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NEIGHBOURS AT WAR: AGGRESSIVE BEHAVIOUR AND SPATIAL RESPONSIVENESS IN THE ANEMONE, ACTINIA TENEBROSA.

This thesis is completed in part of a Masters of Conservation Biology Degree.

Georgia Balfour | Masters of Conservation Biology | July 27, 2017

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“Ehara taku toa ite toa takitahi, engari he toa takimano.

My success is not that of my own, but the success of many”

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GENERAL ABSTRACT:

Habitable space is precious and animals have developed a wide variety of mechanisms to acquire and defend favourable space. Aggression is considered any animal behaviour that involves actual or potential harm to another animal of either the same or different species. Agonistic behaviours must also be considered as it is any social behaviour related to fighting. Both aggressive and agonistic behaviours are observed in many animal species as resources including light exposure, nutrients and mates are often limited. Although agonistic behaviour varies among species, agonistic interactions can be partitioned into three specific types of behaviours: threat, aggression, and submission or avoidance. While any one of these behaviours can be observed in isolation, in an interaction between two animals, there is normally a sequence of behaviours which can culminate in combat. Anemones have unique adaptations such as clubs, fighting tentacles, bundles of stinging cells, sweeper tentacles and acrorhagi that allow them to defend themselves from competitors. Previous research also suggests that anemone populations are a collection of clusters of genetically similar which assemble via limited dispersal and locomotion. In chapter two I examined the effect of aggression on individuals at varying distances and predicted that those anemones that are initially located in closest proximity (<1 centimetre) in the field will be less aggressive towards each other than those anemones found further away from each other. Overall, my results suggest that *Actinia tenebrosa* have an obvious sequence of aggressive behaviours, and that indeed, aggressive behaviours were less common and less severe between nearest neighbours than among individuals sampled at greater distances. My results also show that aggressive behaviours are typically only expressed when individuals are within close proximity of each other <10cm. This behaviour is important to understand as it aids in fully understanding how aggressive behaviours determine dominance hierarchies and the spatial arrangement in *A. tenebrosa*. In chapter three, I investigated whether there was evidence for an ideal spatial arrangement of individuals in the field by testing whether individuals return to a similar spatial arrangement if randomised. The results from this chapter suggest that there is no single ideal spatial arrangement of individuals but rather individuals will find a spatial arrangement that is stable. I also observed that there appears to be an acclimation between individuals that resulted in a favourable position within the cluster. Lastly, I observed that

instead of trying to return to a specific aggregation, individuals acclimate each other and move relative to those individuals surrounding them, much like stars in the sky. The results from this study would suggest that spatial structure of individuals in the field is dependent on intraspecies interactions and the recognition of individuals.

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Chapter One: General Introduction



CHAPTER 1: GENERAL INTRODUCTION

AGGRESSION, A COSTLY ENDEAVOR:

Aggression is ubiquitous in animals (Laidre & Johnstone 2013). Aggression is an animal behaviour that involves actual or potential harm to another animal of either the same or different species while agonistic behaviours are any social behaviour related to fighting (Stuart-Fox, Moussalli & Whiting 2007; Rendall, Owren & Ryan 2009; Laidre & Johnstone 2013). Agonistic behaviours have wider meaning than simply aggressive behaviours they include coercions, shows, retreats, avoidance, placation, posturing and conciliation (Rendall *et al.* 2009). Both aggressive and agnostic behaviours are observed in many animal species because resources including food, shelter, and mating partners are often limited (Stuart-Fox *et al.* 2007; Laidre & Johnstone 2013) (Heppner 2008). Aggression and agnostic behaviours are used to secure resources through the use of displays (growling, barking, tooth displays, raising hackles) or through aggressive acts (biting, kicking, striking, charging, bucking) (Rendall *et al.* 2009; Laidre & Johnstone 2013). Although agonistic behaviour varies among species, agonistic interaction consists of three kinds of behaviours: display threat, aggression, and avoidance, these three behaviours are functionally and physiologically interrelated with aggressive behaviour (Heppner 2008). While any one of these degrees of behaviours can be observed alone, they most commonly are observed in a sequence with a starting behaviour and ending response. (Rendall *et al.* 2009).

Any interaction between individuals can be defined in a four-parts Signal, Intent, Response and Fitness consequences (Laidre & Johnstone 2013). Signals are the acts or structures produced by signallers (Stuart-Fox *et al.* 2007). There are many types of signals including chemical, seismic, acoustic, optical, thermal and tactile, though not all signals are obvious (Stuart-Fox *et al.* 2007)(Laidre & Johnstone 2013).. Intent, conveys information about the display to the recipient and the response is response of the recipient to the signal and intent (Laidre & Johnstone 2013). The response results in fitness consequences that, on average, are neutral or positive for both the signaller and the recipient

(Laidre & Johnstone 2013). An example of this interaction sequence can be observed when a hermit crab raises its claw, performing a threat signal, it provides information about its intent to attack, which a recipient can then respond to by fleeing, which ultimately results in both the signaller and the recipient benefiting by avoiding an escalated fight (Laidre & Johnstone 2013). There are varying degrees of escalation within any interaction (Rendall *et al.* 2009). Depending on the availability and importance of a resource, behaviours can range from brief displays of intent, to a fight to the death, given the fitness costs of a fight to the death, threat displays are the most common form of agonistic behaviours (Riechert 1988).

Many animals have evolved specialized behaviours and corresponding morphology to aid in displaying intent, defence or attack (Guilford & Dawkins 1991). However, these adaptations such as specialised morphological features (e.g. horns) or behaviours (e.g. vocalisations) often come at a cost as they divert energy requirements from other functions (Riechert 1988; Davies, Krebs & West 2012). There are two responses to signals with an aggressive display an avoidance tactic or a fight tactic (Davies *et al.* 2012). Display behaviour tends to express aggression or are in response to confronting aggression or a perceived threat (Seyfarth & Cheney 2017). An example of a display used to show aggression (usually by predatory animals) can be observed in many families such as Canidae (wolves, dogs). When showing aggression or threatened these animals snarl and expose large teeth to intimidate, out of fear or to warn other individuals or prey of how dangerous they can be (Mech 1999). This can be costly as the show can be interpreted as an invitation to engage in combat and can lead to defeat, injury or can potentially be fatal (Mech 1999). Avoidance behaviour is a type of activity, seen in animals exposed to adverse stimuli, in which the tendency to act defensively is stronger than the tendency to attack (Davies *et al.* 2012; Laidre & Johnstone 2013). An example of avoidance is observed in rodents such as rats and mice, that will use cunning and its small body form to quickly evade and hide from potential threats (Nemati, Kolb & Metz 2013). However, these behaviour are context dependent as an individual will attack or retaliate if cornered with no way of escape (Nemati *et al.* 2013). The decision to attack is typically a last resort as the risk of death is high if unsuccessful.

Typically, we distinguish aggressive behaviours into two classes: interspecific (e.g. predatory/antipredatory) and intraspecific (e.g. competition) aggression (Heppner 2008; Davies *et al.* 2012). Interspecific aggression is most commonly observed during predatory encounters, or intimidation. Intraspecific aggression is considered to be a mechanism to establish a hierarchy between individuals for access to material resources such as food and space as well as mating partners (Herczeg & Välimäki 2011). Intraspecific aggressive behaviours are often used to exclude intruders from defended spaces and to avoid competition for resources, and thereby combat among individuals (Herczeg & Välimäki 2011).

AGGRESSION AND ITS EFFECTS ON SPATIAL ARRANGEMENT:

When describing aggression, one must consider whether the animal is sessile or mobile. For sessile or sedentary organisms, attaining and defending habitable spaces is significantly more important than for highly mobile organisms (Okamura & Hatton-Ellis 1995; Svensson & Marshall 2015). Sea anemones are not known for their locomotion; however, this doesn't mean they can't move (Ottaway 1978; Ayre 1982, 1987). Species from the family Actiniidae have an adherent pedal disc that is used in locomotion by contracting and detracting muscles around the foot allowing movement, albeit very slowly (Ottaway 1979a; Brace & Santer 1991; Biewener & Daniel 2010). Anemones are known to move in response to environmental changes such as changes in nutrient flow, competition, predation and light exposure or chemical pressures such as pollution, fertilizer run off, increased salinity and oxygen depletion (Ottaway 1978; Ayre 1987; Ayre & Grosberg 2005; Sherman & Ayre 2008). Studies have also shown that individuals can also detach from the strata and inflate their muscular foot to act as a swim bladder and move with the ocean exchange to a more viable location. (Riemann-Zürneck 1998; Veale & Lavery 2012). Although anemones tend to live a sedentary lifestyle, studies suggest that individuals are capable of sensing their surrounding and adjusting their position to a limited degree (Sherman, Peucker & Ayre 2007).

Habitable space is the most sort after commodity for many sessile or sedentary marine invertebrates species (Sherman *et al.* 2007) as the decision to occupy a particular location is often irreversible or relatively difficult to adjust. Therefore, the location an individual chooses must have an

environment that is conducive to survival and (Grosberg 1988; Sherman *et al.* 2007; Sherman & Ayre 2008). Accordingly, the spatial arrangement of individuals in populations of sessile or sedentary species can have long lasting effects on competition for resources and mating opportunities (Birch 1957; Sherman & Ayre 2008). Accordingly, many animals maintain territories and continual aggression is avoided by the spatial arrangement of these territories. Moreover, not all territorial boundaries are defended equally – some are porous whereas others are fiercely defended (Davies *et al.* 2012) – indicating that there must be some mechanism that allows individuals perceive each other and respond accordingly.

ANEMONES, AN AGGRESSIVE SPECIES:

Anemones have a number morphological and behavioural traits developed specifically for aggressive behaviours (Ottaway 1978; Bigger 1980; Ayre 1982; Ayre & Grosberg 2005; Veale & Lavery 2012). Many anemone species have specialised cells such as clubs, fighting tentacles, acrorhagi and bundles of nematocysts (stinging cells) that are used to sting opposing individuals (Ayre 1982; Ayre & Grosberg 2005; Veale & Lavery 2012). The toxin in an anemone's stinging cells are so potent that the affected area of the stung polyp can undergo necrosis (Norton 2009; Frazão, Vasconcelos & Antunes 2012). This interaction will result in damage to the recipient and either an avoidance response (retraction) or retaliation (Brace & Santer 1991; Foster & Briffa 2014). Importantly, there are a number of aggressive behaviours that are easily observed using the human eye (swelling, exposure of acrorhagi, exposure of mesentery, mucus, tentacle and column damage) (Ayre 1982; Ayre & Grosberg 1995; Foster & Briffa 2014). These adaptations can be costly as individuals not only have to maintain these specialized adaptations but are also open to damage including burning, scarring, tentacle rot during combat (Rudin & Briffa 2012) (Ayre & Grosberg 1995; Foster & Briffa 2014)

Also, The Waratah or Red Beadlet Anemone, *Actinia tenebrosa*, is most commonly found in high rocky intertidal zones along the coasts of Eastern Australia and New Zealand and has been used as a model species to examine aggressive responses (Ottaway 1979; Ayre 1984a; Ayre, Read & Wishart 1991; Veale & Lavery 2012). The Waratah anemone is often the most common anemone in these regions and they tend to have quite large populations and geographical range (Ottaway 1979; Ayre

1984a; Ayre *et al.* 1991; Veale & Lavery 2012). This species of anemone is found in cracks and crevices, overhangs and under boulders high on rocky shores (Ayre & Grosberg 1995; Sherman *et al.* 2007; Veale & Lavery 2012). At low tide, the tentacles retract and the anemone closes to resemble a dome shaped red-brown jelly (in varying sizes-,5cm up to 40cm) (Ayre 1985; Veale & Lavery 2012a). At high tide or when submerged in rock pools the anemone opens to display its feeding tentacles, these tentacles are located around the three rings around the edge of the anemones aperture (Veale & Lavery 2012). The colouration of the anemone is dependent on the amount of light that reaches the external surface of each anemone, and anemones found sheltered under rocks and hangings tend to be a richer red or brown (Ottaway & Kirby 1975; Ayre 1988; Veale & Lavery 2012). While those anemones that are exposed to long periods of sunlight tend to be dark red or green in colour (Ottaway 1972). *Actinia tenebrosa* can reproduce both asexually and sexually and are a viviparous species that often brood their young inside its body cavity (Black & Johnson 1979; Ayre 1984, 1988; Veale & Lavery 2012a). When the offspring are fully developed or in response to stress, small fully developed juvenile anemones are expelled through the mouth (Ottaway & Kirby 1975; Black & Johnson 1979; Ottaway 1979a). The juveniles often attach themselves to rocks in close proximity to the “parent” forming clusters of clones (Grosberg 1988; Veale & Lavery 2012). The Waratah anemone can also reproduce sexually with planktonic larvae settling far away from the parent individual (Ottaway & Kirby 1975; Sherman *et al.* 2007).

