type affected counts, while in females the results were more complex. Among males, pit sea urchins had lower proportions of spermatogenic columns and new sperm than flat individuals (Figs 10, 11). These low proportions of mature and developing gametes in male pit sea urchins were offset by higher proportions of nutritive phagocytes than male flat sea urchins.

Among females, microhabitat similarly affected primary oocytes (Table 1), with a higher proportion of primary oocytes in flat block than pit individuals (Figs 10, 11) In contrast to males, however, there was an interaction between microhabitat and spine clipping for mature ova with

PC and FNC females exhibiting the fewest mature ova counts. Female nutritive phagocyte production was not significantly affected by either factor.

4. Discussion

Conditions in the rocky intertidal can change over short time and spatial scales and phenotypic plasticity allows individuals to adapt to the stressors of this habitat (Denny, 2006; Ebert, 1996; Ferrier and Carpenter, 2009; Flowers et al., 2002; Fowler-Walker et al., 2006; Kitzes and Denny, 2005; Russell, 1987; Russell, 1998). Changes in resource allocation can drive plasticity (Black et al., 1982; Denny, 2006; Ebert, 1996; Flowers et al., 2002; Nicastro et al., 2010; Russell, 1998; Walker, 1981). The results of this experiment demonstrate that microhabitat and spine repair have important impacts on sea urchin morphology, physiology, and behavior, and therefore could have indirect impacts on the intertidal community.

In the field, these factors are found in a range of combinations as pits vary in size, shape, and orientation, and therefore do not provide a uniform level of protection from waves and/or predators. By simplifying the complexities of microhabitat (pit vs flat) and wave impact (clipped vs not clipped) the direct effects of these factors on resource allocation were quantified. Clipped sea urchins had thicker spines with more repair cycles than unclipped sea urchins, confirming that cycles are added during breakage and repair (Carpenter, 1984; Ebert, 1967, 1984, 1986; Edwards and Ebert, 1991). Despite this direct impact on spine morphology, spine clipping did not affect test growth or any somatic component indices (Figs 6, 7). This is in contrast to what Edwards and Ebert (1991) found, as their clipped sea urchins exhibited increased body wall index and test calcification. Their experiment lasted ten weeks longer (three more clippings) and they did not vary microhabitats. The results here suggest that test growth is not affected by spine regeneration.

Although some have found that sea urchin spines continue to grow in vitro after removal (Dubois and Ameye, 2001; Heatfield, 1970; Lewis et al., 1990) the results here indicate spine repair is not independent of reproductive growth. Spine clipping decreased the relative gonad mass in sea urchins (Fig. 7). This result supports previous findings that indicate that gonad index is higher in sea urchins at sheltered sites (Ebert, 1968; Sellem and Guillou, 2007). Conversely, the stereological counts reveal that the only reproductive component affected by spine clipping was female mature oocytes (an interaction effect with microhabitat, Fig. 10). This is not entirely surprising, however, as Iliffe and Pearse (1982) and Meidel and Scheibling (1998) found that gonad quantity (GI) is not always indicative of quality (stereological counts). Therefore, the results suggest that sea urchins with damaged spines are able to reallocate resources to aid in spine repair without too much disruption to gametogenesis. Viewed in the context of wave shock, sea urchin populations in areas of high wave exposure could have reduced reproductive potential.

Despite the effect of spine repair on gonad index, the majority of the results presented suggest that microhabitat is the most important factor affecting resource allocation and life history. The most obvious results of living in a pit were the effect on allometry (Fig 8), and overall growth (Figs 5, 6, and 8). Sea urchins in pits grew more spheroid in shape while those on flat blocks became more vertically compressed (Fig 6). These changes in H:D ratio reflect the effects of microhabitat and may be driven by tube-feet-attachment area (Hernandez and Russell, 2010), which is perhaps the most important adaptation in the intertidal (Denny and Blanchette, 2000; Gianguzza et al., 2010; Hernandez and Russell, 2010; Santos and Flammang, 2008; Santos et al., 2005; Tuya et al., 2007). Without a secure connection to the substrate, intertidal organisms risk dislodgement and death (Denny and Blanchette, 2000; Tuya et al., 2007). For sea urchins,

the increased attachment area of pits can also provide refuge from predators (Clemente et al., 2013; Gianguzza et al., 2010)

