University of Alberta

Plastic phenotypic responses of the sea star *Pisaster ochraceus* to spatial and temporal variation in wave exposure

by

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A thesis submitted to the Faculty of Graduate Studies and Research

in partial fulfillment of the requirements for the degree of

Master of Science

Department of Biological Sciences

Fall 2011

Edmonton, Alberta

The Artist is he who detects and applies the law from observation of the works of Genius, whether of man or Nature. The Artisan is he who merely applies the rules which others have detected.

- Henry David Thoreau

ABSTRACT

The intertidal sea star *Pisaster ochraceus* lives in a wide range of wave-exposure conditions in the northeastern Pacific. Its body form and tube foot tenacity are both highly correlated with wave exposure. On wave-exposed shores sea stars attach to the substrate with higher tenacity, weigh significantly less per unit arm length, and have a narrower body and arms. Water velocity explained over 99% of the variation in average body shape and 92% of the variation in average tenacity. Reciprocal transplants revealed that both traits responded plastically to changes in wave exposure, so the variation is therefore primarily ecophenotypic. This plasticity is likely also adaptive, allowing *Pisaster* to respond to variation in flow in both space and time. Both phenotypic responses probably act to reduce dislodgement risk – changes in tenacity influence attachment force and changes in body form modify the drag and lift experienced, which are the main effectors of dislodgement.

ACKNOWLEDGEMENTS

These past three years have been an amazing experience and one that would not have been possible without the support and guidance of many friends, my family, colleagues and mentors.

First off I would like to thank my supervisor Rich Palmer for the guidance and knowledge he passed on to me throughout my thesis. I also owe him many thanks for providing the initial spark and introducing me to the allure of marine invertebrates during my undergraduate. One of his greatest assets is an ability to inspire curiosity, a curiosity he has fostered within me and many others.

Thanks to my committee members, Dr. Sally Leys and Dr. Lindsey Leighton for providing guidance and help with the formulation of this thesis. I would also like to thank the support staff at the University of Alberta who made my life much easier during this degree.

Ron Koss and Gerry Hilchie ran a great lab; thanks to them, my fellow TAs and my students for giving me an incredible teaching experience.

The Bamfield Marine Sciences Centre deserves many thanks and praise. The staff were amazing facilitators and would lend a helping hand whenever needed. Everyone at the centre and the rest of the town were inspiring and provided me a great community in which to live and learn, some of my fondest memories will remain in Bamfield.

Many thanks to everyone who helped with my field work, as this project was quite field-intensive it required a lot of help and there were many who readily and graciously lent a hand. In particular need of thanks are my field assistants Katie Gale and Emy Montgomery who endured many early mornings and late nights in the field without much of a complaint. Thanks also to Dr. Chris Neufeld who risked life and limb to pick us up from Seppings Island in a snowstorm and large December swell. Dane Stabel helped out tremendously with data collection for the second trial for PIT tag retention (Chapter 2). Thanks to everyone else who helped out in some way or form including Tao Eastham, Carly Janusson, Tetsuto Miyashita, Marjan Berezandeh, Dr. Brad Anholt, Beth Rogers, Dr. Dave Riddell, Kelley Bartlett, Sarah Tyne, Shirley Pakula, Bob Baden, Kaal Haugen, John Richards, Janice Pierce, Siobhan Gray, Cliff Haylock, Rick MacLeod and all of the kitchen and housekeeping staff at BMSC.

I would like to thank Tracy MacKeracher who has provided an immeasurable amount of support and help. Thanks so much for all of the time helping with field work, particularly with the multiple camping trips out to the islands in the cold and snow to collect my transplants. Also, thanks for all of the help with writing and providing support when I needed it most.

Finally, I would also like to thank my family and friends for lending me support over the last three years. The life of a master’s student is not particularly “normal”, and all of you understood and helped me out when I was being particularly abnormal. Thanks so much to my parents who have always been supportive and encouraging of anything I do. By letting me explore you gave me an interest of all things living and set me on my path.

This study was supported by a NSERC CGS-M, a Queen Elizabeth II graduate scholarship and a Graduate Research Assistantship to K.H. and a Natural Sciences and Engineering Research Council of Canada operating grant A7245 to my supervisor Dr. A. R. Palmer.

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CHAPTER 1

1. General Introduction

> Pisaster *neither has nor seems to need protective coloration. Anything that can damage this thoroughly tough animal, short of the "acts of God" referred to in insurance policies, deserves respectful mention. To detach a specimen from the rocks one heaves more or less mightily on a small crowbar, necessarily sacrificing a good many of the animal's tube feet but doing it no permanent injury thereby, since it will soon grow others to replace the loss. The detached tube feet continue to cling to the rock for an indefinite period.*
>
> -*Ed Ricketts, "Between Pacific Tides"*

West side of Seppings Island – Photo by K. Gale

1.0 Habitat: The Wave-Swept Intertidal Shore

The exposed headlands of Seppings Island in Barkley Sound, British Columbia are dominated by vast beds of mussels. These sessile animals fasten themselves to the rock using multitudes of byssal threads and gain support against the onslaught of the crashing waves from their tightly packed neighbors. Sea stars, *Pisaster ochraceus* – seemingly out of place – dot these beds and despite a lack of support by their compatriots, are also able to withstand the maelstrom. The wave swept intertidal shore can be one of the most stressful environments on Earth (Denny, 1988). When the tide recedes, intertidal organisms are faced with the rigors of terrestrial life as they are exposed to extreme heat and desiccation stress. As the tide rises, these organisms can experience some of the most stressful flow conditions on Earth; drag and lift forces imposed by waves are significant, as water velocities can exceed 15 ms^{-1} (Helmuth and Denny, 2003). On the other side of Seppings island – only 300 meters away – *Pisaster* also thrive in a sheltered cove where, in contrast to the exposed headlands, a small child could safely swim. How is *Pisaster* able to live and thrive over such a wide range of exposure conditions?

Intertidal shores, in spite of their extreme stresses, are home to a diverse assemblage of animals and seaweeds. In fact these extreme physical stresses of flow and desiccation are responsible for shaping intertidal species assemblages and their interactions. At low tide, a striking pattern commonly seen is zonation, which refers to the predictable vertical banding of intertidal species based on predation, competition for space and interspecific differences in tolerance to desiccation and flow (Colman, 1933; Paine, 1966). In the northeast Pacific, barnacles are the most heat and desiccation-resistant animals, and thus form the highest band (Foster, 1971a). Mussels occupy an area just below the barnacles; although they are better space competitors, they are less resistant to desiccation than barnacles. The common intertidal sea star *Pisaster ochraceus* eats both barnacles and mussels, and thus enforces a lower boundary for the mussels, which form the lowest band (Paine, 1969). Although a common and formidable predator on many intertidal species, *Pisaster* is restricted to the lower intertidal zone as this sea star is less resistant to flow and desiccation than its higher-intertidal prey (Paine, 1966; Menge, 1978). The importance of flow and temperature in shaping intertidal zonation patterns is obvious when one compares a sheltered shore to a rocky headland. On an exposed headland, the bands are shifted upward, and mussel beds tend to occupy a larger area (Quinn, 1979). The shift in banding is due to both 1) the cooling effects of wave splash in the high-intertidal zone (Harley and Helmuth, 2003), and 2) the increased flow, which constrains *Pisaster* to the lowest areas of the intertidal zone and frees the mussels on the upper shore from predation (Robles et al., 2009). Besides these dominant organisms there is also a very different animal and seaweed assemblage in the two environments. In general, wave-exposed shores are home to more robust species.

1.1 Functional Morphology and Adaptation to Flow

The distribution and abundance of organisms on intertidal shores is ultimately determined by, or at least dependent upon, the functional morphology of the resident species (Denny, 1994; Koehl, 1996). In addition to being variable spatially, intertidal shores show great temporal variation in water flow and heat stress. Adaptation to these temporal changes is likely important for sessile intertidal organisms such as barnacles and mussels, which cannot accommodate change through movement. Within a species, barnacles show many different plastic responses to changes in flow – in high flow, barnacles have more flow-resistant penises (Neufeld and Palmer, 2008) and feeding legs (Arsenault et al., 2001). Mussels show differences in shell shape (Akester and Martel, 2000) and byssal thread strength (Carrington, 2002). Although sea stars may be able to compensate for changes through movement, their ultimate distribution and abundance depends upon their ability to adapt to changes in abiotic factors. Plastic responses to variation in flow have not been described for intertidal sea stars or, for that matter, any echinoderm.

Phenotypic plasticity is a broad term that is used to refer to any modification of an organism's phenotype in response to a change in the environment (Whitman and Agrawal, 2009). The term includes both changes that confer a selective advantage (adaptive plasticity) and those that offer no benefit (non-adaptive plasticity), as well as both reversible and non-reversible changes. A trait that is plastic, or synonymously ecophenotypic, is simply a trait that is determined, at least in part, by mechanisms of phenotypic plasticity. Whether a trait is considered to be genetically or environmentally controlled is also ambiguous because the ability of an organism to express variable phenotypes in response to variable conditions (an organism's reaction norm) is itself genetically determined. It is partly this genetic determination that makes plasticity such an important evolutionary process. It allows for natural selection upon the genetic machinery of plasticity, allowing for both directional evolution of reaction norms and the selection for either a broadening or restriction of the available range of plastic responses (Pigliucci et al., 2006). Complete restriction (genetic assimilation) can result in a constitutively stable trait that no longer responds to environmental perturbations (Waddington, 1942).

1.2 Study Species: *Pisaster ochraceus*

In the northeast Pacific, the dominant intertidal sea star is *Pisaster ochraceus*, which forms dense aggregations on both exposed headlands and sheltered shores. Due to their ability to structure and diversify intertidal communities by removing mussels, they were dubbed the original keystone predator (Paine, 1969). Their distribution across a wide variety of flow conditions and their long planktonic period of up to 32 weeks (Strathmann, 1978) make them an ideal candidate to study adaptations to life under different flow conditions. Although *Pisaster* exhibits high levels of dispersal, local adaptation is not necessarily achieved through plasticity. Phenotypic differences could also arise due to genetic differentiation caused by either selective settlement or differential mortality.

Pisaster's vertical distribution is influenced by vulnerability to dislodgement by waves, so it might be expected to have adaptations that reduce this risk. Dislodgement risk is typically much higher in wave-exposed sites because drag and lift, which increase exponentially with velocity (Denny, 1988), become relatively higher with respect to attachment forces. Common adaptations to minimize dislodgement risk are: 1) reducing wave-induced forces by changing shape or reducing size, and 2) increasing attachment forces to the substrate (Trussell, 1997; Akester and Martel, 2000; Arsenault et al., 2001; Carrington, 2002).

1.3 Thesis Objectives

The purpose of this research was to examine the aspects of the functional morphology of *Pisaster ochraceus* that enable it to occupy such a broad range of wave-exposure conditions and to determine whether any differences were a result of phenotypic plasticity. Differences in both tube foot adhesion and overall body form were studied. For both I determined whether there were differences among different flow regimes in Barkley Sound, British Columbia, discuss the adaptive significance of both sheltered and exposed morphs, and experimentally tested whether differences were due to phenotypic plasticity by performing reciprocal transplants of sea stars between sheltered and exposed sites.

1.4 Thesis Structure

This thesis is composed of five chapters: this introductory chapter (Chapter 1), a chapter examining the effectiveness of the tagging technique used for tagging *Pisaster ochraceus* (Chapter 2), separate chapters examining plasticity of tube foot attachment tenacity and overall body form (Chapters 3 and 4, respectively) and a general conclusion chapter (Chapter 5).

Chapter 2. Testing for phenotypic plasticity in tube foot tenacity required a reliable means of uniquely tagging individual *Pisaster* to conduct reciprocal transplants between low-flow (sheltered) and high-flow (wave-exposed) sites. In Chapter 2, the first data chapter of my thesis, I compare the effectiveness of two methods for tagging *Pisaster*. Specifically, I compare the efficiency of PIT (Passive Inductive Transponder) tag implantation to that of a visual tagging technique employing neutral red that has, despite its effectiveness, not been widely used, (Feder, 1955; Carlson and Pfister, 1999). By double-tagging *Pisaster* with both PIT tags and neutral red, I showed that PIT tags were poorly retained and caused harm to implanted sea stars, while neutral red seemed to be favourable as dye loss was negligible over three months and no harm to sea stars was observed.

Chapter 3. Differences in *Pisaster* body form were documented across a wave-exposure gradient in Barkley Sound, British Columbia, Canada. A precise relationship between body form (aspect ratio) and water velocity suggests that variation in overall body form is adaptive and probably environmentally determined by wave exposure. Exposed sea stars were much lighter per unit arm length and likely experienced reduced drag and lift. Reciprocal transplants suggest that body shape variation is primarily ecophenotypic, as shape changes in response to differences in water flow.

Chapter 4. Differential tenacity of *Pisaster* tube feet was explored across a wave-exposure gradient in Barkley Sound. A precise positive relationship between water velocity and tenacity suggests that tube foot tenacity is adaptive and environmentally determined. Using reciprocal transplants I demonstrated that this relationship is likely due to phenotypic plasticity in tube foot adhesion tenacity.

In chapter 5, I combine and discuss the findings of the experimental chapters, integrating their findings into larger theory and discuss possible avenues of future study.

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CHAPTER 2

Comparison of the effectiveness of PIT tags and a vital stain for individually tagging the sea star *Pisaster ochraceus* (Brandt)[*]

2.0 Introduction

Individual monitoring of animals *in situ* provides a powerful tool for estimating many important life-history traits under natural conditions, including growth rates, survivorship and migration patterns (Woodbury, 1956; Pradel, 1996; Lamare et al., 2009). However, to monitor individual animals in the field effectively, both the tags and the tagging process must not harm the study organism or alter its behaviour in any way, and the tags must be retained at an appreciable rate. Currently, one of the most widely used methods for tagging small bodied organisms are PIT (passive integrated transponder) tags, which are implanted inside an animal’s body (Gibbons and Andrews, 2004). When read with a handheld reader the tags display a unique 13 digit alpha-numeric code. They are popular because they are small, durable, submersible and encapsulated in glass (which reduces tissue irritation). PIT tags have shown merit as a method of tagging several small marine invertebrates including bivalves (Hamilton and Connell, 2009), crayfish (Bubb et al., 2002) and rock lobster (Barrett et al., 2009).

The use of PIT tags in echinoderms, however, has been problematic. PIT tagging had deleterious effects in field studies of both sea urchins (Lauzon-Guay and Scheibling, 2008) and sea cucumbers (Purcell *et al.*, 2008) and the sea cucumbers also displayed poor tag retention. Other proposed methods for tagging

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[*] To be submitted to *Marine Ecology Progress Series,* with author K. Hayne.

echinoderms include branding (Kurihara, 1998) and staining with vital stains (Loosanoff, 1937; Vernon, 1937; Feder, 1955). These methods employ visual tags that can be applied *in situ,* but may be prone to degradation and loss over time (Kurihara, 1998).

Development of an effective tagging method for asteroids is quite important because predatory sea stars can play important roles in structuring marine ecosystems (Menge et al., 1994). This is particularly true of *Pisaster ochraceus*, which is an ubiquitous keystone predator on intertidal shores of the northeast Pacific (Paine, 1969; Paine, 1976). *P. ochraceus* has been extensively studied but because of a lack of an adequate tagging method, little work has been possible at the individual level.

I therefore tested the effectiveness of two tagging methods on *Pisaster ochraceus*: 1) internally implanted PIT tags and 2) dying with the vital stain neutral red to create an externally readable tag on the aboral surface. By doubly tagging individuals using both methods I was able to directly compare their effectiveness and determine which method is more suitable. Also, because vital stains can cause detrimental effects on animals, its effect on *Pisaster* was explored by comparing righting times of dyed and undyed individuals.

2.1 Methods

2.1.1 Collection and PIT Implantation

On September 25, 2010 and February 24, 2011, *Pisaster ochraceus* (N= 100) were haphazardly collected at low tide from Scott’s Bay, Barkley Sound

(48°50’03.16 N 125°05’50.25 W) near Bamfield, British Columbia, Canada. The *Pisaster* were transported to the Bamfield Marine Science Centre where they were placed in water tables connected to a flow-through sea water system. They were then weighed and photographed, and a PIT tag (12 mm Trovan ID100A transponder) was inserted through a small incision made on the side of the arm (Figure 2-1B). The incision, approximately 15-20 mm, was made through a suture in the ossicles near the base of the arm just above the oral surface (Carlos Robles, pers. comm.). Prior to insertion, each tag was placed in a small pouch made from un-dyed, fine-mesh polyester which was heat sealed along all edges (Robles, pers. comm.). This was done because bare tags were readily rejected, often within hours (pers. obs.).