Acrohaial fighting has been observed between pairs of adult *Actinia tenebrosa*, and such fighting almost always results in the withdrawal of the loser (Ottaway 1978; Sherman *et al.* 2007). It is widely recognized that anemones retract their tentacles when threatened allowing them to take shelter using their bodies as protection from any perceived threat (Ayre 1982; Ayre *et al.* 1991; Veale & Lavery 2012). Adults have also be observed to attack and kill juvenile clonemates in laboratory conditions (Ayre 1982). Studies have observed that conflicts in *A. tenebrosa* developed disproportionately more often when adults were genetically more distinct (Ayre *et al.* 1991; Ayre & Grosberg 1995; Veale & Lavery 2012). Studies suggest that neither the sexual maturity nor presence of brooded juveniles affected the probability that conflicts would develop between adults (Ayre 1984; Sherman *et al.* 2007).

Presentation of apparently asexually produced juveniles to adults revealed that both the recognition and effector phases of the acrorhagial response were triggered by genotypic dissimilarity, not by size and that acrorhagial responses were initiated only on contact with juveniles from other adults (Black & Johnson 1979; Ayre 1982; Veale & Lavery 2012). It would seem that relatedness and aggression seems to play an important role in competition for recruitment space and thereby the spatial arrangement of individuals within anemone populations.

This thesis is composed of four chapters, two of which are written in manuscript format (Chapters 2 and 3). Accordingly, there is some repetition of content and methodological details between chapters 2 and 3. Last, chapter 4 aims to synthesise the findings of chapters 2 and 3. Nonetheless, all four chapters contribute to the understanding of aggression and the spatial distribution of the anemone, *Actinia tenebrosa*. More specifically, Chapter 2 investigates the behavioural and interaction responses of *A. tenebrosa* in relation to individuals observed at different distances from each other in the field. I predict that those anemones that are initially located nearest to each other will have less aggressive responses than those anemones found at greater distances. Should this prove true, it would help to quantify behavioural responses across populations and enhance understanding of how and why aggressive behaviours escalate in *A. tenebrosa*. Chapter 3 investigates the spatial arrangement of *A. tenebrosa*, and in particular whether individuals return to similar spatial arrangement to that found initially in the field. I predict that the spatial arrangement of individuals in the field was deterministic and thus individuals would revert to the same spatial arrangement as we found them in the field. It would also suggest that the fine-scale spatial arrangement of individuals is dependent on individual recognition and the response to potential threats.

Chapter Two: Neighbourhood Aggression:



CHAPTER 2: NEIGHBOURHOOD AGGRESSION:

2.0: ABSTRACT:

Aggressive behaviour is ubiquitous in animals and is often associated with competition for resources. In sessile marine invertebrates, habitable space is often the most limiting resource and accordingly, animals have developed a wide variety of mechanisms to defend habitable space from interspecific and intraspecific competitors. Anemones have unique adaptations such as clubs, fighting tentacles, bundles of stinging cells, sweeper tentacles and acrorhagi that allow them to defend themselves, as well as attack competitors. *Actinia tenebrosa* has four key behaviours: initial tentacle interaction, inflated acrorhagi, overtopping and extended mesentery. I predicted that those anemones that are initially located in close proximity (<1 centimetre) in natural populations will be less aggressive towards each other than those anemones found further away from each other. Overall our results suggest that *A. tenebrosa* have an obvious sequence of aggressive behaviours, and that more severe aggressive behaviours are less common between nearest neighbours than among individuals sampled at greater distances from each other. Moreover, we show that aggressive behaviours are typically only expressed when individuals are within close proximity of each other <10cm. The clear sequence of aggressive behaviours and the greater likelihood of aggressive acts towards more distant individuals suggest that there may be a dominance hierarchy underlying the spatial arrangement of *Actinia tenebrosa* in natural populations.

2.1: INTRODUCTION:

Aggressive behaviours are often associated with competition for resources and are common among individuals of the same and different species (Davies *et al.* 2012). In animals living in groups, where interactions among the same individuals are frequent, intraspecific aggression is a mechanism to establish a hierarchy between individuals which can determine access to nutritional resources and mating opportunities (Heppner 2008). Many animals have evolved specialized morphologies and behaviours to aid in the effectiveness of aggressive displays or acts (Williams 1991; Rudin & Briffa 2012). Display behaviour shows aggression or is used in response to confronting aggression or a perceived threat (Laidre & Johnstone 2013; Seyfarth & Cheney 2017). An aggressive display is a

behaviour in which an individual communicates intent (Laidre & Johnstone 2013). The opposite of this, are avoidance behaviours or defensive morphologies. These occur in response to adverse stimuli where those affected animals have the tendency to avoid an interaction rather than to attack (Rendall *et al.* 2009; Laidre & Johnstone 2013). These adaptations can be costly to an individual as the behaviour or morphology may require diverting energetic resources from other functions, or place the individual at greater risk of harm or death (Riechert 1988).

An individual's ranking within a dominance hierarchy determines access to resources such as food, mates, shelter, and other commodities that influence survival and fitness (Brace 1981; Laidre & Johnstone 2013). Dominance hierarchies are best known in social mammals, such as baboons and wolves, and in birds, notably chickens (Laidre & Johnstone 2013). In most cases, the dominance hierarchy is relatively stable from day-to-day, conflict is rare and subordinate animals typically concede when confronted by an animal of higher rank (Brace 1981, 1990). Dominance hierarchies are often used to determine territorial boundaries among individuals, and understanding the dominance hierarchy of a population can help understand the spatial distribution of individuals (Brace, Pavey & Quicke 1979).

Behavioural interactions are often didactic and determine factors such as rank, intent and threat which in turn determine the fitness costs of engaging or avoiding conflict (Laidre & Johnstone 2013). Although cnidarians are a relatively primitive group, they have a surprising repertoire of behavioural responses associated with both interspecific and intraspecific aggression (Ayre 1982; Ayre & Grosberg 2005). *Actinia tenebrosa* is a well-documented example, with strong intraspecies aggressive behaviours. This species has specific fighting tentacles called acrorhagi which consist of concentrated bundles of stinging cells located around the dorsal perimeter of the anemone (Ottaway 1978; Ayre & Grosberg 2005). Studies have shown that inflation of the acrorhagi is a highly aggressive response observed in *A. tenebrosa* (Ayre 1982; Ayre & Grosberg 2005). Acroahagial scarring occurs via tentacle contact and contact to the stinging cells (nematocysts) at the base of the tentacles (Ayre 1982; Ayre & Grosberg 2005). These nematocysts are concentrated bundles of acroahagial tissue that are primarily used for capturing food or combat (Ayre 1984). In extreme cases, anemones will often press

their entire body on top of another individual; a behaviour called overtopping. This ensures that there is a strong likelihood of nematocyst contact with the other individual to cause the greatest damage possible to the opponent (Ayre & Grosberg 1995; Turner *et al.* 2003). Most encounters will result in tentacle scarring on one if not both opponents. Extending the mesentery, is an internal structure pushed out of an individual's oral opening and is thought to be a response to alleviate possible damage to an anemone's anatomy (Ayre 1982; Ayre & Grosberg 1995). This response can often be observed well before direct contact of individuals (Ayre & Grosberg 1995). These biological adaptations and the presence of aggressive behaviour are important as they allow us to effectively study the effects of dominance hierarchies on the spatial arrangement of individuals.

Actinia tenebrosa is most commonly found in clusters with individuals in close proximity to each other (less than 20 cm) (Ayre 1987). These clusters typically consist of upward of ten individuals aggregated in a favourable area such as rocky overhangs, sheltered outcrops or underneath boulders (Veale & Lavery 2012). These clusters can vary in shape depending on the surrounding environment (Ayre 1987) and studies suggest that these clusters often consist of clones or closely related individuals (Ayre & Grosberg 1995, 2005; Veale & Lavery 2012). Clonemates include those individuals resulting from binary fission and are exact clones of the parent (Ottaway & Kirby 1975; Sherman *et al.* 2007). Although these clusters typically contain genetically similar individuals, studies suggest that anemone species can acclimate to the presence of unrelated individuals following extended periods of exposure and direct contact between individuals (Ottaway & Kirby 1975; Ayre & Grosberg 1995). (Ottaway 1978; Turner *et al.* 2003; Foster & Briffa 2014).

In this study, I conducted a laboratory study investigating aggression responses among individuals of *Actinia tenebrosa* collected at different distances from each other in the field. I predicted that those anemones that are located in close proximity to each other in the field (>1 centimetre) will be less aggressive towards each other than those anemones found at greater distances from each other. In investigating these factors, we can determine the effects of aggressive behaviours and dominance hierarchies on the spatial arrangement of individuals in natural populations of *Actinia tenebrosa*

2.2: METHODS:

Sites and Species: Individuals were collected from three different locations (Pilot Bay, Mount Maunganui; Anzac Bay, Bowentown and Waihi Beach, Waihi.) in the Bay of Plenty, New Zealand (Figure.1). The field sites were all semi-sheltered rocky intertidal outcrops with regular tidal exchange. At the field sites, individuals typically occupy sheltered, rocky, cracks and crevices in the high intertidal zone. Individuals are often found in clusters of up to ten individuals, and although the size of individuals in these clusters can vary, we only collected individuals between 10mm and 30mm pedal disc diameter. *Actinia tenebrosa* were the most commonly observed anemone species at these three sites. Seawater from each location was taken at the same time as animal collection and used in laboratory experiments.



Figure 1: Map of the Western Bay of Plenty, New Zealand. The yellow symbol indicates the Waihi Beach study site, the green symbol indicates depicts Bowentown study site and the red symbol indicates the Treasure Island study site.

2.2.1) Pedal disc measurement and weight: Because of the anemones' hydrostatic skeleton, it is difficult to attain accurate and non-invasive measurements of size or mass (Ottaway 1978; Angeli *et al.* 2016). Nonetheless, studies have suggested that pedal disc diameter could be used as a reliable indicator of mass (Angeli *et al.* 2016). Therefore, prior to the commencement of the aggression experiment ten individuals from each site were collected to determine whether pedal disc measurements could be used as non-invasive method to measure anemones mass. Upon collection, wet weight and the diameter of the pedal disc was measured at its narrowest and widest point. The animals were then dried in an 80-degree oven for 24 hours before measuring dry weight.