Perhaps more significant is the fact that pits negatively affected movement and food consumption (Fig 4). These results support findings that when given the option, sea urchins will remain in pits (Grupe, 2006; Hernandez and Russell, 2010; Hyman, 1955). In this study, even when suffering severe spine damage and reduced feeding ability, urchins remained in their pits, rather than leaving to seek alternate shelter or facilitate feeding (Fig 4). Consumption data support the findings of Hernandez and Russell (2010) that remaining in pits makes it more difficult for individuals to deliver food from the aboral to oral region (Fig 4). Despite this difficulty, microhabitat did not affect the growth in diameter of the spine canopy (Table 1) and urchins in pits were observed pointing their spines upward, out of the pit. This continued spine growth likely further limits the ability to deliver food to the mouth when inhabiting a pit. Also, pit sea urchins allocated more energy toward jaw growth than volumetric growth (Fig 8) and exhibited greater Aristotle's lantern index values than sea urchins in the flat microhabitat (Fig 7) a trend exhibited by food limited sea urchins in several studies (Black et al., 1982; Ebert, 1980, 1996; Ebert and Russell, 1992; Edwards and Ebert, 1991; Levitan, 1991; Minor and Scheibling, 1997; Walker, 1981). In addition, Ebert (1996) found that well-fed *S. purpuratus* with relatively small lanterns had similar survival rates to individuals with limited food and larger lanterns. That such an adaptation increases the survival of poorly-fed individuals is interesting in light of the results of this study: if sea urchins show a preference for pits despite the feeding difficulties such microhabitats present, then the cost of dislodgment must be higher than the cost of food limitation. Further, if sea urchins remain in pits regardless of spine damage and by proxy, wave

shock, then it is possible that in providing refugia for sea urchins, friable substrates may indirectly provide relief of grazing pressure for primary producers in the intertidal.

The results of the stereological analysis further emphasize the importance of finding suitable microhabitat. Presumably due to the decrease in feeding, both males and females exhibited altered gametogenesis as a result of living in pits (Figs 10, 11). Males in pits exhibited high NP counts and low spermatogenic column (primary sperm) and spermatocyte counts (mature sperm), indicating that living on a flat block facilitates the transfer of nutrition from somatic reserve cells (NP's) to reproductive cells. Female sea urchins in pits exhibit a similar trend of increased NP counts, but not significantly. Primary oocyte counts were found to be significantly higher in flat block individuals, indicating the same tradeoff of nutrition for reproductive growth. The effect of microhabitat on sea urchin gametogenesis could have indirect effects on their predators. Because removing sea urchins from their pits can be difficult (Clemente et al., 2013) the cost of extracting a sea urchin from a pit may not exceed the benefit of reduced gonad quality.

The plastic responses of the purple sea urchin to the factors examined here are accordant with its life history strategy (Flowers et al., 2002). Long lived organisms like sea urchins tend to place emphasis on growth and repair, spreading reproductive efforts over multiple spawning seasons (Flowers et al., 2002). It should be considered, though, that because *S. purpuratus* has a large geographic range, it also inhabits a wide variety of substrate types and experiences a variety of wave forces. Therefore, *S. purpuratus* in areas of highly friable substrate and low wave exposure likely have different feeding behaviors and reproductive efforts than those in hard bottom, highly exposed sites. In addition, the higher risk of predation at sheltered sites (Clark, 1997; Menge, 1978) could intensify an urchin's preference for pits, thus further reducing

foraging (Green, 2012; Matassa, 2010). The results of this experiment suggest that spine damage negatively impacts relative gonad mass and the production of mature oocytes, which in addition to reproductive output, could also reduce sea urchins' caloric value to predators, potentially negatively impacting local food chains (Trussel et al., 2006). Therefore, by modulating the behavior and physiology of sea urchins, substrate and spine damage likely have vital indirect effects on intertidal communities.