2.1.2 Tagging Using Neutral Red

Following insertion of a PIT tag, each star was given a unique external identification by applying neutral red to create arm bands (Figure 2-1A). The neutral red was mixed with seawater to create a 0.2 g mL^{-1} slurry, and a liberal amount was applied to the aboral surface of the arms. The tagged *P. ochraceus* were left out of water for 15 minutes to allow the stain to set and each individual’s arm code and corresponding PIT tag code was recorded. All individuals were allowed to heal for at least 24 hours and the presence of their PIT tags was verified using the handheld reader before returning them to the field. Stars were returned to Scott’s Bay by placing them on rocks nearby other non-transplanted *Pisaster* at low tide. PIT tag loss was monitored after seven, fourteen and thirty days elapsed. Tagged *P. ochraceus* from the fall trial were also examined after five months. To do this they were identified *in situ* based on the arrangement of their neutral red arm bands and their PIT tag ID. In the case where a PIT tag had been rejected or was unreadable, the arm band code of that individual was recorded and the individual was considered to have lost its PIT tag. No tagged *Pisaster* were removed from the site during the experiment. PIT tag rejection rates were compared between fall and spring trials using a Fisher's exact test in SPSS Ver. 19.

2.1.3 Effect of Neutral Red

The effect of neutral red tagging on the well-being of *Pisaster ochraceus* was explored by comparing activity coefficients of stained and unstained individuals. Activity coefficients are a measurement of an individual's well-being; they are calculated by dividing the righting time (in seconds) by 1000 (Percy, 1973; Lawrence and Cowell, 1996). Righting time was recorded when all arms first attached to the bottom of the water table. Fifty *Pisaster* were collected from Seppings Island, Barkley Sound (48°50'25.92"N, 125°12'28.67"W). Half of the individuals were banded with neutral red, while the other half were left unstained. Righting times of stained and unstained *Pisaster* were tested simultaneously: 1) before staining, 2) seven days after staining, and 3) 30 days after staining. Sea stars were held in the laboratory in running-seawater aquaria and righting times were measured in a single water table in order to reduce tank effects. Activity coefficients were compared between treatments using an ANOVA in SPSS Ver.19.

2.2 Results

2.2.1 PIT Tagging

No tag loss or any other negative effects were observed during the recovery period in the laboratory following PIT tag implantation and all individuals healed before transplantation back to the field. Despite this, 35 percent of individuals lost their PIT tag after seven days in the field in the fall trial (Sept. 2010), and 25 percent lost their tags over the same time period in the spring trial (Feb. 2011) (Figure 2-2). In the fall 17 *Pisaster* were recovered, six of which lost their tags; in the spring 40 were recovered, eight with lost tags. There was no significant difference between the two trials after seven days (two-tailed Fisher's Exact Test, P= 0.31). After 14 days 47 percent of individuals lost their PIT tags in the fall trial (N= 33) and 39 percent lost their tag in the spring trial (N= 32). Again, the difference between the two trials was not significant after fourteen days (two-tailed Fisher's Exact Test, P= 0.62). PIT tag rejection was even greater after 30 days. In the fall trial 54% of recovered individuals (N = 24) had lost their PIT tag by this time, while 47% (N= 47) had lost their tag in the spring trial. There was also no difference in rates of loss between trials at 30 days (two-tailed Fisher's Exact Test, P= 0.45). Even after one month, tag rejection continued and 72% of stars from the fall trial had rejected PIT tags after 150 days (N= 29).

In the spring trial some additional observations were made on the days immediately following initiation of the trial. Tag rejection was observed to occur either through autotomy of the entire arm (Figure 2-3A) or rejection through an opening in the body wall (Figure 2-3B). In one case, a dead sea star was observed, although it was unclear whether death occurred because of the act of implantation or tag rejection. In most cases stars were recovered some time after rejection and the site of rejection could not be discerned; dead stars could not be recovered in all but one case. Out of all stars observed to have lost PIT tags, approximately 12 percent did so by autotomy of an entire arm.

2.2.2 Effects of Neutral Red Tagging

Activity coefficients did not differ significantly between stained and unstained *Pisaster* at both seven days after staining ($Q_{1,51}$= 2.68), or at 30 days after staining ($Q_{1,52}$= 2.08). In addition, activity coefficients did not differ significantly between stars at the start (before staining) and the stained stars at either 7 days (ANOVA on ranks: $Q_{1,85}$= 1.22) or 30 days ($Q_{1,83}$= 0.488).

2.3 Discussion

The high PIT-tag rejection rates observed in *Pisaster ochraceus* may be an underestimate of actual tag-loss rates, due to the potential for tag-induced mortality. Considering that *in situ* rejection of a PIT tag requires that the body wall open to the surrounding environment in particularly fast flow, tag rejection likely poses a risk of injury (e.g., limb autotomy) or mortality to the star. Also, because tag rejection rates can only be discerned from recovered sea stars, any mortality would cause a discrepancy between observed and actual rates of tag loss. Recovery rates were much higher in other long-term transplants using only neutral red tagging (Chapters 3,4). After one week, recovery rates of PIT-tagged sea stars were 32% and 40% for the two trials while recovery of transplanted stars tagged only with neutral red were approximately 50-60% (K. Hayne, unpublished). PIT-tag induced mortality may therefore be a significant and underestimated factor in PIT-tag loss.

Effective tagging techniques require that the tags are retained at a reasonable rate and have no deleterious effect on the individual's health or behaviour. These high rates of loss coupled with possible adverse effects on the sea star probably make PIT-tagging unsuitable for large scale mark-recapture or other *in situ* studies, especially considering the high cost of RFID technology. The effectiveness of PIT tagging has not been well explored in asteroids but has been used for mark-recapture in other echinoderms. Studies of sea urchins yielded contradictory results, including 1) no effect of tagging and complete tag retention in the lab (Hagen, 1996) and 2) overall deleterious effects and tag losses of 20 percent after 80 days in the field (Lauzon Guay and Scheibling, 2008). In sea cucumbers PIT tags appear to have deleterious effects and very poor retention. In one study 75 percent lost their tags in eight days while 50 percent of those showed infection or injury (Purcell et al., 2006). The observed losses and rates of mortality and injury in this study appear to lie between the values previously seen for urchins and sea cucumbers. This may be because body wall stiffness of *Pisaster* lies between the fully calcareous urchins and very sparsely skeletonised body wall of sea cucumbers and the ability to reject tags may be related to the ability to open the body wall. If this is the case, one may expect other asteroids, which tend to be less stiff than *Pisaster*, to show even greater rates of PIT-tag rejection.

In addition to showing deleterious effects and significant tag loss, the use of RFID tags is also logistically difficult. Their use in *Pisaster* requires tagging to be done in a laboratory, to minimize rejection and facilitate healing of the implantation site. This may introduce a bias into long-term studies due to laboratory-induced effects and spatial inaccuracies of transplant back into their native environment. Their use also makes re-identification of study individuals very difficult as there are no visual cues for identification, each individual at a site must be tested with a reader, which may become impractical if many sea stars at a field site are not tagged. Their use with subtidal organisms is also constrained by a lack of submersible reading equipment.

Use of neutral red banding alleviates most of these problems because it introduces an easily identifiable and readable external tag that seems to cause no negative effects in tagged individuals and in intertidal organisms tags can be applied at low tide *in situ* without disturbing the individual. Loss of the neutral red arm bands seems to be negligible, at least in the eight months that dyed stars were kept, and reapplication of the bands is possible if longer term studies are desired. During a multi-year study of *Crossaster papposus*, multiple individuals were tagged with neutral red and monitored over seventeen years with great success (Carlson and Pfister, 1999). Although this study shares a similar method to the present one, it was discovered after completion and methods were arrived at independently. Although activity coefficients suggest no negative effect of neutral red on *Pisaster*, caution should be taken as vital stains can have subtle effects that may be overlooked (Barbosa and Peters, 1971).

Few studies have been conducted on *Pisaster* at an individual level. The use of neutral red for identification may allow for the study of growth rate, migration patterns, mortality and other major gaps in *Pisaster* natural history. Its use for marking other species should be explored as well, particularly in other sea stars, urchins and sea cucumbers as marking them has been problematic.

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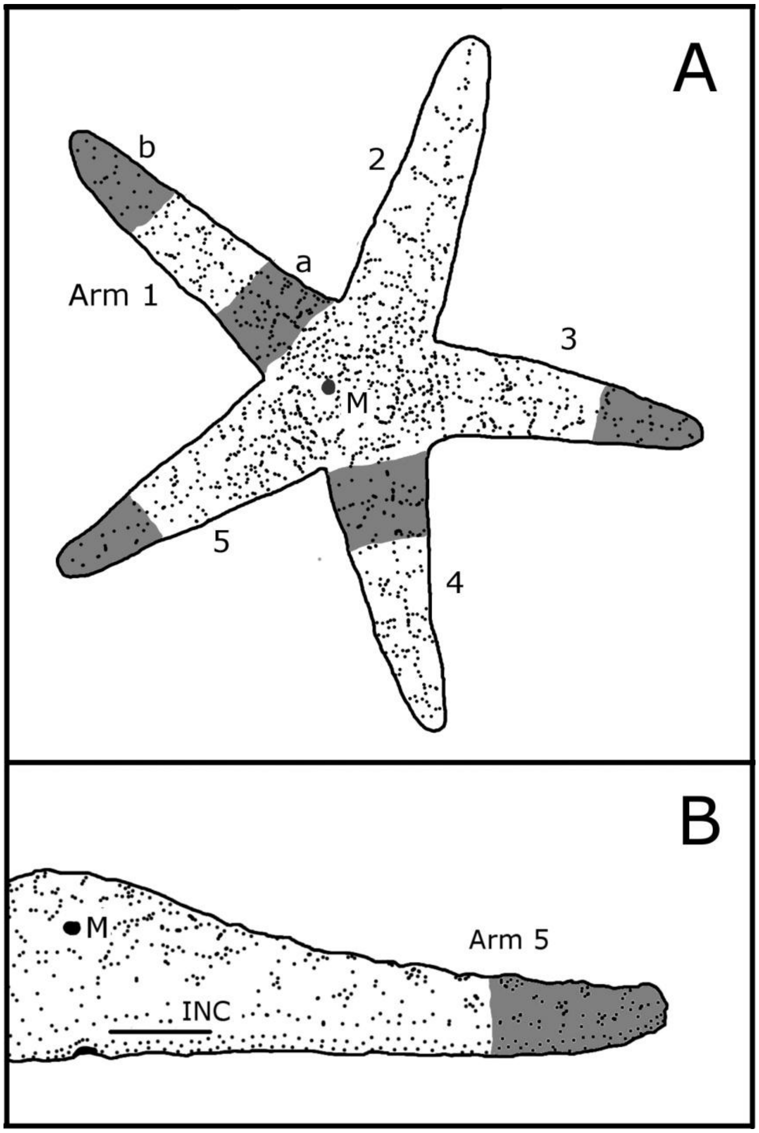

Figure 2-1. Tagging protocols for *Pisaster ochraceus*. A. Using the madreporite (M), arms were numbered in a clockwise direction. Two possible dye positions, a and b, were defined on each arm, and an individual could have any combination on each arm. This allowed for 1024 unique combinations. B. PIT (passive integrated transponder) tags were inserted through a small incision (INC) at the base of arm five. The incision was made through a suture in the ossicles near the aboral surface.

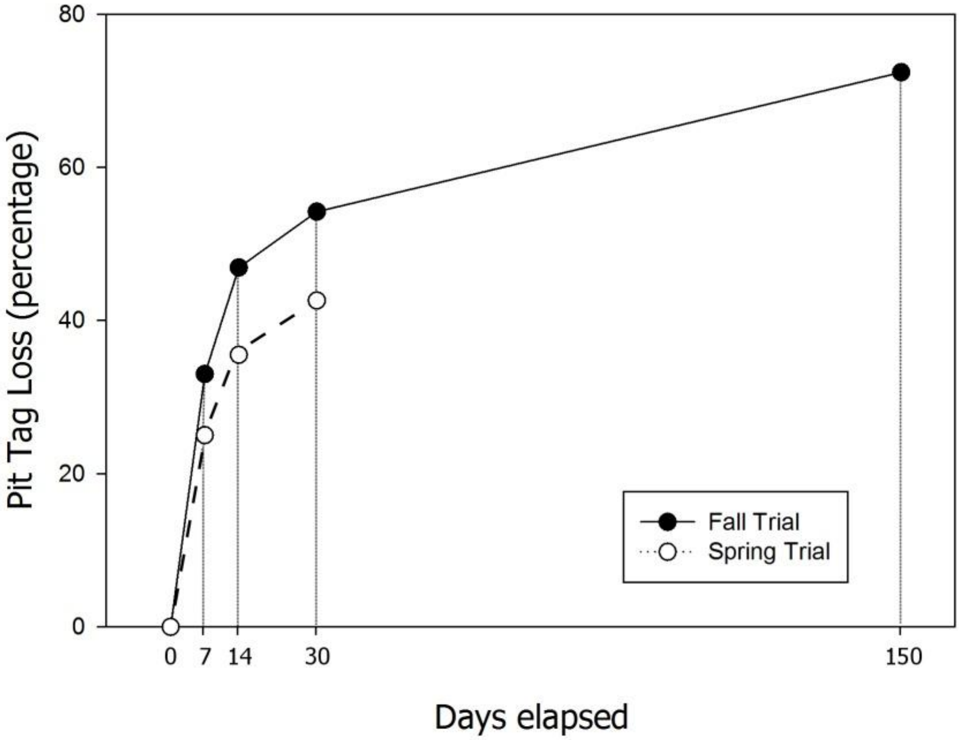

Figure 2-2. PIT tag losses from implanted stars after 7, 14, 30 and 150 days elapsed from trials starting in Fall 2010 (N = 100) and Spring 2011 (N = 100).

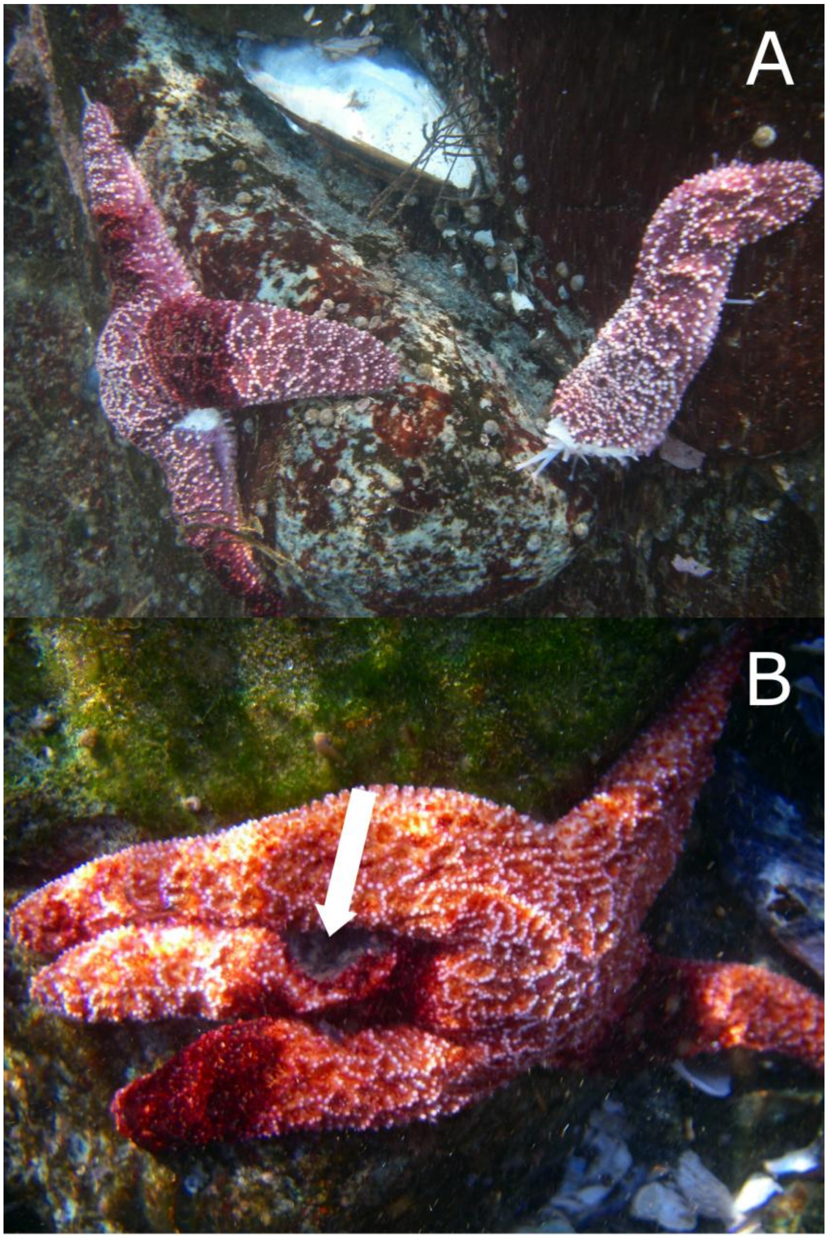

Figure 2-3. Examples of *Pisaster ochraceus* that rejected implanted PIT tags: A. by autotomy of an entire arm; B. through a localized response and opening of the body wall (→).