Experimental design, sample collection and animal husbandry: This study had three orthogonal factors: location considered at three levels (Waihi, Bowentown, Mount Maunganui); Spatial arrangement considered at three levels: nearest neighbour (NN), mid-range (MR), far-range (FR) and the distance between two individuals in the experimental tank which was considered at four levels: touching or 0cm, 5cm, 15cm and 25cm. For each location, there were 15 replicates of the NN, MR and FR levels of the spatial arrangement treatment. For NN pairs we collected an individual and its nearest neighbour (NN), for MR pairs we collected an individual as well as an individual between six and seven metres away and for FR pairs we collected an individual as well as an individual fifteen to sixteen metres away. Individuals were placed in separate plastic containers at the time of collection and maintained in isolation until the experiment to minimize the risk of acclimation to a specific neighbour (Ottaway & Kirby 1975; Ayre & Grosberg 2005). Specimens that were damaged (pedal disc or column rupturing) during collection and transportation were returned to their original location and a new pair was collected. The experimental individuals quickly adhered to the plastic experimental aquaria where they remained for two days prior to the experiment and were supplemented with raw green-lipped mussel *ad libitum*.

The experimental tank was measured and marked with the distances at which we would score behaviours (25cm apart, 15cm apart, 5cm apart and 0cm/touching) (Figure 2). For the experimental trials, we randomly selected one pair of individuals and carefully cut-away the plastic aquaria around each anemone, and transferred the circular, plastic discs with the attached anemones to the isolation chambers placed at opposing ends of the experimental

tank. The experimental individuals were left in the isolation chambers for two minutes to acclimate. After two minutes, we lifted the isolation chamber and began observing behaviours. After two more minutes, we moved the animal's closer together (from 25cm apart to 15cm) and we observed their behaviour for 2mins. Two minutes later we moved the animal's closer again (15cm apart to 5cm) and we observed their behaviour for 2mins. The animals were then moved closer for the last time (5cm-0cm/touching) and we observed their behaviour for 2mins. Once this was completed the animals used were placed back into individual containers. This process was repeated for all 45 pairs of individuals.

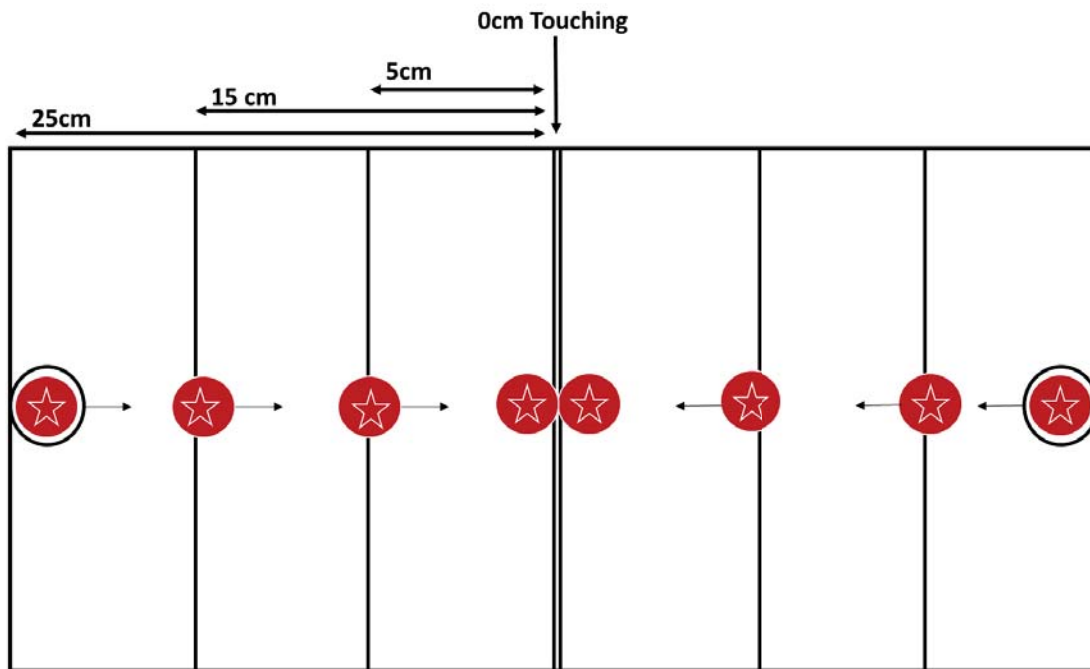


Figure 2: Experimental tank set up. The two experimental anemones examined in each tank are depicted by red stars. At the beginning of the experimental trial the two individuals were at opposite ends (25 cm) of the tank and stayed isolated in chambers (black circle) for 2 minutes. After the 2minutes, individuals were moved closer together to 15cm, after 2 minutes individuals were move closer to 5cm, finally after 2 minutes individuals were moved so that columns were touching (0cm) and left for a further 2 minutes.

2.2.2) Aggressive Responses: This study considered of two different types of aggressive or agonistic responses. First, interaction behaviours capture different degrees of aggressiveness or evasiveness towards another individual. Tentacle interaction (Figure 3A) is often the first behaviour observed and involves the extension of an individual's tentacles toward another individual. Inflated acrohagi (Figure 3B) occurs when the anemone inflates the aperture opening and parapet on the offending side and enlarges its blue nematocysts (stinging cells). Inflating the acrohagi increases the surface area of the acrohagi to either expand exposure of nematocyst or deter the advancement of the invading anemone. Overtopping (Figure. 1, C) occurs when an individual increases its surface area by inflating its parapet (collar), column (outer body wall) and acrohagi and then pushes itself atop another individual. The second types of behaviours were response behaviours which are a direct consequence or response to interaction behaviours. Retraction (Figure 3D) involves retracing all extremities inside its actinopharynx (the tubular throat of the polyp), hence exposing its least vulnerable surface as protection. Extension of the mesentery (Figure 3E) involves the extension of the mesentery (a fold of tissue that attaches organs to the body wall) out of the actinopharynx and in extreme cases can lead to the excretion of partially digested food. This response can be observed not only when individuals are directly attacking or being attacked but has also been observed at distances between individuals of up to 30cm.

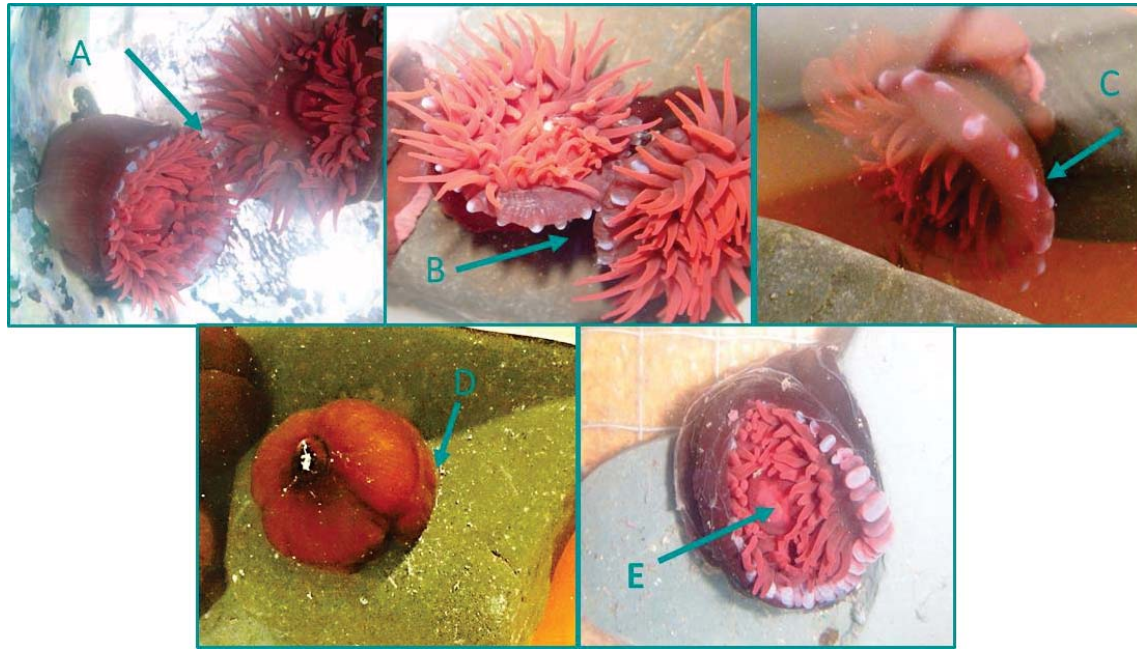


Figure 3: Interaction and response behaviours in *Actinia tenebrosa*: (A) Initial tentacle contact (B) Display of inflated acrorhagi (C) Overtopping (attack with acrorhagi (D) Retraction (E) Extension of the mesentery.

2.2.3) Aggressive Dominance Hierarchy: Some aggressive responses are considered more severe or more aggressive than others (Brace *et al.* 1979; Laidre & Johnstone 2013). Studies suggest that initial interactions tend to be the least damaging with tentacle touching being the least aggressive behaviour, followed retraction, extended mesentery and inflated acrorhagi in escalating order (Ottaway 1978; Ayre 1982). Overtopping is likely the most aggressive response as it involves large surface dominance over another individual (Ayre 1982).

2.2.4) Data Analysis: To determine the effects of spatial arrangement on aggression we used PERMANOVA+ (PRIMER 7) to partition variation in behaviour. Our model considered spatial arrangement as a fixed factor with three levels: Nearest Neighbour (NN), Mid-Range (MR), Far-Range (FR); location as a fixed factor with three levels: Bowentown, Waihi and Treasure Island; and distance as fixed factor with three levels: 0cm, 5cm, and 25cm. We omitted the 15cm distance from formal analyses as no anemones displayed any aggressive behaviours at this distance (Figure 4C). Tank and individual were included as random effects to account for random variation among pairs of individuals within each tank as well as the repeated measures on each individual. If we found that aggressive behaviours were more common for MR or FR pairs than for NN pairs, a significant treatment by distance interaction, this would support our hypothesis that the spatial arrangement of individuals in the field

reflects their aggressive dominance hierarchy. Alternatively, a main effect of the spatial arrangement treatment would indicate that individuals express different aggressive behaviours towards individuals from different distances.

Although location could be considered a random effect, we have chosen to include location as a fixed effect in our analyses for two reasons. First, because we only sampled three locations the precision of the estimate of the dispersion among locations would be very low. Second, and more importantly, although *A. tenebrosa* is distributed from eastern Australia to southern New Zealand we only sampled three populations within a restricted geographical area (35 kms between the furthest locations). If we had considered location as a random factor, we would be assuming that these three locations are a representative sample of all the available populations. We felt that this assumption was difficult to justify given that we know very little about other populations of this widely distributed species. Nevertheless, we acknowledge that the inferences of our study should be interpreted with some caution.

To construct the distance matrix that was used as the response in our analyses, we started by creating a contingency table where the columns were the behaviours and the rows were each observation. The entries in each cell would take on values of either zero or one indicating that behaviour was observed or not. We then χ^2 centred the data and calculated the Euclidian distances among the χ^2 centred behaviours. Because the procedure results in a distance matrix that can be analysed using PERMANOVA, we were able to examine variability in behaviour in detail and in accordance with our relatively complicated experimental design.