This experiment was designed to examine the effects of wave exposure (simulated by spine clipping) and microhabitat on somatic resource allocation. Several caveats should be noted when interpreting the results and/or using similar methods in future studies. First, sea urchin roe maturation is affected by light cycles (James et al., 2007; James and Heath, 2008; Shpigel et al., 2004; Spirlet et al., 2000). Because the intent at the outset of the experiment was to measure only gonad indices and not stereology, sea urchins were not subjected to standardized periods of light and dark. In future experiments of this nature, it would be prudent to synchronize the light cycle to field conditions. Second, the number of experimental individuals (n=44) was selected for analysis of test growth and GI, and limited by available space within the sea table. Because the sex of individuals was not known before assigning them to groups, comparisons were limited to four males/females per treatment. Therefore, in future reproductions, the number of replicates should be increased.

5. Conclusions

This study supports findings that sea urchin spine repair does not impact test growth. Conversely, gonad index results suggest spine repair is not completely independent of reproductive growth. Overall it would appear that microhabitat is the primary factor influencing

sea urchin resource allocation and ultimately, life history. When pit microhabitats are available, purple sea urchins tend to occupy and remain in these cavities. This behavior limits their ability to feed, which reduces gonad quality as measured by stereological counts. This preference suggests it is beneficial for the long-lived sea urchin to prioritize attachment over reproduction. Finally, the factors examined here likely have important cascading impacts on the intertidal community and should be considered in future studies of sea urchin life history, intertidal grazing pressures, and predator/prey interactions.

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List of Tables and Figures with captions:

- I. Table 1. Two way ANOVA results for the effect of microhabitat type (pit vs flat) and monthly spine clipping treatment (clip vs not clipped) on sea urchin feeding, movement, morphometric growth, body component indices, spine regeneration, and gonad stereological proportions. Statistically significant values are in bold.
- II. Figure 1. Examples of sandstone blocks (55 mm x 55 mm x 26 mm) on which sea urchins were housed. The Pit treatments (left) had symmetrical concave cavities $\sim 28.11 \pm 1.48$ mm in diameter and 14.36 ± 1.57 mm deep drilled into the center-surface. Flat blocks were not drilled. All blocks were fenced in with plastic mesh but left open at the top.
- III. Figure 2. Example micrographs of *S. purpuratus* spine cross sections above the milled ring showing breakage-repair cycles and the potential variation between individuals. Spines taken from two different ~ 37 mm (test diameter) urchins from tide pools in Oregon A) Sunset Bay (sheltered site - 2 complete cycles) and B) Gregory Point (exposed site - 6 complete cycles). Scale bar is 1.5 mm. These micrographs are similar to those for sea urchins from the experimental non-clipped and clipped groups, respectively.
- IV. Figure 3. Plastic sections of mature *S. purpuratus* gonad histology (from replicates in 6 month lab experiment, ~ 30 mm test diameter). This female gonad (A), from an unclipped sea urchin living on a flat block, has limited primary oocytes (PO) lining the ovarian wall. The dark spots are the nutrient phagocyte (NP) energy reserves and have been largely depleted to enhance growth of mature ova (O). The testicular wall in the male sample (B), taken from an unclipped sea urchin living on a flat block, is lined with spermatogenic columns (SC) which will migrate toward the central lumen and become new sperm (S). Nutritive phagocytes (NP) have also been depleted in the male to facilitate gametogenesis. Blank luminal space is indicated by L.
- V. Figure 4. Total food eaten (g) and total number of position shifts. Sea urchins were assigned to one of four groups based on two levels each of microhabitat (Pit=P, Flat=F) and clipping treatments (Clipped=C, Not Clipped=NC), (n=11 each, 2 mortalities—1xPC and 1xFNC). Vertical edges of boxes are the 25th and 75th percentiles, the lines extending from the boxes are the 5th and 95th percentiles, and the horizontal lines in the boxes are the medians. Letters indicate significantly different groups. Sea urchins in microhabitat pits preferred to remain within pits, and ate less than those on flat microhabitat.
- VI. Figure 5. Box plots of change in sea urchin weight and volume in response to microhabitat pit refuges and clipping treatments (Pit=P, Flat=F; Clipped=C, Not Clipped=NC), (n=11 each, 2 mortalities—1xPC and 1xFNC). Vertical edges of boxes are the 25th and 75th percentiles, the lines extending from the boxes are the 5th and 95th percentiles, and the horizontal lines in the boxes are the medians. Letters indicate significantly different groups. Urchins on flat blocks grew more in both weight and volume.
- VII. Figure 6. Relative change in sea urchin height to diameter ratios (H:D). Sea urchins were assigned to one of four groups based on two factors (microhabitat and clipping) with two