CHAPTER 3

Plastic responses of the sea star *Pisaster ochraceus* (Brandt) to wave action: Modification of body shape[†]

3.0 Introduction

Wave-swept intertidal shores are among the most extreme flow environments on earth (Denny, 1988). Crashing waves can generate velocities exceeding 15 ms^{-1} on wave-exposed shores (Helmuth and Denny, 2003), and organisms inhabiting these shores commonly suffer wave-induced damage and mortality (Denny, 1995). These extreme forces significantly affect the size, form, behaviour and distribution of intertidal organisms (Dayton, 1971; Paine and Levin, 1981). Despite the extreme forces, some large-bodied mobile animals, like the sea star *Pisaster ochraceus*, not only survive on wave-exposed shores, they also have a major impact on the ecological structure of rocky-intertidal communities over a wide range of wave-exposures (Dayton, 1971; Levin and Paine, 1974). How does a large-bodied mobile animal cope with such a broad range of physical conditions?

Intertidal organisms may be dislodged by either lift or drag forces, and these in turn are directly influenced by body size and shape. Many exhibit changes in form with increasing flow: mussels develop shells with a lower height to width ratio (Akester and Martel, 2000), kelp produce narrower blades (Fowler-Walker et al., 2006) and barnacles have shorter feeding legs (Arsenault et al., 2001; Neufeld and Palmer, 2008) and stouter penises (Arsenault et al., 2001;

[†] To be submitted to *The Journal of Experimental Biology*, with authors K. Hayne & A.R. Palmer

Neufeld and Palmer, 2008). Individuals from wave-swept environments also tend to be smaller and have a more compact form than individuals from calm sites, as seen in many organisms, including encrusting sponges, hydrozoans and scleractinian corals (Denny et al., 1985; Kaandorp, 1999).

Phenotypic variation among sites in such heterogeneous environments can be due to genetic differentiation, a plastic phenotypic response, or a combination of the two (Via and Lande, 1985). However, plastic responses seem to be widespread in intertidal environments probably because they allow for 1) future accommodation to environmental changes (which are common) and 2) adaptation to new local conditions following dispersal (which is usually high). In fact, all of the examples of size and form variation cited above are plastic responses to changes in hydrodynamic environment.

To sufficiently understand the ecological impacts of wave exposure on intertidal organisms one must consider how plastic responses to variation in wave action improve survival in high flow. Although disturbance dynamics were adequately described in mussels using mechanistic approaches involving only wave exposure (Gaylord et al., 1994; Denny, 1995), the description of these dynamics was greatly improved by accounting for seasonal plastic change in morphology and attachment (Carrington, 2002). Wave dislodgement of mussels is an important process that structures rocky intertidal communities (Dayton, 1971; Levin and Paine, 1974). Given the intimacy of the population dynamics of a keystone predator, *Pisaster*, and its preferred prey, the competitively superior *Mytilus californianus*, an understanding of the mechanical constraints on *Pisaster* distribution is equally important for understanding the ecology of northeastern Pacific rocky intertidal communities (Paine, 1969; Paine, 1976).

The ochre star, *Pisaster ochraceus* (Brandt), is an abundant, widespread sea star in the northeastern Pacific and is one of the largest organisms able to withstand the rigors of life on these rocky intertidal shores (Ricketts et al., 1992; Denny, 1999). To better understand the morphological adaptations that facilitate this impressive feat, I studied the ability of *Pisaster* to change body shape in response to variation in wave exposure.

3.1 Materials AND Methods

3.1.1 Collection and Field Survey

Pisaster ochraceus were collected from four sites in Barkley Sound (British Columbia, Canada) that differed in maximum water velocity. In order of decreasing wave exposure they were: Seppings Island exposed (SE) and Helby Island (HE), which were considered relatively exposed, and Dixon Island (DS) and Seppings Island sheltered (SE), which were considered sheltered (Figure 3-1). Maximum velocities at two of the sites (SE and HE) were determined by Arsenault et al. (2001). Maximum water velocities at Seppings sheltered were found using five days of wave drogue data during this study (August 8, 11-14, 2010) that were calibrated to the velocities of Arsenault et al. (2001) by concurrently measuring velocities at Seppings exposed (Bell and Denny, 1994). Water velocity of the last site (DS) was estimated using dissolution of dissimilar metals (McGehee, 1998). Three replicate dissolution rigs were concurrently placed in all four sites for a period of ten days and a calibration curve of dissolution rates and maximum water velocities of known sites (SE, HE, SS) was used to estimate maximum water velocity in the unknown site (DS) ($F_{1,11}$=45.991, P<0.001, R^2=0.87). *Pisaster* (n= 100) were haphazardly collected at low tide from the windward and leeward sides of Seppings Island from August 10-14 (48°50’03.16N, 125°05’50.25W) and Dixon Island and Helby Island on September 8-11, 2010 (48°50’03.16N, 12505’50.25W; 48°51'14.34N, 125° 7'0.50W) near Bamfield, British Columbia, Canada (Figure 3-1). Only sea stars without obvious injury or evidence of past arm autotomy were collected. The *Pisaster* were transported to the Bamfield Marine Science Centre where they were housed in water tables connected to a flow-through sea water system. They were then individually weighed and photographed in planar (aboral) and side view. These photographs were used to measure the length and width of each arm in Image J Version 1.44 (Rasband, W., ImageJ, US National Institute of Health, Bethesda, MA, USA). Arm lengths were measured as the distance from the centre ossicle of the organism to the tip of the arm and arm width was measured at the base of each arm (Figure 3-2). These were used to compute an aspect ratio (length/width) for each arm and a mean aspect ratio across all five arms was calculated for each individual. Aspect ratio can be considered a measure of streamlining in a sea star, as arms become narrower relative to length, aspect ratio — and hence streamlining — increases. Differences in these mean aspect ratios were compared between sites using an analysis of variance (ANOVA) in Systat Ver 13 (Systat Software Inc., Chicago, IL, USA). To test the precision of arm width and length measurements, a subset of sea stars were remeasured (N= 12) and compared to initial measurements of the mean arm length and width for each individual. Percent error in measurement was 0.11% for arm length and 0.48% for width.

3.1.2 Field Transplant Experiment

After sea stars were weighed and photographed, each was then given a unique external identification by applying neutral red to create colored bands at different positions on each arm (Chapter 2). The neutral red was mixed with seawater to create a 0.2 g mL^{-1} slurry, and a liberal amount was applied to the aboral surface of the arms. The tagged *P. ochraceus* were left out of water for 15 minutes to allow the stain to set and each individual's arm code (a combination of mark position on each arm and arm number) was recorded. Stars were returned to each site by placing them on rocks nearby other non-transplanted *Pisaster* at low tide. Sea stars from the exposed side of Seppings (SE) were transplanted to the sheltered (SS) and vice versa on August 11-15, 2010. Stars were also reciprocally transplanted between Helby and Dixon Islands on September 9-12, 2010. After 3 months sea stars from each transplant were collected. Because recovery rates varied, sample sizes differed: 15 were recovered from Helby (HE), 21 from Dixon (DS) and 15 from the leeward side of Seppings (SS). No individuals were recovered from the windward side of Seppings (SE), likely because of their higher vulnerability to dislodgement. To determine natural seasonal change in aspect ratio, 15 non-transplanted stars (seasonal controls) were collected from each of the four release sites at the same time marked sea stars were recovered. All collected *Pisaster* (transplanted and seasonal controls) were taken to the laboratory and immediately photographed and weighed. To ensure that their arms were fully extended sea stars were placed on window screen suspended in flow-through water tables beforehand. Using the neutral red markings, transplanted individuals were identified and aspect-ratio change calculated. Final aspect ratios were computed and compared to initial values using pair-wise T-tests and between sites using an ANOVA in Systat Ver 13.

3.1.3 Drag and Lift Determination

Photographs of the planar view of collected *Pisaster* were used to measure the planar area of a subset of sea stars from the both the exposed (N= 30) and sheltered side of Seppings Island (N= 25). Measurements were done using Image J Version 1.44. Using side profile photographs, the height of each sea star was measured in Image J. Side area was not directly measured because area varies with arm orientation. This height and previously measured values of average arm length were used to calculate an estimate of maximum lateral projected area (MLPA), the side area of a sea star with one arm perpendicular to view and all remaining arms spread equally. This was estimated by equation (1) $MLPA= (1+\cos(36))A_h$ where the long axis of the other arm making up the profile of the sea star was 36 degrees from the long axis of the arm perpendicular to view, and A_h is the projected area of one half of the sea star when viewed from the side and given by, (2) $A_h= [{}^1/_2(h_C - h_T)A_L]+[h_T \bullet A_L]$; h_C is the height of the sea star in the center of the body, h_T is the height of the tip of the sea stars' arm, and A_L in the average arm length of that individual. This was repeated for 38 individuals from the exposed side of Seppings and 32 from the protected. Analysis of covariance

(ANCOVA) was performed for both planar and projected areas with site as a factor and arm length as a covariate (Systat 13).

The lift force experienced by an individual sea star should be directly proportional to its planar area (projected area when viewed perpendicular to flow) according to equation (3) $F_L = C_L(^1/_2\ \rho)V^2(A_{planar})$, where L is the force of lift in newtons, C_L is the coefficient of lift, ρ is the density of seawater in kgm^{-3}, V is the water velocity in ms^{-1} and A is the planar area in m^2. Drag force should be proportional to MLPA according to equation (4) $F_D = C_D(^1/_2\ \rho)V^2(MLPA)$, where C_D is the coefficient of drag. Coefficients of lift and drag were calculated using computational fluid dynamics analysis (ANSYS CFX Ver. 13, ANSYS Inc., Canonsberg, PA, USA) using 3-D models of typical, average-sized sea stars from both the sheltered and exposed site, $C_L = 0.53$ and $C_D = 0.4$ for a sheltered sea star and $C_L = 0.42$ and $C_D = 0.34$ for an exposed sea star (coefficients used were calculated at 5 ms^{-1}) (K. Hayne, M. Warhing and C. Lange, unpublished). Using these force equations and the known relationships of both MLPA and planar areas with arm length for each site (Figure 3-6; Table 3-2) the wave-induced dislodgement force of sheltered and exposed sea stars was calculated as the vector sum of drag and lift.

3.2 Results

3.2.1 Field Survey

I observed differences in aspect ratio among the four sites of different wave exposure. On average, sea stars from the most exposed site had arms that were 12 percent narrower at the base compared to those from the most sheltered site for a given arm length ($P< 0.001$). The aspect ratio was closely correlated with variation in water velocity in breaking waves when all sea stars were included in the analysis individually (Figure 3-3; $R^2= 0.36$, $P< 0.001$). Variation in mean aspect ratio among sites was even more highly correlated with water velocity: water velocity accounted for 99 percent of the variation in mean aspect ratio (Figure 3-3; $R^2> 0.99$, $P= 0.003$).

The water velocity in each site was also significantly correlated with the arm length of adult sea stars from each site (Figure 3-4A; $R^2= 0.042$, $P< 0.001$). Although the relationship was significant, water velocity was a poor predictor of size within each site, explaining only 4 percent of the variation among all sampled individuals. Despite this, on average, sea stars from the most wave-exposed site were 15 percent smaller than those from the most sheltered site.

Mass was not used as a measure of body size because it depended heavily upon aspect ratio: as aspect ratio increased wet mass per unit arm length decreased. As sea stars grow (increase arm length) their mass increases. The slope of this relationship was consistent between sites and intercepts were significantly different: wet masses were consistently higher at more sheltered sites (Table 3-1, ANCOVA $P< 0.001$). Using this common relationship between mass and arm length one can determine the expected mass of a sea star with a typical arm length (Figure 3-4B; Table 3-1). At the overall mean arm length (111.2 mm) a sea star from the most sheltered site was 35 percent heavier than one from the most exposed site. These expected masses were highly correlated with water velocity among sites ($P= 0.035$, $R^2= 0.90$).

3.2.2 Transplant Experiment

During the experimental period (Sept. - Dec. 2010) native (non-transplanted) sea stars at each site showed a seasonal increase in aspect ratio. Aspect ratios of sea stars increased by five percent ($P< 0.001$) on the leeward side of Seppings (SS), by five percent ($P= 0.022$) on Dixon Island (DS), and by six percent ($P< 0.001$) at the exposed site on Helby (HE; Figure 3-5A). This seasonal change in aspect ratio was correlated with increased storm activity in winter compared to early fall. The monthly mean offshore wave height was 1.7 m in September compared to 3.1 m in December (Fisheries and Oceans Canada, La Perouse Bank buoy historical data; http://www.meds-sdmm.dfo-mpo.gc.ca/isdm-gdsi/waves-vagues/index-eng.htm).

Sea stars transplanted from a sheltered site, Dixon Island (DS), to the more exposed site Helby Island (HE) showed a significant increase in aspect ratio ($P= 0.004$) and they were not significantly different from native (non-transplanted) individuals at the end of the experiment ($P= 0.29$) (Figure 3-5B). During this time, wet mass of sea stars decreased by 18% ($P< 0.001$) while arm length did not change significantly ($P= 0.46$). In the reciprocal transplant (HE to DS; Figure 3-5C) aspect ratio was not expected to change because of the effects of the seasonal increase in wave action (Figure 3-5A). The final morphology of transplanted sea stars did not differ from expected ($P= 0.40$) and as a result aspect ratio did not differ from the initial value ($P= 0.59$). Both wet mass and arm length decreased slightly ($P< 0.001$, $P= 0.042$ respectively), however mass per unit arm length did not change significantly ($P= 0.19$). *Pisaster* transplanted from the exposed side of

Seppings Island (SE) to the more protected side (SS) produced stouter arms ($P < 0.001$) and the mean final aspect ratio was not different from the surrounding native stars ($P = 0.40$) (Figure 3-5D). During the experiment, wet mass significantly increased by about six percent ($P = 0.015$) despite arm length decreasing by about five percent ($P = 0.041$). No stars transplanted to the exposed side of Seppings Island (SE) could be recovered, presumably because of their higher vulnerability to dislodgement.

3.2.3 Variation in Shape with Increasing Body Size

Planar area and maximum lateral projected area (MLPA) increased with increasing arm length in sea stars from both the sheltered (SS) and exposed (SE) sides of Seppings Island (Figure 3-6). Slopes of these relationships did not differ between exposure-conditions for planar area ($P = 0.65$) or MLPA ($P = 0.77$), but planar area was negatively allometric ($P < 0.001$) while MLPA was positively allometric ($P < 0.001$) (Table 3-2). Sea star planar area (mm^2) increased with arm length (mm) as $A_{planar} = 2.612(A_L^{1.795})$ for sheltered and $A_{planar} = 2.249(A_L^{1.795})$ for exposed *Pisaster*, where A_L is average arm length of all five arms of an individual. MLPA (mm^2) increased with increasing arm length (mm) as $A_{MLPA} = 0.0750(A_L^{2.290})$ for sheltered and $A_{MLPA} = 0.0615(A_L^{2.290})$ for exposed *Pisaster*. For both planar area and MLPA the sheltered population had consistently and significantly higher surface areas for a given arm length. For planar area it was greater by 16 percent ($P < 0.001$) and for MLPA by 22 percent ($P < 0.001$) (Figure 3-7; Table 3-2). Because of their relatively larger planar and projected areas per unit arm length, sheltered stars would experience a 19 percent higher dislodgement force at a given water velocity (Figure 3-8).