2.3. RESULTS:

2.3.1) Weight vs Pedal area: To test our hypothesis that pedal disc diameter is associated with dry weight and could be used to measure anemone mass, we conducted a regression using the dry weight as the response and pedal disc diameter as the predictor. Although we were able to detect a positive relationship between pedal disc diameter and dry weight ($\text{dry weight} = 0.1764 + 0.0001 \times \text{Pedal area}$) as in previous studies (Ottaway 1978; Angeli *et al.* 2016) (Figure5) the overall fit of the model to the data was poor ($R^2 = 0.2$).

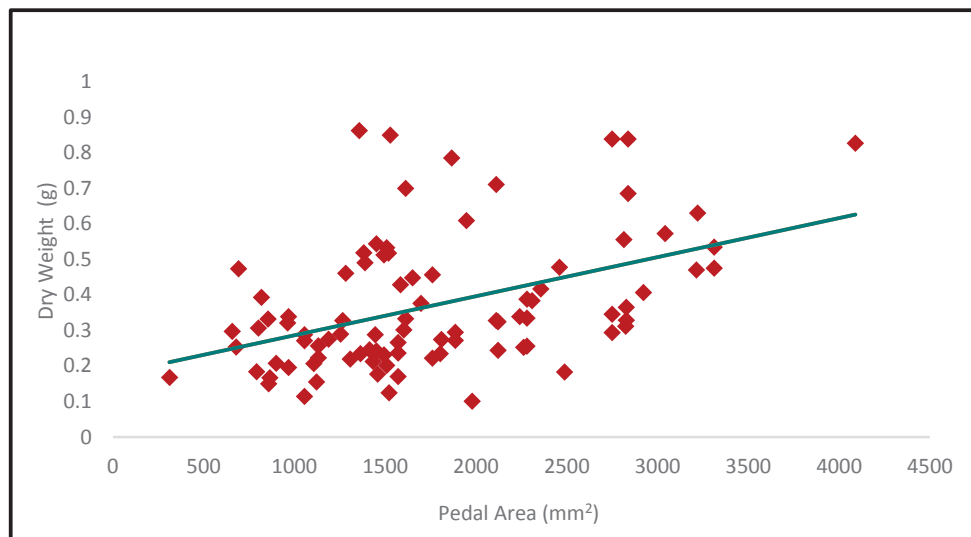


Figure 4: Regression of pedal area and dry weight. Although there is a positive relationship between pedal area and dry weight, the R^2 of the relationships is low ($R^2 = 0.2$).

2.3.2) Aggressive Behavioural Responses: We used PERMANOVA to partition variation in behaviour among the various factors considered in our experiment. I hypothesised that pairs of individuals from close proximity (NN) would be less aggressive towards each other than pairs of individuals collected from greater distances (MR and FR). Figure 5 depicts the frequency of aggressive responses observed at each distance. Firstly, it can be observed that touching (0-cm) individuals had the highest number of responses but also exhibited the most severe responses such as overtopping inflated acrohagi (Figure 5A). This figure also shows that at the 5-cm distance MR and FR individuals exhibited inflated acrorhagi (Figure 5B). Figure 5C also denotes that no behaviours were observed at the 15-cm distance. These results suggest that the most aggressive responses occur when individuals are touching,

however there is evidence to suggest that there may be some recognition and preparation for combat at greater distances (Figure 5D) even when individuals are at some distance. These results also suggest that those individuals located furthest away from each other illicit more aggressive responses than those individuals located nearest each other.

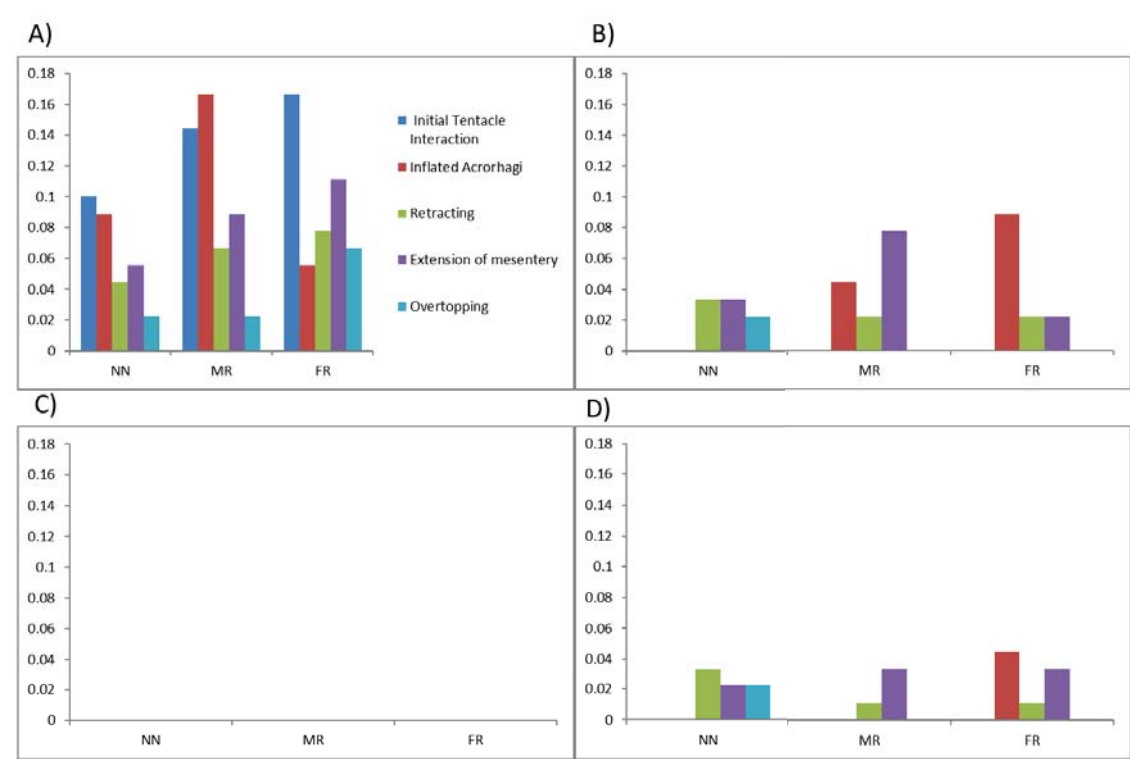


Figure 5: Frequency of aggressive behaviours for individuals at a distance of 0 cm (A) a distance of 5cm (B) a distance of 15cm (C) and a distance of 25cm (D)The principal co-ordinate plot for all distances and the 0cm (touching) distance highlights the sequence of aggressive behavioural responses expected between interacting pairs of individuals. Interactions start with initial tentacle interaction and is followed by inflated acrorhagi, retreating, extension of the mesentery and finally overtopping (i.e. in a clockwise direction in Figure 6A and an anticlockwise direction in Figure 6B). More specifically, we can illustrate this sequence using a path diagram as in Figure 7).

Figure 6 summarises the distribution and clustering of aggressive responses at all possible distances (Figure 6A) and for the individuals at touching distance (Figure 6B). The elicited response sequence (based on the responses that occurred with decreasing distance between pairs) starts with initial tentacle interaction and is followed by inflated acrorhagi, retreating, extension of the mesentery and finally overtopping. A similar response sequence is observed in those touching individuals (Figure.6B) where initial tentacle interaction, is followed by inflated acrorhagi, extension of the mesentery, overtopping and retraction. This suggests that there is a standard sequence of responses observed in each interaction (Figure.7). Retraction may come at the end, middle or at the beginning of a sequence as there can be more than one reason for retraction. Although sequences slightly differ there is still evidence to suggest that there is an obvious order of aggressive responses. Figure 6 also shows that some behaviours although infrequent are more extreme than other behavioural responses: low incidence of overtopping (considered the most aggressive) and high incidence of initial tentacle interaction (least aggressive).

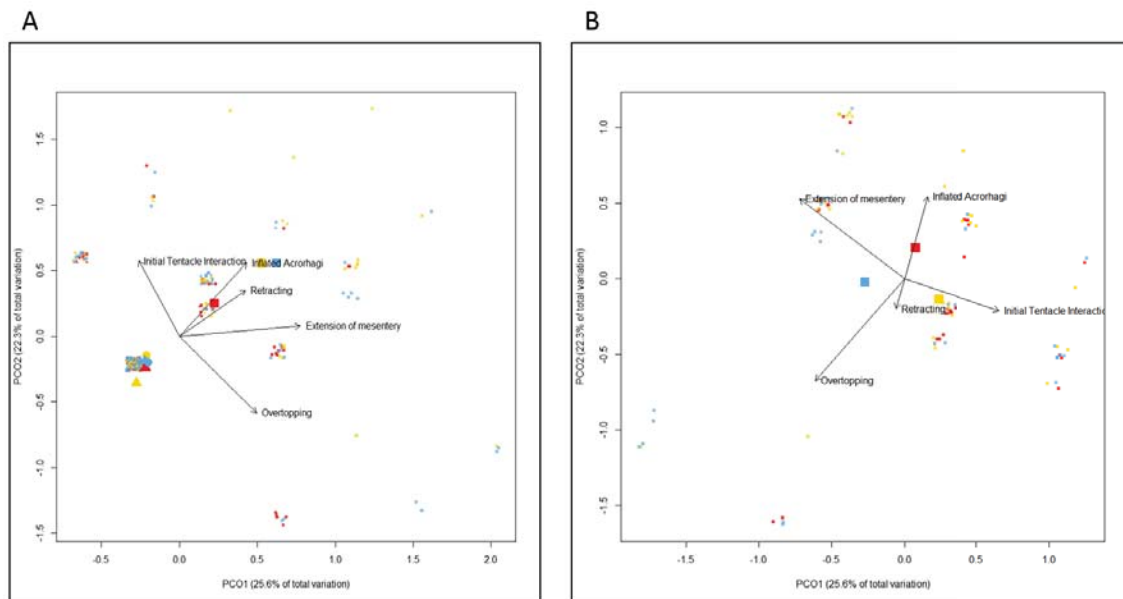


Figure 6: A) The behavioural and escalation of behaviour at all distances (n = 90)). B) The behavioural distribution and escalation of touching (0cm) for the three spatial arrangement levels. For both plots, red symbols indicated nearest neighbours, yellow symbols mid-range and blue symbols far range. The transparent symbols are the observed data while the solid symbols are the group centroids. The 3 distances: 0mm (touching) are indicated by the squares, 15mm = triangles, 50mm = circles. Centroids can be observed as heavily pigmented data plots.

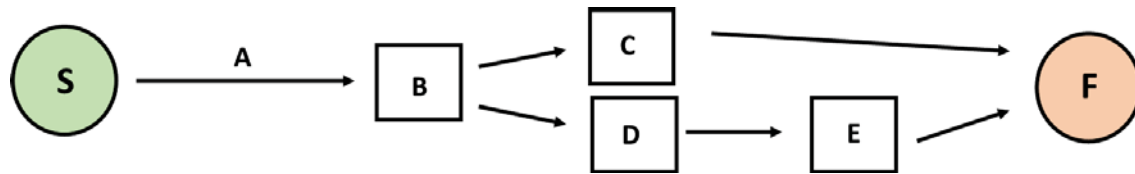


Figure 7: Aggressive response sequence of *A. tenebrosa*. Green circle (S=start) is the starting position of the sequence. The figure shows that from the initial starting position (green circle), interactions begin with initial interaction (A) which motivated one or both individuals to inflate their acrorhagi (B) which leads to either retraction (C) or extension of the mesentery (D) and finally overtopping (E). In natural conditions, we might expect that individuals that have been overtopped would flee the area (orange circle).

We found a significant spatial arrangement by distance interaction which suggested (Figure.4A, Table.1) individuals at the touching distance behaved differently to individual at greater distances. Moreover, those individuals that were most aggressive at the 0cm (touching) (Figure.4B Table.2) distance were the far range individuals.