levels each (Pit=P, Flat=F; Clipped=C, Not Clipped=NC), (n=11 each, 2 mortalities–1xPC and 1xFNC). Vertical edges of boxes are the 25th and 75th percentiles, the lines extending from the boxes are the 5th and 95th percentiles, and the horizontal lines in the boxes are the medians. Dashed line is placed at zero. Positive values indicate greater growth in height, while negative values indicate greater growth in diameter. Letters indicate significantly different groups.

VIII. Figure 7. Box plots of sea urchin lantern and gonad index data. Indices were calculated by dividing wet weight of components by an urchin's total wet weight. Sea urchins were assigned to one of four groups based on two factors (microhabitat and clipping) with two levels each (Pit=P, Flat=F; Clipped=C, Not Clipped=NC), (n=11 each, 2 mortalities–1xPC and 1xFNC). Vertical edges of boxes are the 25th and 75th percentiles, the lines extending from the boxes are the 5th and 95th percentiles, and the horizontal lines in the boxes are the medians. Letters indicate significantly different groups. The Aristotle's Lantern (feeding apparatus) was relatively larger in urchins in pits, indicating a reduction in food consumption. Gonad index was reduced by spine clipping, suggesting that spine growth is not entirely independent of reproductive growth.

IX. Figure 8. Allometric relationship between sea urchin test volumetric growth and jaw growth. With no significant effect of spine clipping, sea urchin allometric growth was compared between microhabitat groups. An ANCOVA of volumetric growth with jaw growth as a covariate revealed significantly different slopes between microhabitat treatments ($F_{3, 38}=8.8929$, $p=0.005$). Circles and dashed line represent pit urchins; triangles and solid line represent flat urchins.

X. Figure 9. Effects of spine clipping and microhabitat on sea urchin spine regeneration cycles. Average numbers of spine rings per sea urchin (mean $\pm$ SE) in four groups based on two factors (microhabitat and clipping) with two levels each (Pit=P, Flat=F; Clipped=C, Not Clipped=NC), (n=11 each, 2 mortalities–1xPC and 1xFNC) as well as pre-experimental controls (n=6). Letters indicate significantly different groups.

XI. Figure 10. Box plots of gonad stereological proportion data from male (left) and female (right) *S. purpuratus*. In both sexes "Blank" indicates empty space within each testis or ovary. Sea urchins were assigned to one of four groups based on two factors with two levels each (Pit=P, Flat=F; Clipped=C, Not Clipped=NC), (n=11 each, 2 mortalities–1xPC and 1xFNC). Vertical edges of boxes are the 25th and 75th percentiles, the lines extending from the boxes are the 5th and 95th percentiles, and the horizontal lines in the boxes are the medians. Letters indicate significantly different groups and absence indicates no significant differences. Microhabitat reduced the relative amount of spermatogenic columns and mature sperm while increasing nutritive phagocytes in males. In females, microhabitat only reduced primary oocytes. Spine clipping only affected mature ova, but also interacted with microhabitat.