3.3 Discussion

3.3.1 Is Body Form in *Pisaster* Plastic?

The close matching of spatial variation in body form to spatial variation in environmental conditions could result from either (i) phenotypic plasticity, where form is directly influenced by environment, or (ii) genetically distinct populations formed by a) low rates of migration from source sites, b) differential mortality of new recruits due to a mismatch between environment and phenotype, or c) selective recruitment of genetically distinct juveniles into suitable sites (Schlichting and Pigliucci, 1998). Only transplant experiments, or studies of seasonal variation of marked individuals, can distinguish among these alternative hypotheses.

My field surveys and experimental transplants revealed natural differences in body form between exposure regimes (in both space and time) and confirmed that this variation arose primarily as a plastic response to local conditions (Figure 3-5), as reported in many other organisms that live on wave-swept rocky shores (Akester and Martel, 2000; Arsenault et al., 2001; Carrington, 2002; Marchinko, 2003; Neufeld and Palmer, 2008). I found that aspect ratios were significantly higher in exposed-shore *Pisaster*. Therefore, for a given arm length, exposed-shore *Pisaster* weighed significantly less than sheltered-shore individuals. *Pisaster* also appeared to undergo seasonal changes in aspect ratio (Figure 3-5A), presumably to better match prevailing seasonal wave-exposure conditions.

This implied phenotypic plasticity was confirmed experimentally (Figure 3-5B-D). When sheltered-shore *Pisaster* were transplanted to a wave-exposed site they developed a higher aspect ratio, while the opposite occurred in transplants from exposed to sheltered sites. The ability of *Pisaster* to actively adapt to wave-exposure conditions may be important for several reasons. First, wave-swept intertidal shores are among the most heterogeneous flow environments on earth, and experience large day-to-day variation in wave activity. Second, seasonal variation in storm activity can be extreme: average wave heights in winter are commonly double those in summer, maximum and daily wave heights can be 10 times higher in winter than summer (Fisheries and Oceans Canada, La Perouse Bank buoy historical data; http://www.meds-sdmm.dfo-mpo.gc.ca/isdm-gdsi/waves-vagues/index-eng.htm). Third, *Pisaster* has a long planktonic period (up to 8 months) and as a result, larvae from one parent may be spread over a wide range of environments (Strathmann, 1978). Fourth, *Pisaster* is a highly mobile predator, so plasticity in body form may facilitate foraging if individuals migrate long distances in search of prey (Robles et al., 1995).

Differences in measured maximum water velocities accounted for almost all (99.7%) of the among-site variation in overall mean aspect ratio but could only account for 36% of the total among-individual variation (Figure 3-3). Such high intra-population variation is not unexpected because most rocky shores are highly heterogeneous topographically, which would create considerable variation in flow conditions within a site. For example, an individual that settled in a crevice probably experiences less flow than one occupying a flat surface oriented into flow, as seen in intertidal snails that use habitat microcomplexity as refugia during periods of high flow (Emson and Faller-Fritsch, 1976; Trussell, 1997a).

3.3.2 Potential Benefits of Higher Aspect-Ratios

Mobile organisms that live on wave-swept shores are vulnerable to dislodgement and death if waves become too large (Denny, 1988). On wave-exposed shores, the probable benefit of the observed changes in *Pisaster* body form is reduced vulnerability to drag and lift. I found that an increased aspect ratio induced by rapid flow was associated with reduced planar and maximum lateral projected areas that, in turn, would yield lower lift and drag forces. Considering the close link between wave exposure and body form, wave activity is probably a major constraint not only on morphology but on behaviour and distribution as well. In addition, larger body sizes appeared to be selected against in exposed sites, as predicted by Denny et al. (Denny et al., 1985; Kaandorp, 1999). Attachment area for a sea star is restricted to the ambulacral groove area (which should be proportional to planar area) in contact with the substratum. The negative allometry observed in this planar area, combined with a positive allometry in projected area, could yield a mismatch between dislodgement force and tenacity as *Pisaster* grow. Although lift and attachment would remain in balance because both are based on planar area, positive allometry in MLPA would mean larger stars would be subjected to a larger drag force relative to attachment force. However, the trend towards smaller body sizes at the more exposed site was not universal across all sites and was probably also affected by the age of the cohorts in each site, as settlement events tend to be episodic (Sewell and Watson, 1993). Small-scale differences in shape between ecotypes affected coefficients of lift and drag for each, exposed sea stars had a lower coefficient of drag and lift than sheltered forms. These differences in shape were obvious – it seemed that exposed-shore sea stars had a more concave shape to the upper margin of the arms from tip to base, while sheltered ecotypes tended to have a straighter margin to the upper arm surface (Figure 3-7).

3.3.3 Potential Costs of Higher Aspect Ratios

The large difference in *Pisaster* form between exposed and sheltered sites implies a cost to higher aspect ratios in quiet waters. Two potential costs seem likely: i) decreased thermal inertia, and ii) decreased gonad volume. Sea stars with an exposed morphology (higher aspect ratio) will be much more prone to overheating than those from sheltered sites, which have much more wet mass per unit body length and therefore a larger surface-to-volume ratio (Helmuth, 1998). Upper zonational limits for intertidal organisms are typically set by temperature and desiccation stresses, as temperature fluctuations during aerial exposure can be extreme and occasionally lethal (Foster, 1971b; Tsuchiya, 1983). In *Pisaster,* body-temperature changes greatly affect physiological processes and feeding rates up to the approximate lethal limit of 35°C (Sanford, 1999; Pincebourde et al., 2008). Because average sheltered sea stars (e.g., SS) were 44% heavier than exposed stars (e.g., SE) for any given arm length, they would be better able to resist desiccation and thermal stress. Therefore, wave-induced changes in body shape must be considered when making predictions about the vertical zonation limits for *Pisaster* (Harley and Helmuth, 2003; Szathmary et al., 2009) and therefore its impact as a keystone predator on rocky intertidal shores (Paine, 1969; Paine, 1976).

Lowered fecundity may be another cost associated with higher aspect ratios in *Pisaster*. In many marine invertebrates, fecundity is proportional to body mass (Giese, 1959), and this is well-known for echinoderms such as sea stars (Farmanfarmaian et al., 1958), sea cucumbers (Conand, 1993) and sea urchins (Conor, 1972). *Pisaster* with higher aspect ratios (thinner arms) have lower body mass per unit arm length (see Field Survey results above). So, other things being equal, they should have lower gonad volumes and hence lower fecundity.

The higher thermal inertia likely associated with lower arm aspect-ratios introduces a possible confounding variable when trying to explain the advantages of higher aspect ratios on more wave-exposed shores (previous section): different wave-exposure regimes may have different thermal stress regimes. Wave action creates wash and spray, which can be significant cooling mechanisms (Harley and Helmuth, 2003). Therefore, increased wash on wave-exposed shores may reduce the selective disadvantage to higher aspect ratios (Szathmary et al., 2009). In *Pisaster*, for example, increased temperatures elicit an increase in water retention and thus thermal inertia (Pincebourde et al., 2009): high-temperature treatments caused an increase of wet weight by about 15 g (approx. 7.6% of total body weight) over times as short as five days, compared to low-temperature controls. However, the increase in mass (per unit arm length) I observed with decreasing wave exposure was much greater than what Pincebourde et al. (2009) induced via increased thermal stress. Therefore, wave forces seem likely to be a more important factor influencing *Pisaster* morphology on rocky intertidal shores. Nonetheless, it would still be interesting to investigate the tradeoffs associated with *Pisaster* shape variation in summer, when thermal stress is high and wave forces are low, and in winter, when the opposite is true.

The mechanism by which *Pisaster* decrease arm aspect-ratio under lower flow conditions is likely similar to the mechanism for increasing mass in response to heat (Pincebourde et al., 2009): uptake of seawater during high tide. I observed little change in arm length during the transplant experiments and the small decreases observed were probably due to decreased feeding during the storm season. Given the large changes in aspect ratio (and therefore mass per unit arm length) over a three-month period (Figure 3-5) and the slow growth rate of *Pisaster* (Paine, 1976), the change in morphology I observed was likely due to increased uptake of seawater rather than tissue growth or restructuring. Changes in aspect ratio may therefore occur much more rapidly than illustrated in this study. On two separate occasions during field sampling, aspect ratios of 50 haphazardly sampled stars were significantly higher than 50 stars sampled from the same location two days earlier and these increases coincided with increased wave action (K Hayne, unpublished). If increased water uptake is the mechanism by which *Pisaster* decreases aspect ratio under conditions of lower wave action, it would represent an elegant method for adapting to local conditions of thermal and wave stress, even over short time periods of hours or days.

Phenotypic plasticity in response to flow has been described in several other intertidal species from the northeast Pacific, including barnacles (Arsenault et al., 2001; Marchinko, 2003; Neufeld and Palmer, 2008) and mussels (Akester and Martel, 2000; Carrington, 2002). This plasticity is likely a major reason mussels and barnacles are among the predominant space-holders on rocky intertidal shores. *Pisaster*, unlike mussels and barnacles, are highly mobile and use this mobility to remove these sessile, dominant space-holders and, as a result, maintain high levels of diversity on intertidal shores (Paine, 1966). The importance of this process is illustrated in the differences between a wave-exposed and a sheltered shore. On exposed shores *Pisaster* are likely constrained to live lower in the intertidal zone because of increased dislodgement risk. This, in turn, would allow mussels to form more extensive beds. *Pisaster*'s ability to alter aspect ratio, and hence ameliorate dislodgement risk, may greatly affect their vertical distribution and hence their impact on intertidal community structure.

In summary, these results suggest that *Pisaster* body shape responds to seasonal changes in wave-exposure in a way that would reduce probability of dislodgement. These shape changes are likely achieved by changing the volume of water stored within the body. Reduced dislodgement risk via increases in aspect ratio may impose a cost in terms of reduced desiccation resistance or fecundity. The ability of *Pisaster* to adapt to seasonal changes in wave-exposure likely facilitates its role as a keystone predator (Paine, 1969) both in winter, when wave forces are high and thermal stress is minimal, and in summer, when the opposite is true.

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Source of Variation	Wet Body Mass		
	df	MS	P
Site	3	0.529	< 0.001
Arm Length	1	23.571	< 0.001
Error	414	0.009	
Equality of Slopes	3	0.001	0.94

Table 3-1. ANCOVA results testing for equality of slopes and differences in intercept among sites for the log-log relationship between wet mass and arm length.

Source of Variation	MPLA			Planar area		
	df	MS	P	df	MS	P
Site	1	0.127	< 0.001	1	0.058	< 0.001
Arm Length	1	2.075	< 0.001	1	1.368	< 0.001
Error	68	0.001		53	0.001	
Equality of Slopes	1	0.002	0.70	1	< 0.001	0.64

Table 3-2. ANCOVA results from tests for equality of slopes and differences in intercept between log-log relationships of arm length to maximum lateral projected area (MLPA) (Figure 3-6A) and planar area (Figure 3-6B) for sea stars from an exposed site (SE) and a sheltered site (SS).

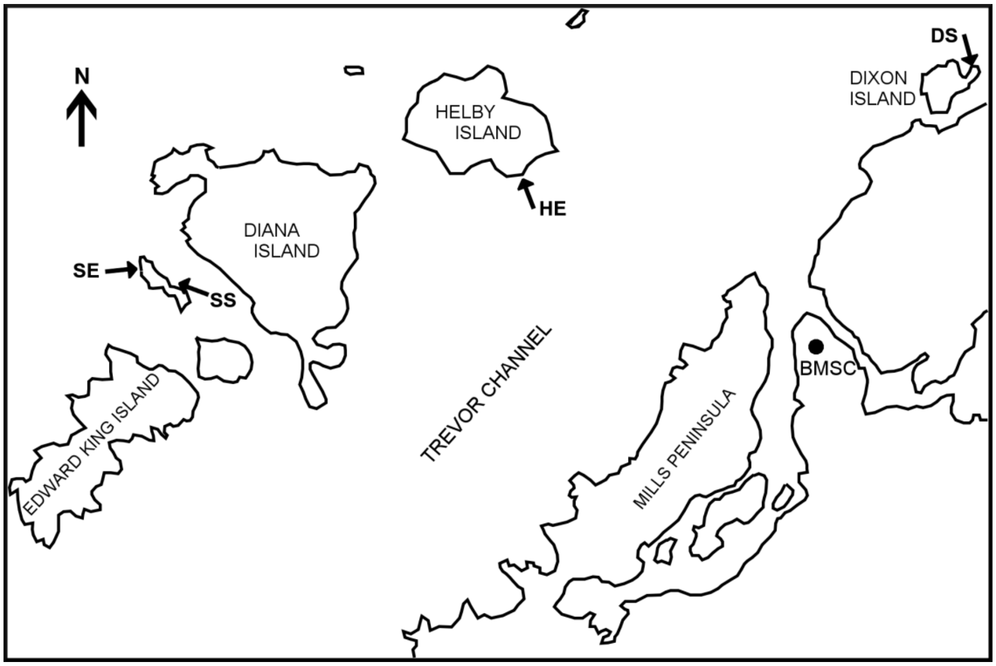

Figure 3-1. Location of study sites near the Bamfield Marine Sciences Centre (BMSC) in Barkley Sound, British Columbia, Canada. SS- Seppings sheltered, DS- Dixon sheltered, HE- Helby exposed, SE- Seppings exposed.

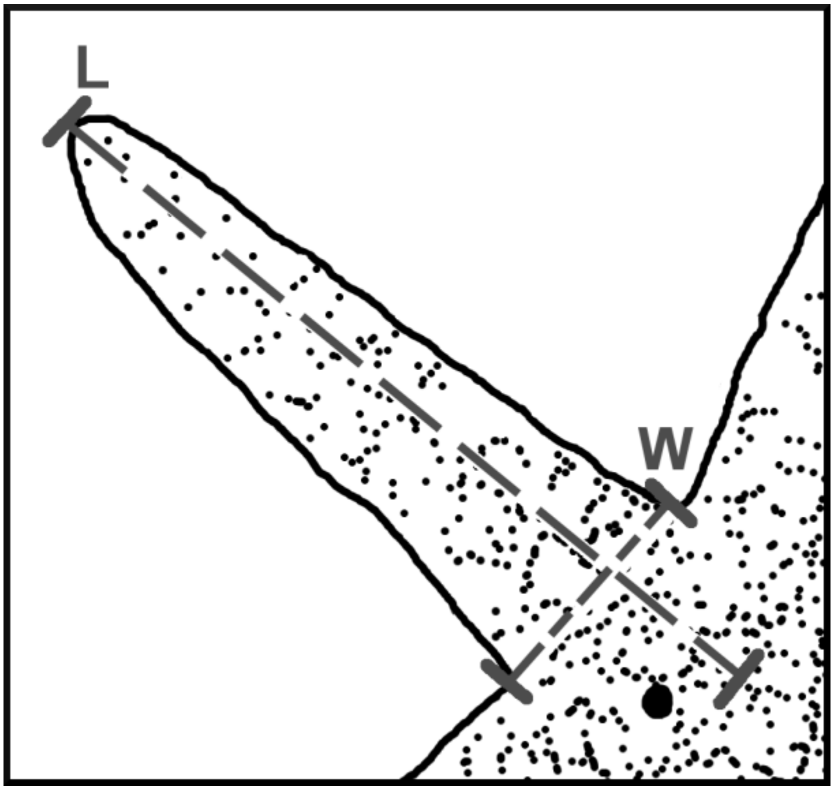

Figure 3-2. Calculation of aspect ratio for *Pisaster ochraceus* (planar view). The length (L) of an individual arm was measured as the distance from the centre ossicle to the tip of the arm. Width (W) was measured as the distance across the arm at its base. Aspect ratios were calculated as L/W for each arm and averaged over all five arms for each individual.