Table 1: PERMANOVA table and pairwise comparisons for all distances.

	DF	SS	MS	Pseudo-F	P(perm)		
Spatial Arrangement	2	2.6805	1.3402	1.8821	0.0703		
Distance	2	29.789	14.894	12.768	0.0001		
location	2	3.9794	1.9897	2.7942	0.008		
Spatial Arrangement x Distance	4	7.8029	1.9507	1.6722	0.042		
Spatial Arrangement x Location	4	3.6687	0.91718	1.288	0.2077		
Distance x Location	4	7.2448	1.8112	1.5526	0.0692		
Tank (Spatial Arrangement x Location)	36	25.635	0.71209	0.97145	0.5738		
Spatial Arrangement x Distance x Location	8	11.702	1.4627	1.2539	0.1544		
Individual (Tank (Spatial Arrangement x Location))	45	32.986	0.73301	0.82289	0.9397		
Distance x Tank (Spatial Arrangement x Location)	72	83.992	1.1665	1.3096	0.0035		
Residual	90	80.171	0.89078				
Total	269	289.65					
B: PAIRWISE COMPARISONS OF Spatial Arrangement x Distance							
Groups	Nearest Neighbour		Mid-Range		Far-Range		
	T	P(Perm)	T	P(Perm)	T	P(Perm)	
	1,2	1.7277	0.0275	2.4448	0.0038	3.0409	0.0004
	1,3	1.751	0.0238	3.5388	0.0005	2.9161	0.0005
	2,3	0.14333	0.9783	1.3144	0.1847	0.9327	0.4728

Table 2: PERMANOVA table and pairwise comparisons for touching (0cm) individuals.

	DF	SS	MS	Pseudo-F	P(Perm)
Spatial Arrangement	2	6.4564	3.2282	1.8912	0.0481
Location	2	7.6562	3.8281	2.2427	0.017
Spatial Arrangement x Location	4	9.424	2.356	1.3803	0.1345
Tank (Spatial Arrangement x Location)	36	61.45	1.7069	0.93494	0.6682
Residual	45	82.157	1.8257		
Total	89	167.14			
2b: PAIRWISE COMPARISONS OF for touching (0cm)					
Groups	T	P(Perm)			
1,2	1.1429	0.2665			
1,3	1.4259	0.0839			
2,3	1.5154	0.0495			

2.4. Discussion:

The overall aim of this study was to investigate the behavioural responses of *Actinia tenebrosa* in relation to those individuals occurring at different distances in natural populations. I predicted that there would be a significant relationship between pedal diameter and dry weight and that pedal diameter could be used to estimate mass of individuals. Unfortunately, although I found a significant relationship the fit of the model to the data was poor and considered it not a sensible estimate of mass. I also predicted that those anemones that are initially located nearest to each other will be less aggressive towards each other than those anemones found upwards of one metre away from each other. The resulting data suggests three key points: first, there is an obvious order of escalation of aggressive response behaviours in *A. tenebrosa*. Second, we found that some behaviours, although infrequent tend to be the more extreme behaviours that can be observed. Third, we found that individuals collected from greater distances away from each other were more aggressive towards each other than individuals collected at closer distances.

An accurate estimate of a mass would have been helpful in determining if size of the anemone influenced aggressive dominance. Although pedal measurements and weight measurements have been used effectively in other studies (Chomsky *et al.* 2004) we were unable to find a useful predictive association between pedal disc area and dry weight in our populations. In a study comparing several morphological parameters to determine which field measurements (column height, column diameter, limbus diameter, pedal disc diameter, pedal disc area and pedal disc perimeter) could accurately and reliably reflect the size of an anemone measured in the laboratory Angeli *et al.* (2016) found pedal disc diameter was considered the most accurate way to estimate the size of sea anemones in the field, and literature recommends its use as an effective, non-destructive tool (Karges *et al.* 2003; Angeli *et al.* 2016). Future studies should consider biases caused by inflated discs that can result from stress and injury at the time of collection. Moreover, subsequent studies should also consider the water displacement method which has often been used for odd shaped, relatively small organisms with a relatively large internal water volumes as a tool for measuring anemone mass (Karges *et al.* 2003). This method is based on the Archimedes'

Principle, that the water volume displaced is equal to the volume of the object immersed in the water (Karges *et al.* 2003). Some studies suggest that methods to quantify the size of individual animals and features of biological communities are important for understanding behavioural variability (Angeli *et al.* 2016).

Our study suggests that there is an obvious sequencing and escalation of aggressive behaviours among *A. tenebrosa* individuals. The observed sequence of behaviours we have provided could be used to compare and contrast with other aggressive anemone species where a dominance hierarchy has been established (Sherman & Ayre 2008). For instance, there have been numerous studies into agnostic behaviour among clones of the anemone species *Anthropleura elegantissima* (Sebens 1981; Ayre & Grosberg 2005). One such study showed that fighting ability to be an important determinant of interclonal competition for space and shows that clonal diversity is very high in populations of *A. elegantissima* (Ayre & Grosberg 2005). The results of this study showed that dominance rank was statistically correlated with the difference in the number of fighting tentacles per clone, and argues that dissimilarity in the number of fighting tentacles provides a simple explanation for variation in dominance rank (Ayre & Grosberg 2005). Overall Ayre and Grosberg (2005) found that intraspecific competition for space among *A. elegantissima* clones may be decided by a combination of both behavioural and morphological features of competing clones, as well as previous exposure to other clones (Ayre & Grosberg 2005). Our results were similar in the fact that they both shared initial tentacle interaction at the beginning of the sequence (Ayre & Grosberg 2005). Behavioural sequencing in anemones is important as future research into this area of study could lead to a universal dominance aggression scale, for not only this species but also other species of aggressive anemones with different weaponry and ecology.

We also found that the most aggressive behaviours tend to have low prevalence. This suggests that it is highly costly to the individual to utilise these aggressive behaviours during conflict/interaction (Riechert 1988; Laidre & Johnstone 2013). Therefore, there is not only a cost to the recipient of this behaviour but that there may also be a survival cost to the aggressor (Laidre & Johnstone 2013). It has been observed in large clonal anemone aggregations that very few of the anemones are able to engage in interactions at any given time (Ayre 1982; Ayre

& Grosberg 2005). One would therefore expect the costs of intraspecific aggression to be distributed unevenly in clustered groups, with those individuals at or near an interclonal cluster boundary bearing more of the behavioural response costs (Ayre & Grosberg 2005). In understanding the costs of aggressive behaviour and responses, we can better understand the severity and survival cost to not only an individual anemone but also the complexity of the spatial distribution of individuals within populations.

This study found that, under laboratory conditions, far-range individuals displayed a greater frequency and severity of aggressive responses than individuals located closer together. Based on the assumption that nearest neighbours are genetically related, greater aggression towards individuals occurring at greater distances suggests that their genetic dissimilarity promotes aggression (Ayre 1985; Grosberg 1988; Veale & Lavery 2012). The results of my study are consistent with this hypothesis. Allorecognition in anemones is the ability of an individual to distinguish its own tissue or self from those of other individuals (Grosberg 1988; Rogers & Lechler 2001). Previous studies suggest that intraspecific aggression between clonal cnidarians is not random with respect to genotype; however, aggressive behaviour is revised according to the relatedness of individuals (Grosberg 1988; Sherman & Ayre 2008). In doing so, close relatives behave passively, whereas distant relatives behave aggressively. Such behavioural restriction requires the existence of both an internal allorecognition system and a sequence of cues that provides specific identifiers of relatedness (Grosberg 1988). More evidence of this can be observed in studies where neighbouring individuals that are placed close to each other in laboratory experiments will move and spread, but are not often out of tentacle range of other neighbouring individuals, suggesting they recognize genetically similar individuals (Ayre 1987; Ayre & Grosberg 1995). Investigation into this type of behaviour is important as *A. tenebrosa*'s ability to locally adapt (Ayre 1982, 1985) and its intricate reproductive approach (Black & Johnson 1979; Ayre 1984) is likely to create a distinct pattern for the genetic structure among populations.

Chapter Three: Spatial Distribution



CHAPTER 3: SPATIAL DISTRIBUTIONS

3.1. ABSTRACT:

Habitable space is often the single greatest resource limiting the potential for growth, survival, and ultimately reproductive output in sessile marine invertebrate communities. Previous studies suggest that anemone populations on rocky shores are a collection of clusters of genetically related individuals. These clusters vary in the number of anemones within a cluster and cluster size is often dependent on the characteristics of the environment in which they live. Populations of *Actinia tenebrosa*, are comprised of consistent and prevalent clusters of individual polyps. Here, I examined whether *Actinia tenebrosa* individuals can recreate the spatial pattern of clusters found in the field under controlled conditions in the laboratory, which might suggest of an optimal spatial arrangement in the field. As a whole, my results suggest that there is no single optimal arrangement of individuals, but rather individuals converge on a stable aggregation within 48 hours that is maintained for a further 10 days in the laboratory. Importantly, although individuals converged on these stable spatial arrangements, like the stars across the night sky, individuals continue to move while maintaining the same spatial arrangement. The results from this study suggest that the spatial arrangement of individuals within clusters is deterministic; however, there appear to be multiple stable arrangements rather than a single optimal arrangement.

3.2. INTRODUCTION:

In a terrestrial environment, mobility is important as it provides the ability to forage for resources distributed across the landscape (Alexander 2001). However, in a marine environment, the ability to move is not necessarily as important, due to delivery of resources via tidal exchange (Ottaway 1979; Veale & Lavery 2012). For many sessile and sedentary marine organisms, habitable space is often the single most important resource limiting the potential for growth, survival and ultimately, the reproductive output of individuals (Ayre 1982; Ayre & Grosberg 2005). It would seem that the spatial arrangement of individuals is critical for overall fitness of the majority of anemone species. (Veale & Lavery 2012).

Anemones are widely described as sessile benthic organisms (Ayre & Grosberg 1995). However, do have the ability to move to locate to a more viable environment (Brace *et al.* 1979; Brace & Santer 1991; Ayre & Grosberg 2005). Anemones exhibit varying types of locomotion, the most typical being pedal locomotion which involves contracting internal muscles to contract and detach a muscular foot in order to translocate (Ottaway 1978; Ayre 1987). Moreover, studies suggest that they can also inflate their pedal disc, detach from the strata and to move significant distances by floating in the water column, perhaps to escape predation or unfavorable conditions

(Riemann-Zürneck 1998). Although rare, substrate detachment and pedal locomotion have been observed among other similar anemone species including *Actinia equina* and *Actinia tenebrosa* (Ottaway 1978; Ayre 1987; Turner *et al.* 2003; Foster & Briffa 2014).

Anemone populations are often made up a collection of clusters of genetically related individuals (Ayre 1984, 1987; Veale & Lavery 2012). Clusters are typically comprised of up to ten individuals but the size of clusters can vary depending on the surrounding environment (Veale & Lavery 2012). The identities of individuals found within clusters tend to be stable (Ayre 1984b) and it is generally agreed that clusters are maintained by the fitness costs of intraspecific aggression (Ayre 1982, 1984b, 1987), towards unrelated or more distantly related individuals (Ayre 1982, 1987; Sherman & Ayre 2008). Nonetheless, previous studies suggests that some anemone species will imprint or acclimate to unrelated individuals after long periods of exposure (days, months) and aggressive interactions among unrelated individuals become less frequent (Grosberg 1988). Imprinting is considered to be rapid learning during a brief receptive period, typically soon after birth or hatching, and establishes a long-lasting behavioral response to a specific individual or object (Bartolomei & Ferguson-Smith 2011; McCabe 2013). This can be observed as an attachment to a parent, offspring, site and in some cases different species (McCabe 2013) . Acclimatization is described as the process in which an individual organism adjusts to a change in its environment (such as a change in altitude, temperature, humidity, photoperiod, or pH), allowing it to maintain routine functions across a range of environmental conditions (Chomsky *et al.* 2004). Understanding, acclimation and or imprinting behaviours in anemones may help uncover the drivers of the spatial distributions of anemone species.