XII. Figure 11. Plastic sections of mature gonads from *S. purpuratus* males (A-D) and females (E-H). Photos are in order of treatment groups (A, E = PC; B, F = PNC; C, G = FC; D, H = FNC). Males in the pit groups (A and B) show decreased spermatogenic columns and new sperm, but increased nutritive phagocytes. Females gonads (E-H) showed some similar results, with counts of primary oocytes in females negatively affected by the pit microhabitat treatment (E), but PNC and FC treatments showed an increase in ova (F and G). Scale bar (in H) for all photos = 0.2mm.

Variable	Source	Df	F Ratio	P value
Feeding (total pieces of kelp)	Pit	3,38	5.1652	0.0288
	Clip		0.6283	0.4329
	Pit x clip		0.0938	0.7611
Movement (movements/ recordings)	Pit	3,38	251.7376	<0.0001
	Clip		0.9653	0.3321
	Pit x clip		0.2289	0.6351
Growth in diameter (mm)	Pit	3,38	22.8119	<0.0001
	Clip		0.0156	0.9014
	Pit x clip		0.016	0.8999
Change in H:D	Pit	3,38	31.6724	<0.0001
	Clip		0.4632	0.5003
	Pit x clip		0.2137	0.6465
Weight change (g)	Pit	3,38	11.0727	0.0020
	Clip		0.0001	0.9944
	Pit x clip		0.3720	0.5455
Volumetric growth (cm ³)	Pit	3,38	8.7739	0.0052
	Clip		0.1937	0.6623
	Pit x clip		0.2201	0.6416
Growth in diameter of the spine canopy (mm)	Pit	3,38	2.1966	0.1466
	Clip		35.4919	<0.0001
	Pit x clip		0.5037	0.4822
Body Wall Index (body wall and peristomal membrane wet weight/total wet weight)	Pit	3,38	0.093	0.7621
	Clip		1.6196	0.2109
	Pit x clip		2.1334	0.3770
PVF Index (Pervisceral fluid weight/total wet weight)	Pit	3,38	0.0110	0.9169
	Clip		0.2123	0.6476
	Pit x clip		0.9550	0.3346
Lantern Index (lantern wet weight/total wet weight)	Pit	3,38	14.6068	0.0005
	Clip		0.5723	0.4540
	Pit x clip		0.7991	0.3770
Gonad Index (gonad wet weight/total wet weight)	Pit	3,38	1.6103	0.2122
	Clip		4.9230	0.0325
	Pit x clip		0.0995	0.7542
Gut Index (gut wet weight/total wet weight)	Pit	3,38	1.9979	0.1657
	Clip		0.0001	0.9919
	Pit x clip		1.2293	0.2745
Gut Content Index (gut content wet weight/total wet weight)	Pit	3,38	0.0252	0.8746
	Clip		0.4727	0.4959
	Pit x clip		0.7916	0.3792
Jaw Growth (mm)	Pit	3,38	4.2581	0.0459
	Clip		0.2244	0.6284
	Pit x clip		0.8512	0.3620
Spine Rings	Pit	3,38	0.5011	0.4833
	Clip		67.49222	<0.0001
	Pit x clip		0.0252	0.8747
Spine Base Diameter (mm)	Pit	3,38	0.4284	0.5167
	Clip		4.9197	0.0326
	Pit x clip		0.2515	0.6189
Spermatogenic Columns	Pit	3,76	18.8041	<0.0001
	Clip		0.0102	0.9200
	Pit x clip		1.6264	0.2061
New Sperm	Pit	3,76	12.5867	0.0007
	Clip		0.1530	0.6967
	Pit x clip		0.1868	0.6668

Male Nutritive Phagocytes	Pit	3,76	34.9152	<0.0001
	Clip		0.2858	0.5945
	Pit x clip		0.6939	0.4075
Primary Oocytes	Pit	3, 76	8.6404	0.0044
	Clip		0.9242	0.3394
	Pit x clip		0.1517	0.6980
Mature Oocytes	Pit	3,76	1.1703	0.2828
	Clip		0.3493	0.5563
	Pit x clip		10.3457	0.0019

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832 Figure 1

Figure 2

836 Figure 3

Figure 4

Figure 5

Figure 6

Figure 7

846 Figure 8

848 Figure 9

Figure 10

Figure 11