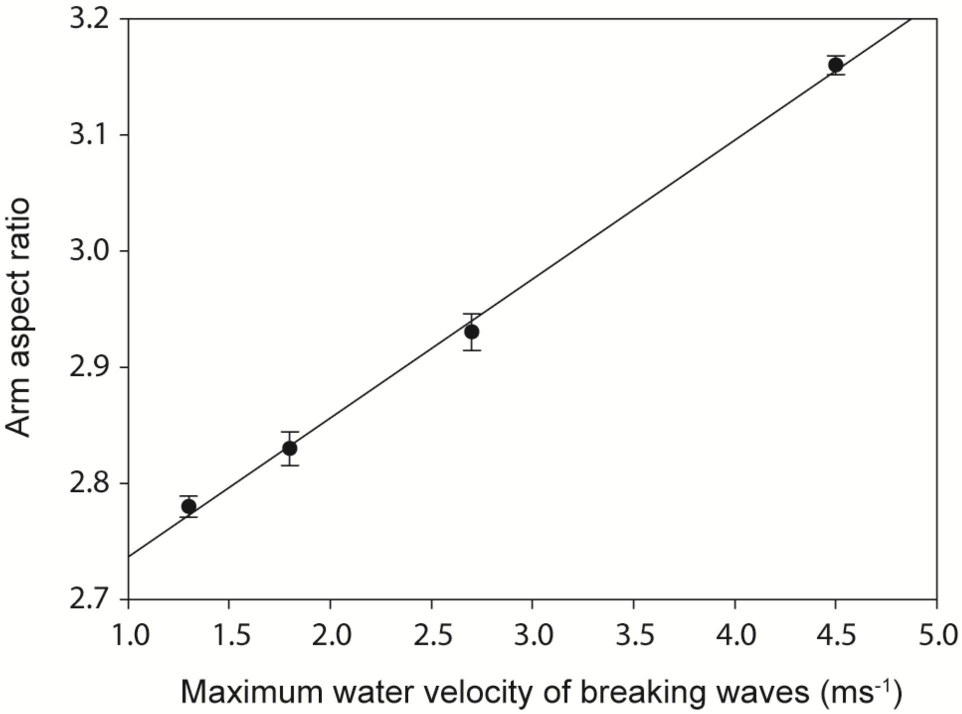

Figure 3-3. Variation of arm aspect-ratio (L/W) in *Pisaster ochraceus* as a function of wave exposure among four intertidal sites in Barkley Sound (see Figure 3-1). Maximum water velocity is a relative measure of the observed maximum velocities experienced in each site during late summer. The sites are (left to right) Seppings Island sheltered (SS), Dixon Island (DS), Helby Island (HE) and Seppings Island exposed (SE). All points are mean±s.e.m. OLS regressions: raw data, $F_{1,417}$= 232.8, P< 0.001, R^2= 0.36; population means, P= 0.003, R^2= 0.998.

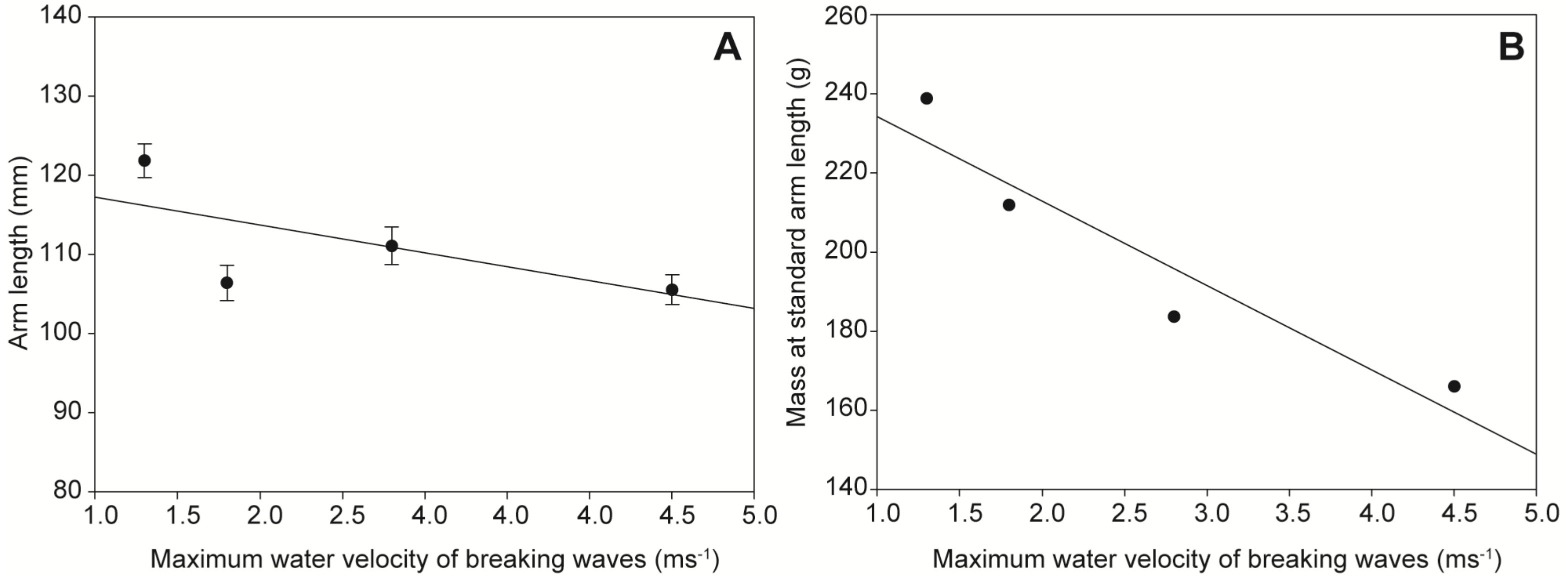

Figure 3-4. Body form of *Pisaster ochraceus* as a function of wave exposure from four sites in Barkley Sound (sites are the same as in Figure 3-3). (A) Mean arm lengths. All points are mean±s.e.m. OLS regression: raw data, $F_{1,417}$= 18.4, P< 0.001, R^2= 0.042. (B) Wet weight standardized to a common arm length of 111.2 mm by analysis of covariance (ANCOVA performed with both axes on a log scale). OLS regression for population means, $F_{1,3}$= 27.5, P= 0.034, R^2= 0.898.

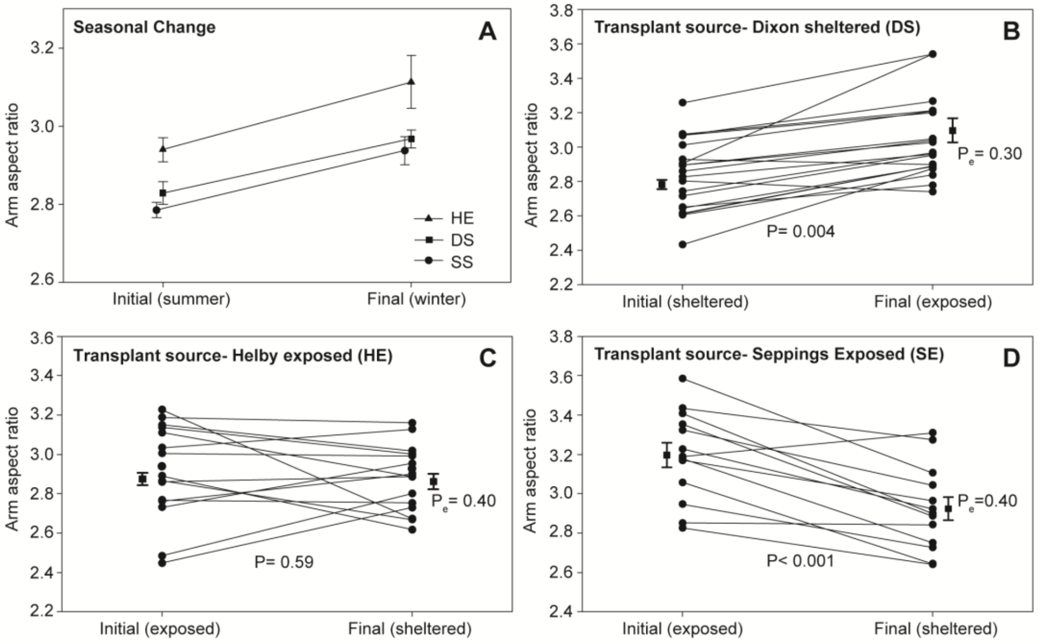

Figure 3-5. Arm aspect-ratio of *Pisaster ochraceus* from three different source populations before and after a three-month period from summer to winter 2010. (A) Mean±s.e.m. aspect ratio of individuals haphazardly sampled from the transplant sites at the beginning and end of the transplant experiment to characterize natural, seasonal change in aspect ratio. Closed circles in B-D correspond to individually marked sea stars. Closed squares in B-D represent the mean (±s.e.m.) initial aspect ratio (on the left) and the expected mean final aspect ratio (on the right) assuming that the final aspect ratio was the same as that of native (non-transplanted) sea stars sampled in winter (from A). (B) Individuals (closed circles) transplanted from a sheltered site (DS) to an exposed site (HE). (C) Individuals transplanted from an exposed site (HE) to a sheltered site (DS). (D) individuals transplanted from an exposed site (SE) to a sheltered site (SS). (P) P-value for the difference between initial and final aspect ratio of transplanted stars. (P_e) the P-value for the difference between the final aspect ratio of transplanted stars and the expected final aspect ratio.

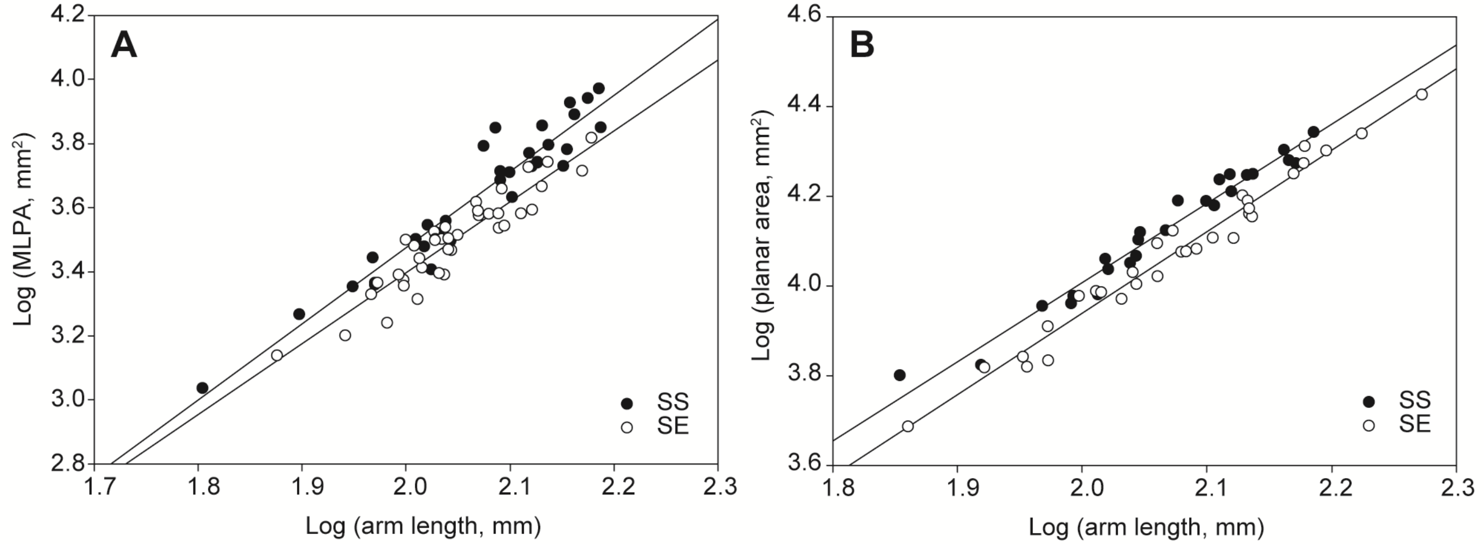

Figure 3-6. Morphological differences between *Pisaster ochraceus* from the exposed (SE) and sheltered (SS) sides of Seppings Island. Each point represents a single individual. (A) Planar area as a function of arm length. The difference between slopes was non-significant (P= 0.65), the differences between intercepts was highly significant (P< 0.001). (B) Maximum lateral projected area (MLPA) as a function of arm length. The difference between slopes was non-significant (P= 0.77), the difference between intercepts was highly significant (P< 0.001). See Table 3-2 for complete statistics.

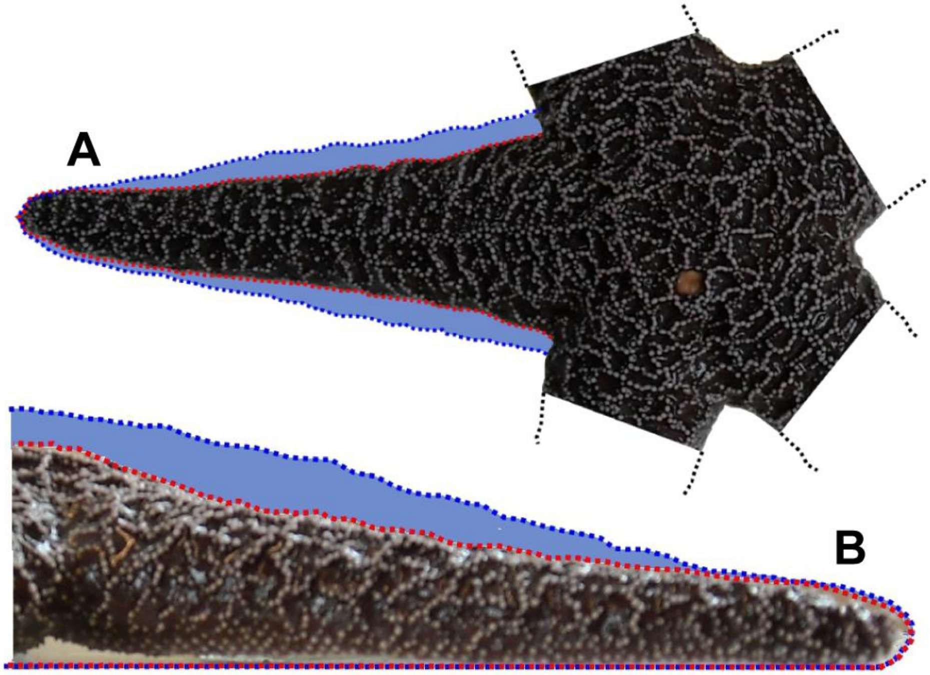

Figure 3-7. Comparisons of (A) planar and (B) lateral projected areas of a single arm of an approximately typical *Pisaster ochraceus* between the exposed (SE; narrower arm) and sheltered (SS, fatter arm) sides of Seppings Island. Arm length= 120 mm.

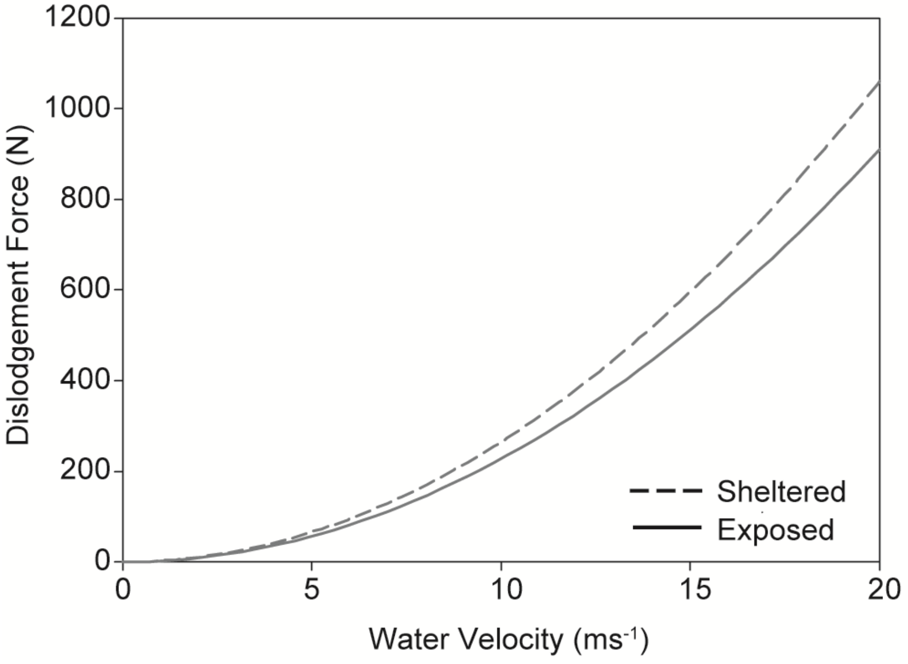

Figure 3-8. Expected wave-induced dislodgement force as a function of water velocity for an average *Pisaster ochraceus* with an arm length of 111.2 mm. Dislodgement force was calculated as the vector sum of both lift and drag expected from the planar and maximum lateral projected areas shown in Figure 3-6.