In this chapter, I investigate whether the spatial arrangement of *A. tenebrosa* in the field is reproducible in the laboratory and thereby indicative of an optimal arrangement of individuals in the field. I predicted that after randomisation, individuals would revert back to their original arrangement. If my hypothesis proves true it would suggest that the small-scale spatial structuring of individuals in the field is fixed and dependent on the costs of aggressive intraspecific interactions.

3.3 METHODS

Sites and Species: All individuals were collected from three different locations (Pilot Bay, Mount Maunganui; Anzac Bay, Bowentown and Waihi Beach, Waihi.) in the greater Bay of Plenty, New Zealand (Figure.8). Anemones at these sites preferred cracks and crevices with low wave action. In these locations, individuals occupy a twenty-square meter area in the high intertidal zone. The anemones tend to be clustered in clonal groups of up to ten individuals and are regularly distributed over this area. All experimental individuals were collected at the same time. The size of individuals was kept between 10cm and 30cm pedal disc diameter to examine a representative range of sizes for adult anemones found in the field. Seawater from each location was taken at the time of collection and used in the laboratory aquaria over the two-week period of our experiments.



Figure 8: Map of the Western Bay of Plenty, New Zealand. The yellow symbol indicates the Waihi Beach study site, The green symbol depicts the Bowentown study site and the red symbol indicates the Treasure Island study site.

Collection and laboratory Methodology: At each site two clusters of at least five individuals were identified along the high rocky intertidal zone. The maximum distance between any two anemones collected in the field was 50cm as this was the maximum dimension of our laboratory aquaria. Photos including a ruler for scale were taken of each cluster to determine original spatial arrangement of individuals in the field. Within each cluster, the individual closest to the center of the cluster was identified as the focal individual (Figure 9) and numbered as

individual 1, Individual 2 was the nearest neighbour to individual 1; individual 3 was the second nearest neighbour to individual 1 and so on up to individual 5.

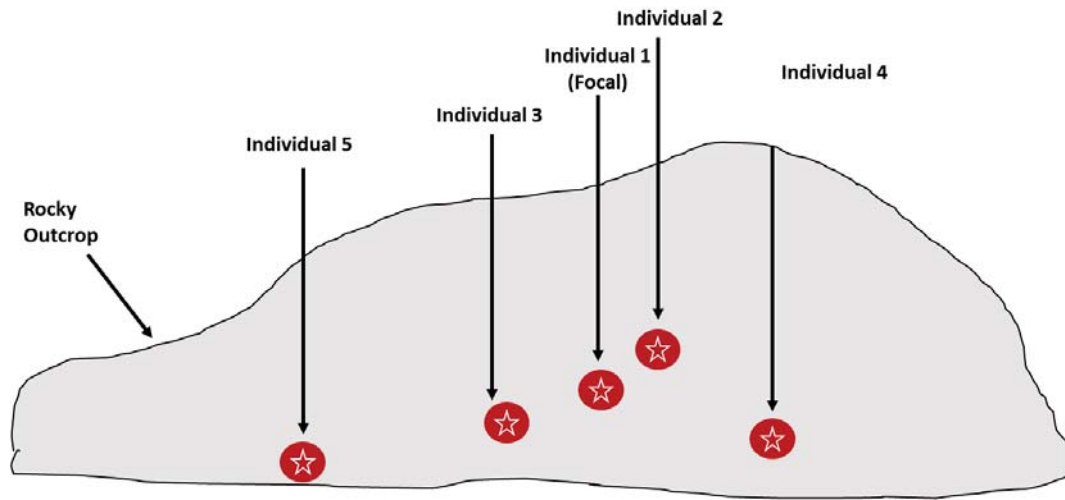


Figure 9: Sequence for collecting anemone individuals on a rocky outcrop. The focal individual is established by identifying the centermost individual of the cluster, this individual becomes individual one. Individual two is the nearest individual to one. Individual three is the next nearest individual after two, while individual four is the next nearest individual after three to the focal individual. Individual five is the furthest individual away from individual one.

The individuals were then removed from the substrate, measured across their widest and narrowest points to determine size and placed in their own labelled containers. Each individual was kept in isolation in a clear plastic container (with continuously oxygenated seawater) for transport and left to acclimatize for two days before the start of the experimental period. For each experimental trial ($n = 12$), we filled a 60-liter aquaria with fresh seawater, we then placed a set of five isolation chambers arranged as a rosette in the middle of the tank (Figure 10). The five experimental individuals, were then allocated to a numbered isolation chamber and placed in the center of the chamber. (Figure.10).



Figure 10: Lab photograph of initial anemone arrangement in clear isolation aquaria. Clear aquaria indicate individual isolation chambers. Dark masses depict anemone individuals.

A GoPro Hero 4 camera was then arranged beneath the aquaria and set to time-lapse mode capturing one photo every 60 seconds (Figure 11). The camera was propped up with a stand in order to ensure the field of view captured the entire tank (Figure 11). After two minutes, the isolation chambers were removed and the movements of these anemones were recorded continuously over a 12-day period. The water in the aquaria (tank) was continuously aerated and we exchanged half the volume of water in each aquaria every two-days. Over the experimental period individuals survived on the zooplankton within the exchanged seawater, no additional nutrients were supplied.

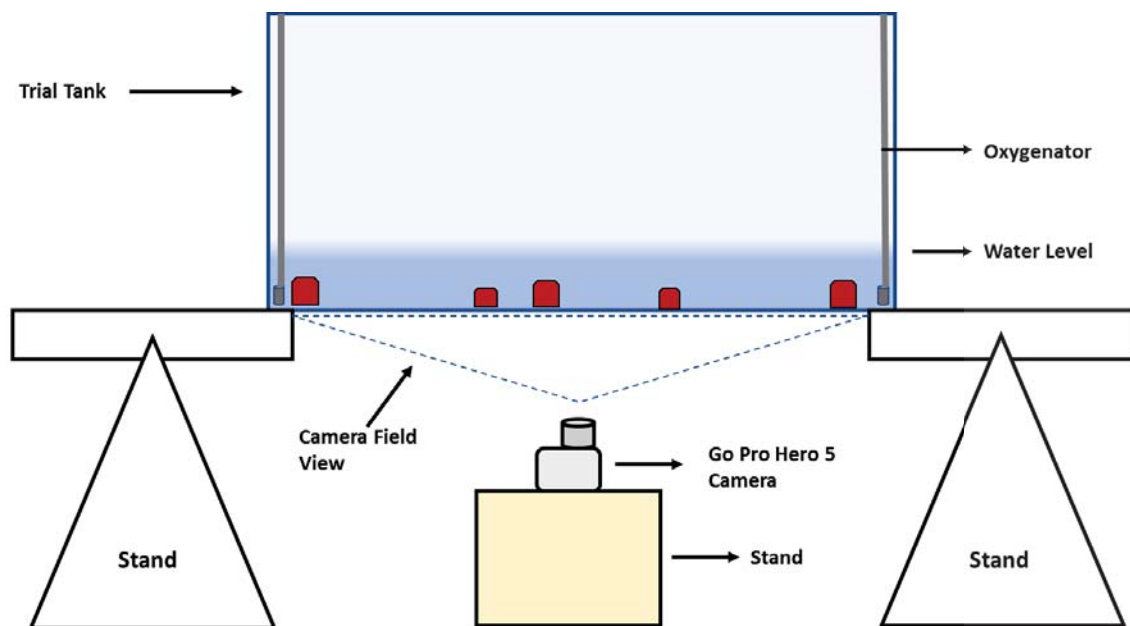


Figure 11: Experimental manipulation set up. An aquaria (tank) was set across two stands and filled with 20cm of water. A GoPro Hero 4 camera was elevated underneath the aquaria to capture stills over the experimental period (12 days). Oxygenators were mounted on each side of the aquaria to ensure even oxygenation over the aquaria for the entire study period. Test individuals are depicted as red domes.

Video Analysis and Photo Mapping: All image analysis was completed using the photo imaging software ImageJ. For each photo, either the original arrangement in the field or each of the time-lapse photos in the laboratory, I calibrated the image and calculated the linear distances between the centers of the pedal discs of every individual. The time-lapse produced 17,280 images per experimental trial; however, we limited our effort to only 90 images. I processed an image every three minutes for the first two days. I processed an image every 6 minutes for the following two days, 9 minutes for two days, 27 minutes for two days, 81 minutes for two days, 243 minutes for one day and 729 minutes for one day.

Statistical analyses: The image processing procedure described above produced a matrix of pair-wise distances among individuals in each photo. If my original hypothesis that individuals would revert back to their original arrangement in the field was true, we should expect that the distances among individuals in the laboratory would become more similar to those found in the field over time. Accordingly, in our first analysis we calculated the Pearson's correlation between the among-individual distances in the field and the among-individual distances calculated for each time lapse photo in the laboratory. If the correlation became increasingly positive over time this would support our hypothesis that individuals are moving towards the original arrangement in the field. Given

that the Pearson's correlation values calculated above ignore the inter-dependencies in the among individual distances, for instance, the distances between individuals 1 and 2 and individuals 2 and 3 is not independent of the distance between individuals 1 and 3, in our second analysis used Procrustes rotation to find the optimal alignment between the arrangements of individuals in the field and the arrangements of individuals in each time lapse photo. The metric of interest in our second analysis was the sums-of-squares errors between the Procrustes rotated time lapse photos and the original arrangement in the field. In this case, a decrease in the sums-of-squares errors would be indicative of movement towards the original arrangement in the field.

A somewhat surprising result of our study was that individuals did not remain stationary for any significant period of time (Figures 12 and 13). Moreover, examining Figure 8 it appears that the movements of individuals between time lapse photos were not independent of each other: movement by one individual was mirrored by another individual and often these movements were of similar magnitudes. Accordingly, for our final analysis we used a time-series analysis to examine the temporal autocorrelation (correlating an individual's movements in one-time period with its movements in the next time period) and cross-correlation (correlating an individual's movement in one-time period with the movement of a different individual in the next time period) among anemones in each tank. Here, a significant positive cross-correlation would support our observation that individual movements are not random and instead are a response to movements by neighboring individuals.

3.4 RESULTS

There was no strong indication that the anemones were recreating the spatial arrangement in the field (Figure 12). Nonetheless, to investigate further, I decided to test if the linear distances among individuals (Figure 13) or the overall arrangement (Figure 14 and 15) of individuals become progressively more similar to that found in the field. Instead, my data suggests that independent of whether the arrangement at the beginning of the experiment was similar or dissimilar to that in the field, after a period of two days the anemones in each tank did not recreate their arrangement in the laboratory, rather the anemones found a different arrangement where the distances among individuals (Figure 14) and their spatial arrangement (Figure 15) did not change.