CHAPTER 4

Hanging tough: The intertidal sea star, *Pisaster ochraceus* (Brandt), modifies tube foot form and tenacity in response to wave action[‡]

4.0 Introduction

Rocky intertidal shores include some of the most stressful flow environments on earth, and yet they support a diverse array of life. Wave-induced disturbance is an important process for maintaining diversity on these shores, it influences both community structure (Dayton, 1971), and the morphology and physiology of individuals living in these communities (Etter, 1989). Wave exposure can influence the growth rate (Steffani and Branch, 2003), behaviour (Trussell, 1997b), maximum size (Denny et al., 1985), reproductive output (Etter, 1989) and body morphology (Denny et al., 1985) of an intertidal organism. Many of these effects are a result of the increased dislodgment risk in high flow (Trussell, 1997a). For any benthic aquatic organism, the probability of dislodgement is determined by the hydrodynamic forces imposed by water flow and attachment force resisting this flow.

Attachment and wave-induced disturbance have been well examined for many different intertidal organisms such as kelp (Bell and Gosline, 1997; Milligan and DeWreede, 2000; Denny and Gaylord, 2002), mussels (Price, 1982; Bell and Gosline, 1997; Hunt and Scheibling, 2001), snails (Denny, 1984; Trussell, 1997a), limpets (Branch and Marsh, 1978; Denny, 2000) sea stars (Paine, 1926; Flammang et al., 1994; Santos et al., 2005c) and urchins (Santos

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[‡] To be submitted to *The Journal of Experimental Biology*, with authors K. Hayne & A.R. Palmer

and Flammang, 2007). Intertidal shores are spatially heterogeneous, and seasonal and daily variation in wave-exposure can be extreme (Denny, 1988). Snails and mussels both increase attachment under higher flow conditions through phenotypic plasticity (Trussell, 1997a; Carrington, 2002). The ecological impacts of wave exposure on intertidal organisms therefore depend upon how plastic responses to variation in wave action may improve attachment in high flow.

Most large intertidal organisms survive the extreme flow on intertidal shores by attaching themselves permanently to the substrate – mussels use byssal threads, barnacles use cement, and kelp use holdfasts. However, some of the largest animals capable of dealing with the extreme stresses of wave-swept shores are sea stars, which are mobile organisms. Their tube feet are used for attachment, locomotion and feeding (Flammang, 1996). These tube feet — a unique feature of the Echinodermata — are a collection of independently operated tubular appendages that attach to the substrate via chemical adhesion (Thomas and Hermans, 1985; Flammang, 1996). Their impressive attachment capabilities come from the use of many tube feet in unison. A complex cycle of attachment and detachment allows sea stars to move while maintaining constant attachment to the substrate (Flammang, 1996). Tube feet also show great variation in form that appears to be suited to the environment in which a particular species resides (Sharp and Gray, 1962). For example, sea stars from sandy environments have pointed tube feet, while those from rocky environments have suckered tube feet for attachment (Santos, 2004). In addition, sea stars with suckered tube feet from more exposed environments generally have higher tenacity (Sharp and Gray, 1962). Although interspecific differences in tube form and function have been relatively well explored, studies of intraspecific differences in attachment are few (Siddon and Witman, 2003; Santos and Flammang, 2007) and nothing is known about whether echinoderms can alter tube-foot form or performance via phenotypic plasticity.

The most common intertidal sea star in the northeast Pacific, *Pisaster ochraceus*, is a keystone predator that on exposed headlands can reach up to 40 cm in diameter and weigh well over 1 kg (K Hayne, unpublished). At this size an individual would be exposed to a dislodgement force of 238 N when hit with a crashing wave travelling at 10 ms^{-1} (Chapter 3). Despite showing great affinity for exposed shores and the mussels found there, *Pisaster* occur in a wide range of environments from the most exposed headlands to sheltered coves.

Wave-dislodged *Pisaster* are often observed on exposed shores (Hayne & Palmer, pers. obs.) and this dislodgement may represent a significant source of mortality. For dislodgement to occur, all attached tube feet must simultaneously fail. Tube-foot failure may occur in one of two ways: 1) the tube foot could break along the podial wall leaving the basal disc attached to the substrate, or 2) the chemical adhesion could be the point of failure so the whole tube foot would detach from the substrate. Plastic changes in either would increase tenacity and reduce the risk of failure: 1) an increase in the tensile strength of the podial wall by modifying load bearing tissue layers, or 2) an increase in the chemical adhesion of the tube foot via increased secretion or changes in properties of the adhesive mucus.

I investigated how *Pisaster* cope with such a wide range of flow environments in three ways. First, I examined the relationship between attachment strength and maximum water velocity experienced by *Pisaster* over a wide range of wave-exposure conditions. I predicted that tube foot tenacity, the attachment force per unit area of attached tube feet, should increase with increasing wave exposure. Second, I tested whether *Pisaster* exhibited phenotypic plasticity in tenacity by reciprocally transplanting them between low-flow (sheltered) and high-flow (wave exposed) environments. Third, I examined histological cross-sections of tube feet taken from individuals before and after transplant into a more exposed site, to test whether the fine structure of tube feet varied in response to transplantation and to elucidate the mechanism of tenacity modification.

4.1 Methods

This research was conducted at the Bamfield Marine Sciences Centre (Bamfield, British Columbia, Canada) and nearby sites in Barkley Sound. All statistical analyses were performed in Systat V.13 (Systat Software Inc., Chicago, IL, USA).

4.1.1 Collection and Field Survey

To determine how adhesion strength varied with wave exposure in *Pisaster*, tube foot tenacity (force per unit area) was measured *in situ* at sites spanning a wide range of wave exposure conditions. *Pisaster* were sampled from four sites (in order of increasing maximum water velocity: Ross Islet (RS; 48°52'26.22N, 125° 9'37.96W; N= 17), Dixon Island (DS; 48°50’03.16N, 125°05’50.25W; N= 18), Helby Island (HE; 48°51'14.34N, 125° 7'0.50W; N= 17), and Seppings Island (SE; 48°50'26.54N, 125°12'34.27W; N= 15) (Figure 3-

1). Ross Islet and Dixon Island were considered relatively sheltered while Helby and Seppings Islands were considered relatively exposed. Water velocities for RS, HE and SE were obtained from a prior study (Arsenault et al., 2001). Water velocity of the last site (DS) was estimated using dissolution of dissimilar metals (McGehee, 1998). Three replicate dissolution rigs were concurrently placed at all four sites for a period of ten days and a calibration curve of dissolution rates and maximum water velocities of known sites (SE, HE, SS) was used to estimate maximum water velocity in the unknown site (DS) ($P< 0.001$, $R^2= 0.87$). Tenacity was measured concurrently at all four sites throughout the summer (May-August) of 2009 and 2010.

To measure tube foot tenacity, an individual *Pisaster* was selected haphazardly during low tide and detached from the substratum. Only sea stars without obvious injury and no evidence of past arm autotomy were collected. It was then allowed to attach to a Plexiglas plate with the middle section of one arm placed directly over an approx. 2.5 cm removable, polished glass disc nestled within a slightly larger circular hole in the plate (Figure 4-1). Attachment to the glass disc was facilitated by immersing the individual in a basin filled with seawater while maintaining its position on the plate. This method was successful presumably because placing the sea star in water simulated re-immersion following natural dislodgement. Once attached, the aboral surface was photographed with a calibration scale, so arm length measurements could be obtained later from pictures, and the oral surface of the glass disc was photographed to determine the number and total surface area of attached tube feet.

The plate and sea star was then secured horizontally at the top of a stand and a Wagner FDX 25 electronic force meter (Wagner Instruments, Greenwich, CT) was connected to the glass disc and pulled straight down at a consistent rate of approximately 0.2 cm s^{-1} until the tube feet detached from the glass disc. Following detachment, the sea star was weighed and broken tube feet, which remained attached to the disc, were photographed. Tenacity (T) was then calculated by dividing the maximum attachment force (F_a) by the total area of attached tube feet (S):

$$T= F_a / S$$

Arm length, attached tube foot area and counts of attached and broken tube feet were measured in Image J Version 1.44 (Rasband, W., ImageJ, US National Institute of Health, Bethesda, MA, USA). Arm lengths were measured as the average distance from the centre ossicle of the oral disc to the tip of the arm.

A least-squares linear regression was performed to test for a relationship between tenacity and maximum water velocity, and among-site differences in tenacity were tested using an analysis of variance (ANOVA).

4.1.2 Field Transplant Experiment

To determine whether tenacity and tube-foot form were phenotypically plastic traits, *Pisaster* were reciprocally transplanted between a sheltered (low flow) and an exposed (high flow) site. Sea stars were haphazardly collected at low tide from Dixon Island (N= 100) and Helby Island (N= 100) from September 8th to 11th, 2010 and transported to the Bamfield Marine Science Centre where they were placed in water tables connected to a flow-through sea water system. Each sea star was given a unique external identification by applying neutral red (a vital stain) to create arm bands at either the tip or the base of the arm (Chapter 2). Briefly, neutral red was mixed with sea water (0.2 g mL^{-1}) and the slurry was applied to the aboral surface, creating a unique combination of arm bands on each sea star. They were left out of water for 15 minutes for the vital stain to set and then placed back in the water table. The next day sea stars were transplanted between each site by placing them in the intertidal at low tide. After approximately three months, tenacity was reliably measured for 15 recaptured stars at each of the two sites. Within-site, natural seasonal change in tenacity was measured by sampling native (non-transplanted) individuals at both Helby Island (N= 14) and Dixon Island (N= 14).

Differences between initial and final tenacity in both transplanted and native (non-transplanted) *Pisaster* were tested with a T-test.

4.1.3 Morphological Comparisons

To test whether plastic changes in tube-foot tenacity were associated with changes in tube-foot morphology, a subset of tube feet were removed from sea stars both before and after transplantation and quantified using histological measurements. Tube feet were collected from all sea stars transplanted and also those recaptured. To ensure a standardized extension of tube feet at the time of collection and preservation, tube feet were flash frozen when the sea star was attached to a substrate (Shur Freeze Cryogen Spray, Triangle Biomedical, Durham, NC, USA). To do this, each sea star was allowed to attach to a Plexiglas plate, five tube feet were flash frozen using through a hole centered on one of the arms, and these were immediately dissected off and placed in chilled buffered 10% formalin. They were allowed to fix for 24 h and then stored in 70% ethanol. A subset of these were analyzed for morphological differences. Four tube feet from eight individuals both before and after transplant were dehydrated in a sequence of graded ethanols, embedded in paraffin wax using a Tissue-tek II embedding station (Sakura Finetek, Torrance, CA, USA) and cut in cross-section using an AO Spencer 820 microtome (American Optical, Buffalo, NY, USA). Sections were collected at 150 µm intervals starting at the center of the basal disc and were mounted on clean glass slides. Sections at distances of 300 and 600 µm from the basal disc were stained using hematoxylin and eosin and Masson's trichrome (Humason, 1979) (See Fig.4-2). These sections were photographed using a Luminera Infinity 1 digital camera (Luminera, Ottawa, ON, Canada) on an Olympus CX-41 microscope (Olympus Canada, Richmond Hill, ON, Canada) and measurements were made using Image J. Total area of the tube foot was measured, as was the area of the epidermis, connective tissue and longitudinal muscle (Figure 4-2). The measurements from these replicate tube feet (N=2-4, owing to differences in quality) were averaged for each individual and initial and final morphologies were compared by a two-way repeated-measures ANOVA using time period and distance from disc as factors and each individual as a subject. To account for variation in tube foot area between samples morphological comparisons were done using the proportion of the total cross-sectional area that each tissue layer composed and not raw values.

4.2 Results

4.2.1 Field Survey

I observed differences in tenacity among the four sites of different wave exposure (P< 0.001). Mean tenacity of the most exposed individuals (SE) was 91% greater than that of the most sheltered individuals (RS) (Figure 4-3; P< 0.001). Tenacity was positively correlated with maximum water velocity across the four sites (Figure 4-3; P< 0.001). Maximum water velocity explained 55% of variation among all individuals (P< 0.001) and 92% of variation in tenacity among sample means (P= 0.027). Among-site differences in the number of tube feet attached to the disc were not significant (P= 0.56).

4.2.2 Transplant Experiment

In both sites native (non-transplanted) *Pisaster* showed a seasonal increase in tenacity over the three-month period of the transplant experiment (Figure 4-4). Sea stars from Dixon Island (DS) had a winter tenacity 54% greater than the summer tenacity (P< 0.001) and *Pisaster* from Helby Island (HE) had a winter tenacity 29% greater than their summer tenacity (P= 0.005). When interpreting the results from transplanted *Pisaster,* seasonal change had to be considered because complete plasticity would be a change from the initial source-site tenacity to the tenacity of the final destination-site (after correction for the expected effect of seasonal change).

Stars transplanted to the more wave-exposed site showed an 85% increase in tenacity (P< 0.001), however this increase was not significantly greater than the increase seen in non-transplanted sheltered stars due to seasonal change (Figure 4-

4A; P= 0.14). The final tenacity of these transplanted stars was also not different from surrounding native (un-transplanted) exposed stars (P= 0.14), the expected morphology under complete plastic change.

Stars transplanted to the more sheltered site showed a slight, 10%, decrease in tenacity. This decrease was non-significant (P= 0.33) because of seasonal change. However, the tenacity of transplanted *Pisaster* was significantly lower than that of non-transplanted exposed stars (Figure 4-4B; P= 0.019); it was also not different from surrounding native (non-transplanted) sheltered stars (P= 0.68).

4.2.3 Histology

Stars transplanted into a more exposed site increased the proportion of the total podial cross-sectional area composed of epidermis over the transplant period (Table 4-1; Figure 4-2; Figure 4-5A). Proportional areas of connective tissue, longitudinal muscle and the total body wall showed no significant change during transplantation (Figure 4-5 C-D; P= 0.59; P= 0.13; P=0.45; N= 8). All tissue layers had significantly different relative areas at 300 µm and 600 µm from the basal disc both before and after transplantation – epidermis, connective tissue and the overall body wall was proportionally thicker closer to the disc (P< 0.001, P< 0.001, P= 0.002 respectively) while longitudinal muscle was greater further away (P= 0.003) (Table 4-1; Figure 4-5). There were no significant interactions between factors (P> 0.34 in all cases).

4.3 Discussion

4.3.1 Adaptive Nature of Variation in Tube Foot Tenacity

The risk of dislodgement for an intertidal organism is much higher on wave-exposed than on protected shores. For *Pisaster*, the probability of dislodgement is determined by drag and lift forces according to its body size and shape (Chapter 3) and the attachment strength of the tube feet. Attachment strength is governed by the number of tube feet the sea star uses to attach to the substrate and their adhesiveness. The benefit of increased tenacity in high flow is obvious: more tenacious individuals have a reduced probability of wave-induced dislodgement and death.

The close positive correlation between water velocity and tenacity suggests that increased tenacity is beneficial in high flow but disadvantageous in quiet waters. Probable mechanisms for increasing tenacity in *Pisaster* may include: 1) increasing the attachment force at the point of attachment to the substrate by increasing or modifying adhesive mucus secretion, 2) strengthening the tube foot wall to prevent breakage, or 3) a combination of the two. The most likely disadvantage to greater tenacity is the metabolic cost of producing stronger tube feet or adhesive mucus. When sea stars move over a hard substrata they leave a trail of mucus “footprints” (Flammang et al., 1998), and production of adhesive mucus can be costly, as seen in snails (Denny, 1980). Stronger tube feet may also be metabolically costly as intertidal organisms in high flow have higher metabolic rates when maintaining attachment (Donovan and Taylor, 2008).

However, for *Pisaster* these metabolic costs may be offset by higher food availability in exposed sites (Paine, 1966; Paine, 1974).

4.3.2 Is Tube-Foot Tenacity Plastic?

The observed phenotypic plasticity in tenacity would permit *Pisaster ochraceus* to optimize attachment strength in a changing environment. I found that sea stars underwent a seasonal increase in tenacity from summer to winter: their attachment strength increased in response to seasonal increases in wave heights. This plasticity could also be experimentally induced (Figure 4-4). Sea stars transplanted into a more sheltered site showed a much lower final winter tenacity than those sea stars that remained in their natural exposed site. Those sea stars transplanted into a more exposed site showed increases in tenacity but the increase was not significantly higher than those stars not transplanted, despite being 31% more tenacious. Perhaps rates of plasticity were not fast enough to differentiate them from the non-transplanted stars because both were increasing in response to the seasonal increases in flow. Alternatively, sample sizes may not have been sufficient to differentiate between the two, despite actually having quite different winter tenacities (31%). Tenacity measurements were time-consuming to obtain, which limited sample sizes, and were variable between stars as tenacity probably depended upon many factors, including physiological condition and behaviour of the star being tested. With an increased sample size the difference between the two would probably approach significance.

The plastic change in tenacity following transplantation is assumed to be a response to changes in water flow, as most other abiotic factors were similar between sites. However, the change in wave-exposure may also cause changes in food availability that could confound the observed results, for example mussels in the exposed sites were generally much larger (pers. obs.). In these sites there may be selection against sea stars with weak tube feet that are unable to open large mussels. This potentially confounding effect of transplantation was not likely important for two reasons: 1) *Pisaster* feed very little during fall and winter, the period during which transplants were done (Mauzey, 1966), and 2) non-transplanted sea-stars also showed seasonal change in tenacity. Therefore, the observed modification of tube foot tenacity is likely primarily a response to changes in water flow directly.

Although *Pisaster* adapted to seasonal change in wave exposure, precise rates of plasticity remain unknown. However, differences between the variability of tenacity and aspect ratio can reveal relative rates of plasticity for the two traits - the more plastic trait will respond more quickly to short-term or micro-spatial differences and show greater variability within a site. Although water velocity explained more of the among-site variation in mean aspect ratio (99% versus 92%), aspect ratio had much higher within-site variability causing it to explain less within-site variation (36% versus 55% for tenacity). Although probably dependent upon a greater variety of confounding variables (e.g., nutritional state, mobility), tenacity showed less among-individual variation than aspect ratio, suggesting that it had a slower rate of plastic response (Chapter 3). This slower plastic response in tenacity is not unexpected, other studies examining rates of morphological plasticity in response to wave exposure have found that form responds to seasonal changes but probably not to isolated small-scale perturbations in wave exposure (Carrington, 2002; Marchinko, 2003). Rates of plasticity are probably directly determined by the metabolic cost of change – if change is metabolically costly, as with a morphological change, a slower response time will minimize the risk of wasteful overreaction to minor environmental perturbations and will better track the mean optimal ecotype.

4.3.3 How Does *Pisaster* Change Tenacity?

Histological examination of morphological differences in tube feet of sea stars before and after transplant into a more exposed site revealed that morphological change was limited to an increase in epidermal thickness, particularly in the region close to the adhesive disc of the tube foot (Figure 4-2; Figure 4-5). The structural strength of a tube foot is primarily determined by the thickness of the connective tissue and longitudinal muscle layers, and not epidermal layers (Santos et al., 2005a; Hennebert et al., 2010). The increase in tenacity I observed seems likely not to be a result of increasing the structural strength of the tube foot. However, tenacity may also be increased by increasing the chemical adhesion to the substrate. Adhesive mucus is produced by epidermal tissue on the basal disc of the tube foot (Flammang et al., 1994; Flammang, 1996). The increases in epidermal thickness seen in the tube-foot wall, despite being non-adhesive in nature, could signal a similar increase in the epidermis of the adhesive disc as they both form one continuous layer in the tube foot (Santos et al., 2005b). If chemical adhesion increases in response to flow one would expect increases in tube foot breakage if sea stars were not also increasing the mechanical strength of their tube feet. The transplant data suggest such a response. For sea stars from both Dixon and Helby, tube-foot breakage was greater in winter compared to summer ($P= 0.032$, $P= 0.022$), and within each period, high attachment strengths were correlated with high proportions of tube foot breakage ($P= 0.005$, $R^2= 0.46$; $P= 0.012$, $R^2= 0.35$).

4.3.4 Broader Implications of a Change in Tenacity

The results offerered here reveal the first evidence that intertidal sea stars plastically modify attachment strength in response to variation in hydrodynamic conditions. Such plasticity should come as no surprise because *Pisaster ochraceus* has very high levels of dispersal, both as adults (Robles et al., 1995) and larvae (Strathmann, 1978), and experience large seasonal changes in wave exposure (Harley and Helmuth, 2003). Plastic responses in tenacity may allow *Pisaster* to function effectively despite wide variation in abiotic conditions, which may explain why *Pisaster* can be such an important keystone predator (Paine, 1969). These results, when combined with studies of the detachment forces a *Pisaster* experiences (Chapter 3) and the probabilities of storm events (Denny, 1995), may help us better predict the probability that an individual *Pisaster* might be dislodged during its lifetime. Although intertidal disturbance and its effect on community organization is well studied (Dayton, 1971; Sousa, 1979; Paine and Levin, 1981), little is known about how vulnerable *Pisaster* is to detachment during storm-events. Natural dislodgement does happen, as dead or dying individuals are sometimes observed high on the shore following storms (Hayne & Palmer, pers. obs.). An understanding of dislodgement and disturbance processes in *Pisaster* is of particular importance given its disproportionately large ecological effect on the surrounding intertidal community.

In summary, attachment strength in *Pisaster* appears to vary in response to both spatial and seasonal variation in water velocity in a way that would reduce probability of dislodgement. Changes in tenacity may be achieved by an increase in the strength of chemical adhesion to the substrate. However higher adhesive strengths are likely offset by higher metabolic costs, so the balance of this trade-off will likely depend upon the wave exposure experienced. This plasticity probably allows *Pisaster* to cope with environmental heterogeneity and persist as a keystone predator despite large seasonal differences is water velocity.

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Source of Variation	Epidermis %		
	df	MS	P
Distance From Disc	1	0.0672	< 0.001
Distance x Individual	7	0.0008	
Time Period	1	0.0215	0.011
Time Period x Individual	7	0.0019	
Distance x Time	1	0.0001	0.863
Residual	7	0.0015	
	Connective Tissue %		
	df	MS	P
Distance From Disc	1	0.0174	< 0.001
Distance x Individual	7	0.0004	
Time Period	1	0.0004	0.592
Time Period x Individual	7	0.0012	
Distance x Time	1	0.0003	0.344
Residual	7	0.0002	
	Longitudinal Muscle %		
	df	MS	P
Distance From Disc	1	0.0155	0.003
Distance x Individual	7	0.0008	
Time Period	1	0.0073	0.127
Time Period x Individual	7	0.0025	
Distance x Time	1	0.0001	0.831
Residual	7	0.0057	
	Total Body Wall %		
	df	MS	P
Distance From Disc	1	0.0620	0.002
Distance x Individual	7	0.0029	
Time Period	1	0.0039	0.450
Time Period x Individual	7	0.0061	
Distance x Time	1	0.0002	0.755
Residual	7	0.0066	

Table 4-1. Results from a two-way repeated measures ANOVA testing for differences in relative cross-sectional areas for several tissue layers in the tube feet of *Pisaster* before and after transplant into a more exposed site (time period) and at sections taken from 300 µm and 600 µm from the basal adhesive disc (distance from disc).

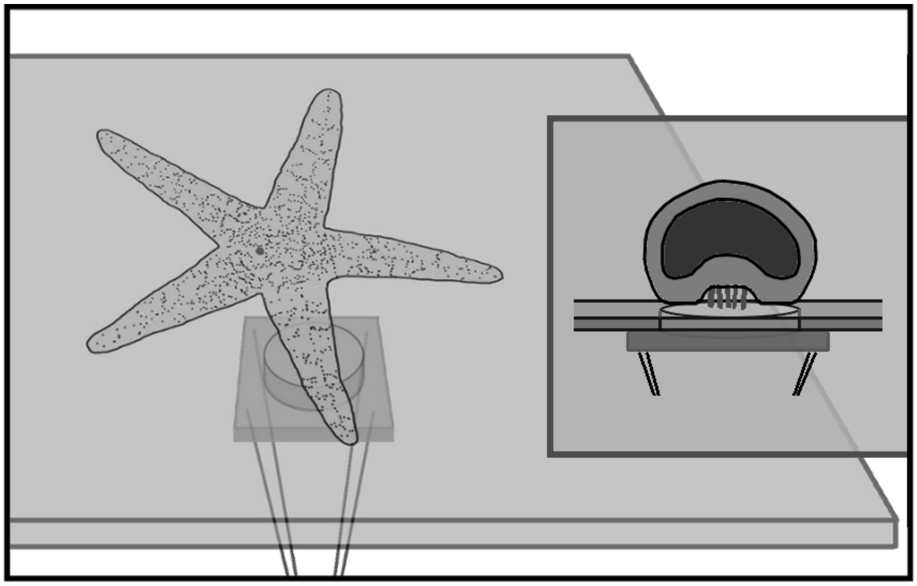

Figure 4-1. Diagram of apparatus to measure tenacity of attached tube feet in top view and side view (inset). The apparatus consisted of a glass disc nestled flush into a large Plexiglas plate. The sea star attached tube feet onto the glass disc, which was pulled down with a recording force meter.

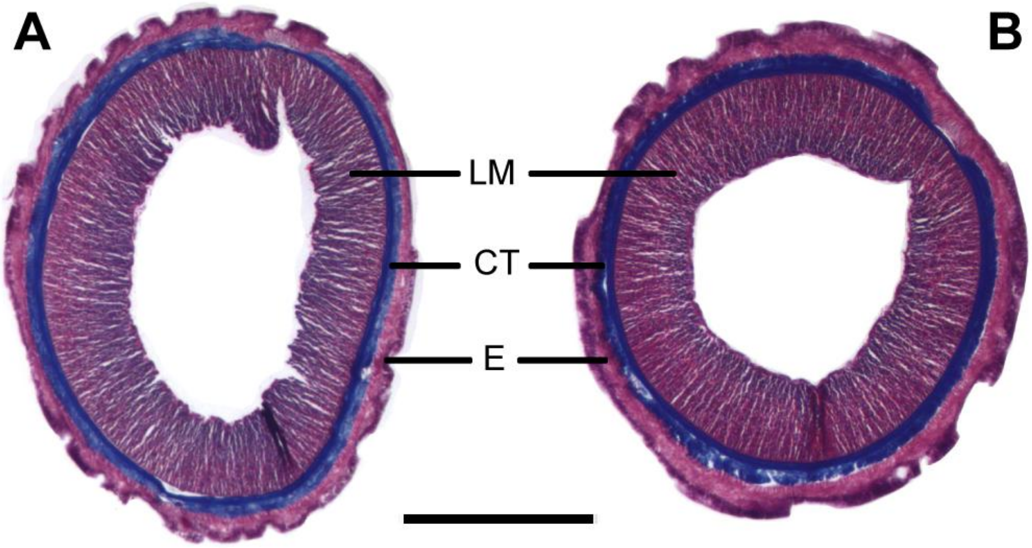

Figure 4-2. Comparison of tube foot cross-sections of a sea star before (A) and three months after (B) transplantation into a more exposed site, on average there was a 30% increase in epidermal area (E) and no significant change in connective tissue (CT) or longitudinal muscle (LM) areas. Section was taken at 600 µm from the basal disc of the tube foot; sea star mass: 234 g, arm length 106.5 mm; scale bar: 100 µm.

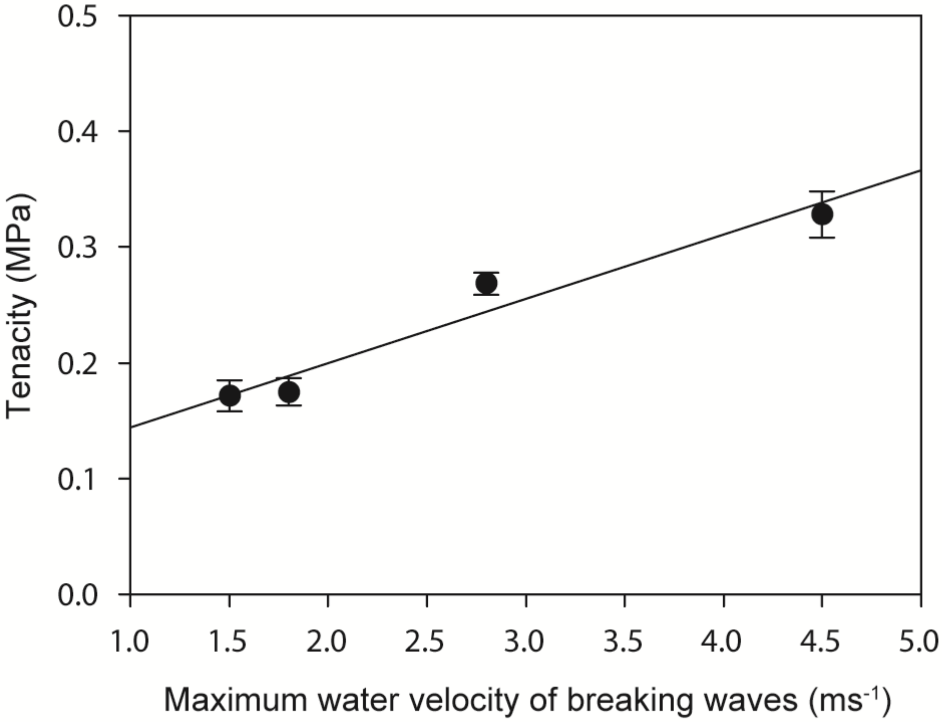

Figure 4-3. Variation in tube foot tenacity of *Pisaster ochraceus* as a function of wave exposure among four intertidal sites in Barkley Sound, British Columbia, Canada. Tenacity is the attachment force per unit area of attachment. Maximum water velocities were relative measures of the maximum velocities experienced at each site. The sites are (left to right) Ross islet sheltered (RS), Dixon Island (DS), Helby Island (HE) and Seppings Island exposed (SE). All points are mean±s.e.m. (OLS regressions: raw data, $F_{1,65}$= 80.8, $P< 0.001$, R^2= 0.55; population means: $P= 0.027$, R^2= 0.92).

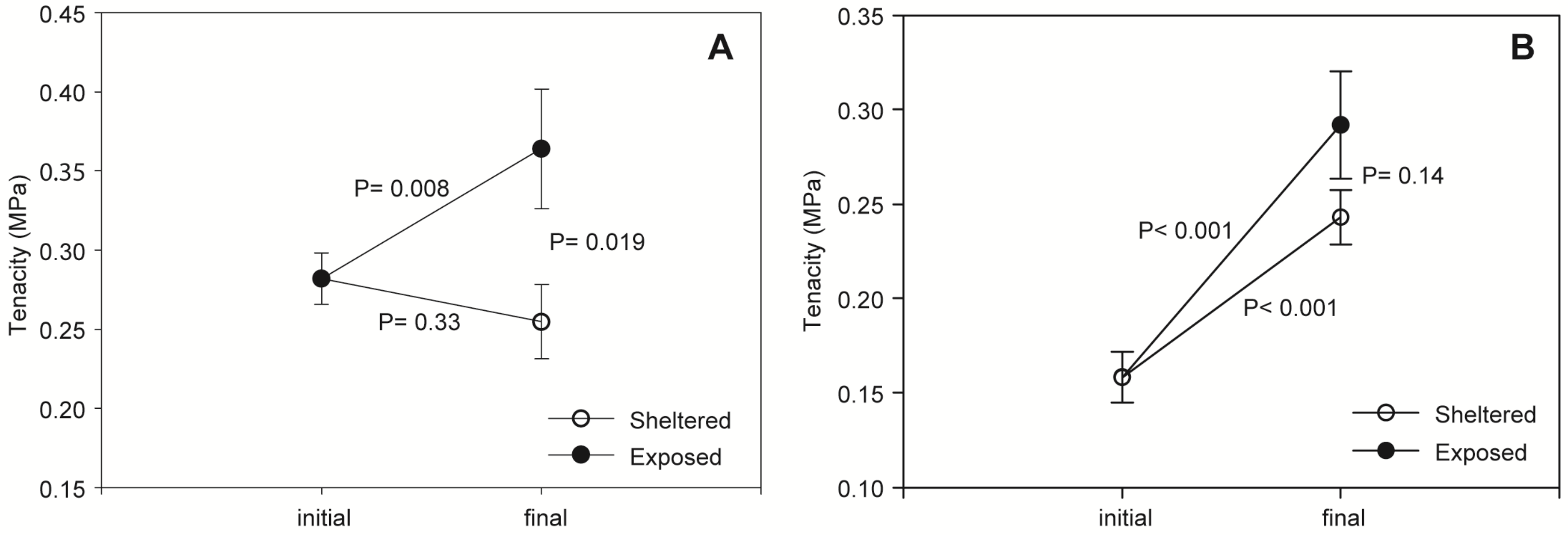

Figure 4-4. Changes in tenacity of *Pisaster ochraceus* from two source populations before and after a three month period from summer to winter 2010. (A) Mean±s.e.m. tenacity of individuals from a sheltered site (DS) before (open circle, left) and after (closed circle, right) transplant into a more exposed site (HE) and winter tenacity of native (non-transplanted) sheltered individuals (open circle, right). (B) Mean tenacity of individuals from an exposed site (HE) before (closed circle, left) and after (open circle, right) transplant into a more sheltered site (DS) and winter tenacity of native (non-transplanted) exposed individuals (closed circle, right). P-values correspond to: the P-value for the difference between initial and final tenacity of transplanted stars, the P-value for the difference between the initial and final tenacity of non-transplanted stars, and the P-value for the difference between the final tenacity of those transplanted and those not.