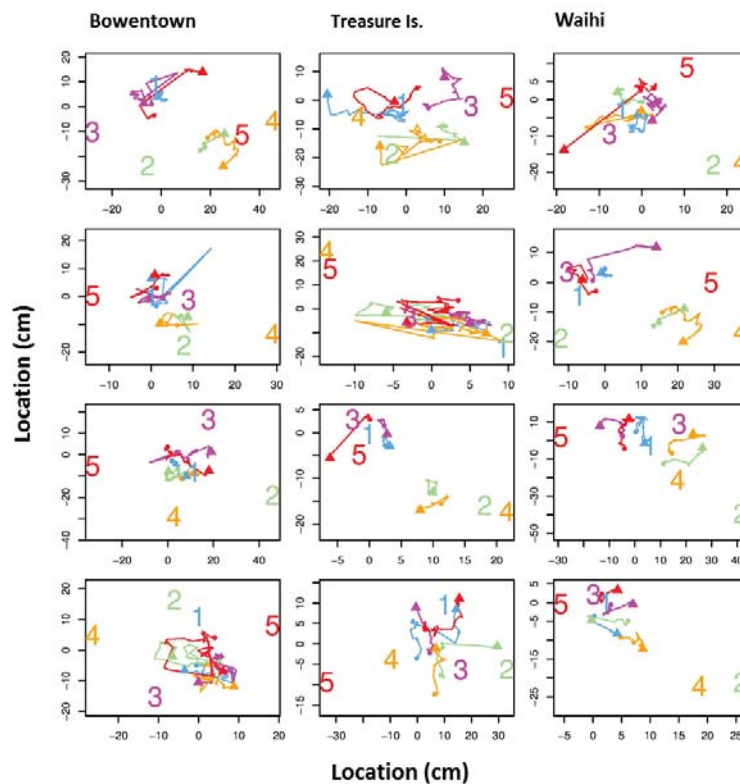


Figure 12: Raw Tracks: Movements of each animal over the experimental period for each replicate (row) cluster collected from each site (columns). Each individual's movements are indicated by the different colours; Blue-1, Green-2, Purple-3, Orange-4 and Red-5. Circles indicate the arrangement at time zero in the laboratory, triangles indicate the arrangement at the end of the experimental period. The numbers indicate the arrangement of individuals in the field. If individuals were recreating the arrangement in the field we would expect the triangle symbols to be placed in close proximity to the number of the corresponding colour.

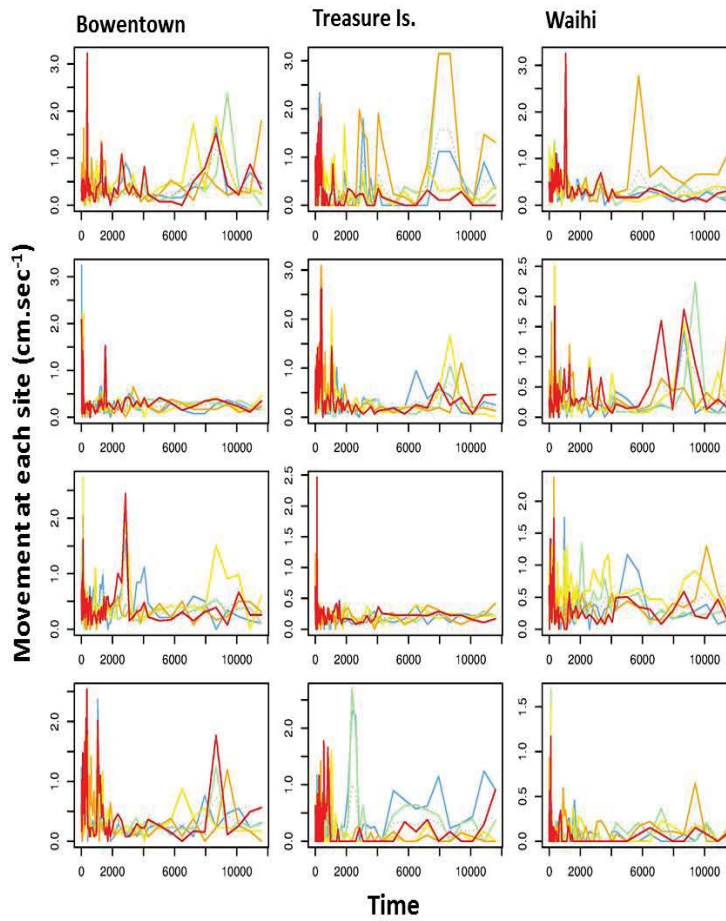


Figure 13: Relative rates of movement of all individuals over the study period. Each individual's movements are segregated by a different colour; Blue-1, Green-2, Purple-3, Orange-4 and Red-5. (five individuals per

After observing that the distances among individuals and their spatial arrangement became stable after a relatively short period of time, I investigated whether this was an indication that movement had ceased, and the anemones had become sedentary. Much to my surprise, I found that the rate of movement was still relatively high (Figure 13) despite there being no appreciable change in the distances among individuals or their overall spatial arrangement (Figures 14 and 15).

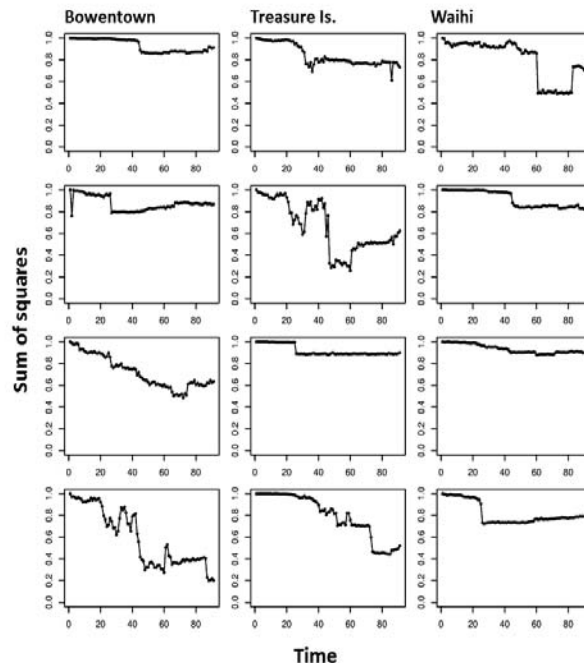


Figure 14: Procrustes rotation to find the optimal alignment between the arrangements of individuals in the field and the arrangements of individuals in each time lapse photo. No evidence to suggest individuals were striving to maintain an optimal alignment.

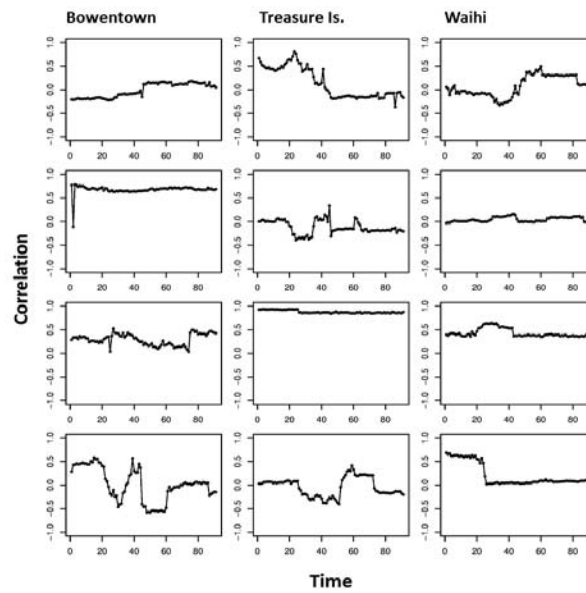


Figure 15: Pearson's correlation between individuals distances in the field and among individual distances in the laboratory for each time period. Despite initial period of rearrangement most replicates found a stable arrangement that persisted throughout the

Examining the temporal autocorrelation and cross correlations among individuals I found that individual movements were often significantly positively correlated (Figure 16). Moreover, I found that although the mean cross-correlation at the individual level was relatively similar to the magnitude of the auto-correlation, the maximum cross-correlation at the individual level was significantly higher than the auto-correlation (Figure 17). In other words, given there was a lower probability of predicting the movement of the same individual in successive time periods (auto-correlation) than the movement of at least one neighbouring individuals (maximum cross-correlation), individuals were able to perceive the movements of their neighbours and respond accordingly.

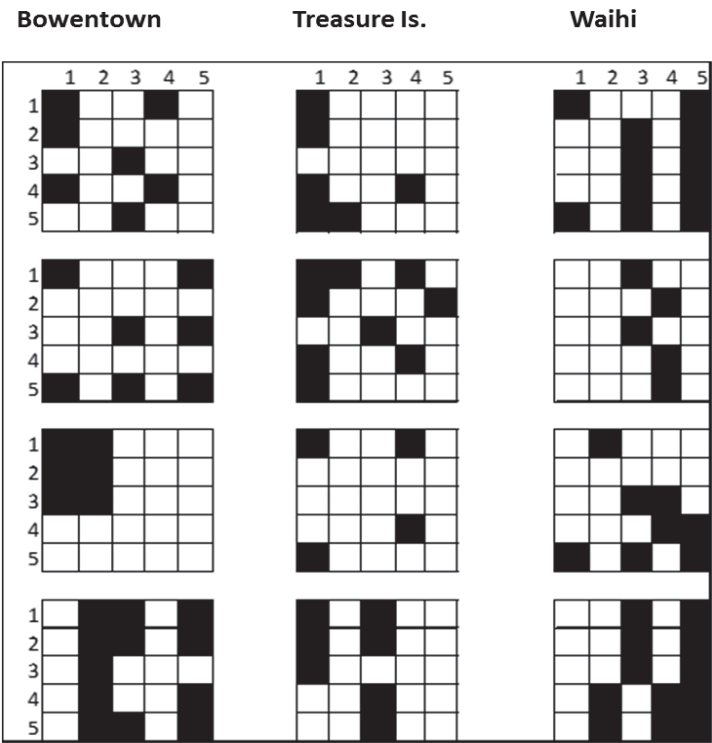


Figure 16: A Prevalence of significant autocorrelation (diagonal entries in each 5×5 matrix) and cross correlation (off-diagonal entries in each 5×5 matrix) values. Filled squares indicate comparisons where the time series analysis found a significant correlation between the movement of a focal individual in one-time period (rows of each 5×5 matrix) and the movement of the same individual or a different individual in the next time period (columns of each 5×5 matrix). Note, the 5×5 matrices are not symmetric because the values used for each correlation differ depending on which individual is used as the focal.

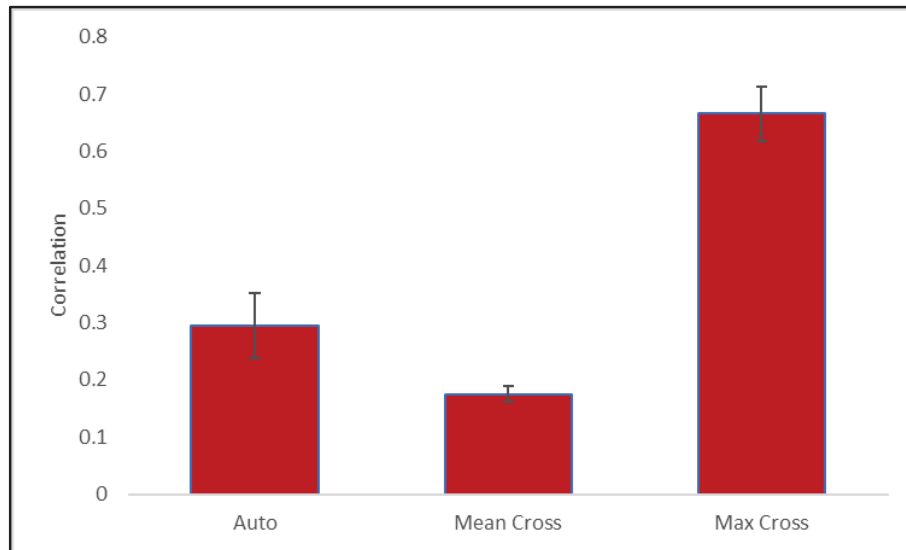


Figure 17: Plot depicting mean auto-correlation vs mean cross correlation and maximum cross correlation. Above we can observe that the averages of both auto and cross correlation are not significantly different. However, between maximum cross correlation and auto-correlation there is a significant difference in favor of maximum cross correlation. This would suggest that individuals correlate their movement in response to another individual's movement.