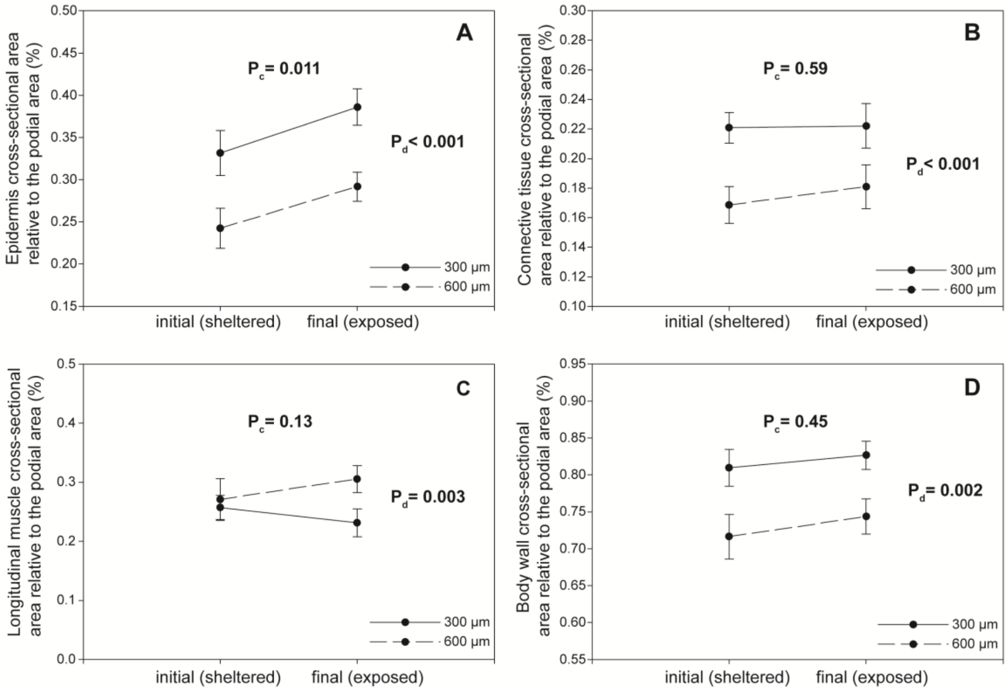

Figure 4-5. Changes in relative tissue layer cross-sectional areas of histological sections taken at 300 µm (solid line) and 600 µm (dashed line) from the basal disc of tube feet removed from sea stars (N= 8) before (initial) and three months after (final) they were transplanted into a more exposed site. (A) Epidermis cross-sectional area relative to total podial area. (B) Connective tissue area relative to podial area. (C) Longitudinal muscle area relative to podial area. (D) Total tube foot body wall area relative to podial area. (P_c) P-value for the difference between initial and final relative areas. (P_d) the P-value for the difference in relative areas between the sections taken at 300 µm and 600 µm.

5. General Conclusions

5.0 Response of Tenacity and Body Form to Changes in Flow

In marine invertebrates, levels of phenotypic plasticity are generally greater in species with extensive larval dispersal (Hollander, 2008). Heterogeneous environments, created through either spatial or temporal variability, also foster high levels of plasticity (Levins, 1968). Although prevalent in variable environments, plasticity is not a necessary solution. A well-adapted, genetically fixed phenotype could also withstand conditions year-round, but a fixed phenotype bears the cost of often being mismatched to the surroundings because it cannot respond to environmental change. Plastic traits also carry the costs of monitoring environmental change and the metabolic expenses of changing the phenotype expressed (DeWitt et al., 1998). Clearly, to understand the evolution of plasticity one must understand both the costs and benefits of plastic responses. Furthermore, because selection acts on the phenotype, and in plastic organisms phenotype is determined by both genotype and environment, plasticity amplifies the range of phenotypic variation upon which natural selection can act, which may influence genetic adaptation to the environment (Via and Lande, 1985) as well as speciation (Agrawal, 2001).

Plasticity is favored in heterogeneous environments because it better allows the phenotype of an organism to track and adapt to changes in the environment (Levins, 1968). Wave-swept intertidal shores are some of the most heterogeneous environments on earth. This heterogeneity is largely caused by seasonal variation in storm activity and differences in spatial orientation among sites (Denny, 1988). Differences in local topography can also cause variation in water velocities experienced over small distances within a site (Denny et al., 2004). The ability to adapt plastically to environmental variation would afford many advantages to a mobile intertidal invertebrate (such as a sea star) by allowing it: i) to adapt to a new area following larval dispersal, ii) to maintain a suitable phenotype as wave exposure varies seasonally, and iii) to adapt to new environments during migration as an adult.

In *Pisaster*, tube-foot tenacity and arm aspect-ratio responded plastically to changes in wave exposure: both increased with increasing exposure. Furthermore, *Pisaster* responded plastically both to experimentally induced changes in exposure during transplantation and to natural seasonal increases in flow at the field sites. Water velocity explained 92% and 99% of the variation in mean tenacity and mean body form, respectively, suggesting that the relationship between exposure and morphology is incredibly precise. This is likely because wave exposure is a major abiotic stressor affecting the distribution and abundance of intertidal organisms (Dayton, 1971). In fact, even the other dominant stressor, desiccation, depends highly upon wave exposure and the resulting splash (Harley and Helmuth, 2003).

5.1 Functional Morphology of *Pisaster* and its Larger Implications

The precision of the relationship between water velocity and both aspect ratio and tenacity in *Pisaster* also suggests that departures from optimum form exact strict penalties. This probably occurs because both tenacity and body form are highly adaptive traits. Other intertidal organisms also show plastic morphological change in response to changes in wave exposure. For example, in high flow kelp and seaweeds develop narrower blades and stronger holdfasts and stipes (Sjnrtun and Fredriksen, 1995; Fowler-Walker et al., 2006), mussels develop more streamlined shells and increased byssal-thread tenacity (Akester and Martel, 2000; Carrington, 2002), and littorine snails produce a more streamlined shell and increased foot size (Trussell, 1997a, b). Alterations in size, shape, or tenacity of attachment under higher flow all likely reduce the risk of dislodgement by minimizing lift and drag forces. The adaptive significance of the plastic changes observed in *Pisaster* is likely the same – increases in aspect ratio and tube foot tenacity reduce the risk of dislodgement. Because of body shape differences, sea stars from the most exposed site would experience a 19 percent lower dislodgement force than sheltered stars in the same flow conditions (Chapter 3). Exposed sea stars also showed greater tube-foot tenacity even though they did not adhere with more tube feet (Chapter 4), suggesting that adaptation to flow is primarily morphological and not behavioral. The combination of these two adaptations represents a significant reduction in dislodgement risk for exposed sea stars.

The tight correlation between water velocity and the state of these plastic traits suggests significant tradeoffs are associated with each morph, as flow-resistant states were only formed when needed. Increased tenacity is likely energetically costly and increased aspect ratio probably increases the risk of desiccation and may reduce fecundity. These costs likely arise from the probable mechanisms that give rise to the plasticity. Increased tenacity appears to be achieved by increased chemical attachment to the substrate through an increase or modification in the production of mucus, which probably has associated metabolic costs, both for the maintenance of secretory structures and for secretion itself. To change aspect ratio sea stars probably change the amount of water stored in the perivisceral coelom, thereby changing wet weight and shape. Increased aspect ratio likely results in lower fecundity and higher desiccation risk, a major stressor and occasional cause of mortality for *Pisaster*. Although more prone to hydrodynamic loading, sheltered stars have a much higher wet weight per unit arm length. This decreases risk of desiccation and increases coelomic volume thereby increasing the available area for gonads.

The costs of these plastic responses may also be relevant to their rate of response. Although the exact rates of the plastic responses for the two traits are unknown, differences in the variability of tenacity and aspect ratio suggest that the rates differ. For plastic traits, those capable of responding to change more rapidly would tend to be more variable within a spatially heterogeneous site. If plasticity is rapid a sea star could adapt to local microenvironments, whereas if the plastic response is slow, movement of the sea star and heterogeneity of wave conditions would yield a morphology that would more accurately reflect "average" flow. Water velocity explained more of the among-site variation in mean aspect ratio than tenacity (99% versus 92%). However, within a site, only 36% of the total among-individual variation in aspect ratio was explained by water velocity compared to 55% for tenacity, suggesting that aspect ratio is the more plastic of the two traits. This makes sense considering the probable mechanisms. Tenacity change involves morphological changes that are likely metabolically costly to carry out. High rates of plasticity for an expensive trait would be disadvantageous in a variable environment because responses to ephemeral variation would be wasteful. Understanding how the costs of plastic change influence their rates is important. Little is known about how rates of plasticity are determined and how differences in rates of plasticity may affect the fitness of a particular species. Presumably, rapid rates of plasticity and small lag times for response would always be favored if they were not associated with higher costs because it would allow the organism to remain locally adapted in spite of sporadic environmental change. An organism like *Pisaster*, which has two traits that respond to one environmental cue, represents a useful system with which to test these hypotheses.

The mechanisms of plasticity in *Pisaster* appear to be different than those seen in other intertidal organisms. All other described plastic changes in form in response to wave-exposure involve tissue growth and restructuring (Trussell, 1997a; Akester and Martel, 2000; Arsenault et al., 2001), but *Pisaster* appears to change its aspect ratio by varying the uptake of water into the perivisceral coelom, the main coelomic compartment that occupies nearly all of the arm of a sea star. Although not connected to the perivisceral coelom, the madreporite is thought to be the primary structure responsible for modifying coelomic fluid volume (Ferguson, 1992). This is done by either increasing or decreasing water uptake through the madreporite, which is directly connected to the water vascular system and then to the perivisceral coelom through a permeable membrane (Ferguson, 1992; Pincebourde et al., 2009). Being able to change its internal volume in a physiological manner likely allows *Pisaster* to respond much more quickly to changes in wave exposure than if tissue growth or restructuring were needed. Although exact rates are not known, *Pisaster* seems likely to respond to changes in flow within several days. Necessary elements for this mechanism include a large coelomic cavity surrounded by a pliable body wall, so cnidarians, sea stars and possibly sea cucumbers may be some of the few animals capable of this mechanism. Some anemones modify their height in response to ambient flow. They actively change shape in high flow thereby minimizing drag (Koehl, 1977).The mechanism of change in tube-foot tenacity by sea stars may also be novel, as most other modes of plasticity in the intertidal zone involve an increase in size or increase in mechanical strength of attachment structures (Trussell, 1997a; Carrington, 2002). Snails and limpets seem able to increase the tenacity of their pedal mucus, as they can secrete a different, stronger type of mucus when attaching rather than gliding (Smith et al., 1999; Smith and Morin, 2002). However, snails from exposed shores increase attachment by increasing foot size and apparently not through modification of pedal mucus (Trussell, 1997a). For *Pisaster* the precise mechanism of modifying attachment is unclear, although it may involve either an increase or modification in production of adhesive mucus rather than a change in breaking strength of the tube feet themselves.

5.2 Ecological Impact of Plasticity in *Pisaster*

Despite being subject to large amounts of wave-induced disturbance, rocky intertidal shores are surprisingly structured. Evidence of this structure is seen in the zonation of dominant species in sequential horizontal bands (Colman, 1933). These bands are ultimately formed by different species outcompeting other species in their band. However, whether these species compete or not depends on predation and tolerance of abiotic factors such as wave exposure and desiccation stress (Paine, 1966). Spatial and temporal heterogeneity in these abiotic factors may have profound effects on the community structure of intertidal shores (Sanford, 1999; Helmuth et al., 2002), as apparent in the radically different zonation patterns seen on wave-exposed and sheltered shores. The future success of any other intertidal organism depends on its ability to adapt to changes in the abiotic factors that govern this zonation (Bradshaw and Holzapfel, 2006). *Pisaster*'s success as a keystone species probably depends heavily on its ability to adapt to changes in a highly heterogeneous environment. As a consequence it plays a key role structuring both sheltered- and exposed-shore communities.

In addition to the benefits plasticity may confer in current heterogeneous environments, plasticity may also influence an organism's ability to cope with impending environmental change. Climate change includes changes not only in average temperatures but also in storm activity and offshore wave heights, particularly through extreme events (Young et al., 2011). This has been seen in both the Pacific (Bromirski et al., 2003) and Atlantic oceans (Bacon and Carter, 1991) and is predicted to continue in the future. The effects of changing temperatures and wave exposure on species distributions have been separately addressed by many researchers. However, the value of studying the integrated effects of climate change has been recently recognized, because studies of responses to just one abiotic factor are of less value (Harley et al., 2006; Helmuth et al., 2006) . The plasticity seen in *Pisaster* illustrates the need for a comprehensive study of factors influencing adaptation. *Pisaster* may be able to accommodate future increases in wave exposure through plasticity in aspect ratio, but increases in aspect ratio would also reduce thermal inertia which may be needed to cope with concurrently increasing temperatures. The impact of climate change on such keystone species could cause widespread community level changes. Although *Pisaster* is plastic in response to both wave exposure and temperature (Pincebourde et al., 2009) the direction of plasticity for each will be in conflict.

5.3 Future Directions

The identification of a new system that exhibits dramatic adaptive plasticity provides many opportunities for future research. This study presents the first evidence of phenotypic plasticity of an echinoderm in response to flow. Considering the ubiquitousness and ecological significance of other echinoderms, a great opportunity exists to study similar adaptations and processes in other species. The plastic responses seen in *Pisaster* are of interest not only for understanding the species' widespread abundance and effect on the surrounding community, but also as avenues for evolution of the species as well. Selection can act not only on new genetically fixed phenotypes but also on variants created through phenotypic plasticity in a manner where phenotype proceeds genotype (Palmer, 2004; Crispo and Rausher, 2007). In the face of future climate change the current range of phenotypes *Pisaster* can generate plastically may not be enough to prevent extinction or at the least range shrinkage, directional evolution of reaction norms may be needed to accommodate unabated climate change.

The results presented in this thesis also reveal a need to be conscious of weight variation due to wave-induced plasticity in future studies of *Pisaster*. Other studies examining growth have used only wet weight as a measure of body size (Paine, 1976; Sanford, 2002). In reality a combination of both weight and arm length are needed to minimize the effect of plastic, flow-induced changes in weight. In both lab and field studies one should expect weight to change due to this plasticity, the former because of a reduction of flow from field conditions and the latter because of seasonal changes in exposure and aspect ratio. Intriguingly, laboratory-reared *Pisaster* have been described as “fatter” than their field counterparts (Morris et al., 1980), but these differences were thought to be due to food availability rather than a plastic response to flow.

Although the association between water velocity and aspect ratio seems clear, more research is needed to account for confounding variables such as temperature, which also affects water retention, and thus possibly shape, in *Pisaster* (Pincebourde et al., 2009). It would be interesting to examine body shape in mid-summer when temperatures are much more extreme. Rates of change in tenacity and aspect ratio should also be examined to gain a better understanding of the spatial and temporal scales over which plasticity occurs. Finally, *Pisaster* may represent a reliable biological indicator for measuring and comparing wave exposure, as aspect ratio can be easily measured and is tightly correlated with water velocity. This is particularly promising given the wide range of habitats in which *Pisaster* occurs, although the pattern seen in Barkley Sound must be verified for other sites.

5.4 Literature Cited

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