3.5 Discussion:

The overall aim of this study was to investigate if there was some determinism to the spatial distribution of *Actinia tenebrosa*. I tested this by examining whether individuals return to similar spatial arrangement after being disturbed. If my hypothesis proves true it would suggest that the spatial structure of individuals was dependent on intraspecies interactions and the recognition of potential threats. My data indicate three key results: first, I found that there is no evidence to suggest an optimal spatial arrangement for this species but rather that individuals can form multiple stable aggregations. Second, I found significant evidence to suggest that acclimation occurred over a two-day period resulting in the formation of an arrangement of individuals that was stable. Third, I discovered a significant correlation between the relative rate of movement of one individual the relative rate of movement for the surrounding individuals, indicative of the perception and response to the behaviours of surrounding individuals.

I found no evidence to suggest that there is an ideal spatial distribution among individual anemones in the field. Instead, we found that there may be multiple stable spatial arrangements among the same individuals. Following an initial period of reorganization, I observed a definite plateau in the arrangement of individuals. The result suggests that *Actinia tenebrosa* individuals must be able to recognize surrounding individuals and establish a spatial arrangement that minimizes aggressive interactions. Recognition in anemone species has been observed in species other than *Actinia tenebrosa*. A highly specific cellular recognition system, capable of distinguishing between syngeneic and allogeneic tissue, exists in *Anthopleura elegantissima*, a clonal sea anemone with well-known aggressive responses toward other individuals (Ayre & Grosberg 1995, 2005). In this species, nematocyte excitation required direct contact of the acrorhagus with foreign tissue and is presumably mediated by cell surface receptors (Ayre & Grosberg 1995, 2005). Although the above study is based on the direct contact of nematocysts, my study has suggested that the decision establish a stable spatial arrangement did not require direct contact. This result is evident in the largely overlapping tracks in figure 13 and the dyadic movements of individuals. Therefore, it appears that individual recognition and the avoidance of aggression may directly responsible for the fine-scale spatial distribution of this species.

Acclimatization is the process in which an individual organism adjusts itself to a change in its environment (such as a change in temperature, altitude, salinity, humidity, or pH), allowing it to maintain or exceed previous routine function across a range of environmental conditions (Falkowski & LaRoche 1991). Imprinting however is the rapid learning that occurs during a brief receptive period, typically soon after birth or hatching, and establishes a

long-lasting behavioral response to a specific individual or object (such as attachment to parent, offspring, or environmental site) (McCabe 2013). Previous literature suggests that anemones that are kept in close quarters for an extended period of time can acclimate or imprint on each other and this can affect their behavioural responses (Sherman & Ayre 2008). Studies suggest that it can take an extended period (weeks, months) for acclimation among unrelated individuals, and once acclimatized individuals and before a stable arrangement is accomplished (Ottaway & Kirby 1975; Veale & Lavery 2012). Because, in my study I collected individuals from already established clusters, it is likely that individuals were already either acclimated or genetically related. Accordingly, the shorter (approximately two days) imprinting or acclimatisation period we found may reflect that there was some degree of familiarity among individuals which allowed them to more rapidly form a stable arrangement. Laboratory studies in the same study species revealed that the most important factor contributing to the spatial arrangement of individuals was habituation to non-clonemate contact (Ayre & Grosberg 1995). Their results suggest that habituation to non-clonemate contact occurs *in situ*, and that loss of habituation must occur occasionally to account for the occurrence of fighting tentacles (Ayre & Grosberg 1995). Both previous studies along with this study highlight the importance of small scale spatial structure that is likely driven by fine-scale genetic structuring of populations in mediating the likelihood of aggressive encounters among individuals.

Although the spatial arrangement of individuals stabilised after 2-days, significant movement occurred throughout the 12-day experimental period. Accordingly, instead of settling on a stable arrangement and then remaining sedentary, the individuals were constantly moving and responding to the movement of the individuals around them. This would then suggest that the anemone species *A. tenebrosa* must have allorecognition of surrounding individual's, and perhaps a memory of previous interactions, to respond to the movement of its neighbours. Previous studies suggest that inducible defenses are congruent with historical biological interactions and can affect the probability of individual survival and growth, as well as affecting population dynamics of specific species (Harvell 1990; Brace & Santer 1991). It also suggests that the benefits of inducible defenses (such as acrohagial fighting) are often balanced by fitness costs, including reduced growth, reproductive output and survivorship. A more integrated approach would involve examining aggressive (fighting) as well as more passive (avoidance) inducible defenses. Previous research, along with the results of this study suggest there are mechanisms at work in the biology of anemones in regard to recognition and identification that are important in comprehensively understanding the population dynamics of *A. tenebrosa*.

I did not find evidence for a single optimum or ideal arrangement, instead my data suggests that there may be multiple stable arrangements and remarkably these stable arrangements are maintained despite the constant movement of individuals. It would be interesting to observe if the patterns detected under constant conditions in the laboratory are also replicated in the field under constantly changing conditions (e.g. tides, water motion, competition of other species, predators). Nevertheless, my data clearly shows that the fine-scale arrangement of individuals in the field is deterministic and presumably maintained by the anticipated fitness costs of engaging in combat with neighbouring individuals.

Chapter Four: General Discussion



Chapter 4: GENERAL DISCUSSION

4.0 GENERAL CONCLUSIONS

As a whole, this study examined the influence of complex aggressive interactions among individuals of the anemone, *Actinia tenebrosa* on their spatial arrangement. In my first chapter, I showed that there was an obvious sequencing of aggressive behaviours starting with initial tentacle interaction, then inflated acrorhagi followed by either retraction or extension of the mesentery, and finally resulting in overtopping. I also observed that aggressive responses were more common and more severe among individuals which would naturally occur at greater distances from each other relative to individuals which were naturally nearest neighbours. Last, I observed that aggression responses are most commonly observed when individuals were touching. In my third chapter, I showed that there was no evidence of an ideal spatial distribution but rather, individuals will conform to a different but stable arrangement. These alternate stable arrangements developed after approximately two days and were maintained for the remainder of the experimental duration (12 days). Last, I observed that although the arrangements were stable the individuals were not sedentary, instead individuals move in relation to those individuals surrounding them while maintaining the same spatial arrangement. These facts are important to the coherent understanding the spatial distribution in this species.

4.1 CHAPTER 2 CONCLUSIONS

The manipulative study presented in chapter 2 has produced a number of results important for the understanding of how aggressive behaviours determine dominance hierarchies and the spatial arrangement of the sea anemone, *Actinia tenebrosa*. I predicted that those individuals that were located closer together would show less aggressive behaviours than those individuals at greater distances. At the conclusion of this study it was observed that indeed individuals that are located nearest each other most often express less aggressive behaviours (most typically retraction) than those at greater distances. There have been similar studies conducted in the species *Anthropluera elegantissima* (Ayre *et al.* 1991; Ayre & Grosberg 1995, 2005). That would suggest that that many anemone species have a set sequence of behaviours which are expressed depending on the identity of those individuals around them. It has been stated that interactions among individuals is determined by the genetic similarity among individuals (Ayre *et al.* 1991; Veale & Lavery 2012a). My result demonstrates that similar to *A. elegantissima*, there is a definitive sequence of aggression behaviours for *A. tenebrosa*. This suggests that there must be recognition mechanisms among individuals and a dominance hierarchies may be a common occurrence for many anemone species (Ayre 1982; Brace 1990; Turner *et al.* 2003). There may even be the possibility that there may be a universal aggression scale for aggressive anemone species, this information could be instrumental in

understanding the mechanisms that drive anemones to be aggressive and possibly why some of these behaviours are so extreme.

It is widely accepted that individuals that are more genetically related tend to be located closer to each other than those genetically dissimilar individuals (Black & Johnson 1979; Ayre *et al.* 1991; Veale & Lavery 2012a). The ability to naturally determine the spatial distribution of a species suggests that mechanisms that allow individuals to discriminate their neighbors must be present. There is some debate in the scientific community as to what exact mechanisms are involved in allorecognition in anemone species; however, most studies suggest that substances on the surface of the cells determine self and non self (Brace 1990; Frank & Rinkevich 2001). Future research into this subject would determine the exact science as to why specific anemones can identify other and also further understand the local adaption and potential growth of this species.

At the conclusion of this study it was also observed that the highest numbers of aggressive responses and the most severe responses were observed when individuals were touching. It can be suggested that individuals considered that is not worth the cost of aggression when individuals were too far away to inflict any damage. If this were true, it would lead to further research into the possibility of chemical compounds being released into the surrounding water as a deterrent or precursor to aggression. Some research on the chemical make-up of liquids has already been started about a different anemone species (Goldstone 2008; Anctil 2009). A study was conducted on the anemone species *Anthropluera elegantissima* regarding the reaction of individuals to the nematocysts of assumed rival individuals of the same species (Ayre & Grosberg 1995, 2005). This study suggests that in the field, those anemones that appear to “lose” an interaction more often than not, will translocate via pedal locomotion, and in some cases when unable to move would die as a result of prolonged aggression (Ayre & Grosberg 1995). Further research into this behaviour could give answers for this same question in *A. tenebrosa*.

4.2 CHAPTER 3 CONCLUSIONS

The results from the manipulative study presented in chapter 3, challenged my prediction that individuals of *A. tenebrosa* would return to the same spatial arrangement as they had in the field. Instead, I observed that individuals will acclimate to the anemones surrounding them and form alternate stable arrangements. These results would suggest that individuals must have some internal recognition system that allows them to identify and recognise certain individuals. Recognition in anemones has been observed in the other species (Lubbock 1980; Williams 1991; Zeh & Zeh 1997). These studies suggest that an anemone must be capable of distinguishing between syngeneic and allogenic tissue at distances greater than immediate contact. Previous studies suggest that the most

important factor contributing to positive interaction of individuals is habitation of anemones to non-clonemates (Ayre & Grosberg 1995). These results suggest that there is considerable potential for local adaption to fine scale environment variation in *A. tenebrosa*.

4.3 FUTURE DIRECTIONS

Overall, my results suggest that the spatial distribution of *A. tenebrosa* is determined by aggressive interactions and the recognition of specific individuals in the immediate environment. Consequently, these behaviours have the potential to influence population growth and the persistence of populations at any given geographic location.

Unfortunately, examining genetic differences among individuals was not within my masters' budget. In future, I would run genetic analysis to determine genetic similarity amongst individuals therefore ensuring tests would be based on the accurate genetic relatedness as opposed to an assumed genetic relatedness. In future, I would also investigate a larger geographical area within New Zealand and Eastern Australia. The detailed manipulative experiments I conducted were only possible because it was easy to maintain *A. tenebrosa* in the laboratory and because *A. tenebrosa* had a range of easily identifiable aggressive behaviors. The characteristics make *A. tenebrosa* a great model system to examine as the influence of aggressive behaviours on the spatial arrangement of individuals and the development of dominance hierarchies in organisms with limited capacity for movement.

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