Reproductive success and factors affecting sexual selection in a fiddler crab, *Uca mjoebergi*

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Declaration

All research presented in this thesis is my own independent work except where specified in the text. I am the senior author and principal researcher for all works presented in the thesis. Patricia Backwell is a co-author on all chapters and she contributed to project designs, analysis and writing in each of the chapters. Each chapter has been written as manuscripts for publication. Chapters 1 - 4 have been accepted for publication and chapter 5 is currently in review for publication. For the sake of continuity and consistency, some minor editorial changes have been made to the published articles. No part of this thesis has been submitted for a previous degree.

Huon L Clark

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Abstract

Producing offspring is the most important aspect of an animal’s life. Reproductive success is the cornerstone of evolution, but successful mating is a complex process that we are only starting to understand. This thesis examines five aspects of reproductive success in a fiddler crab, *Uca mjoebergi*. This is an ideal study species since the crabs are tractable, abundant and amenable to manipulation. In the first chapter, I examine the natural mate-searching behaviour of females and show, for the first time in any species, that there is a mosaic of preferences: females prefer males of different sizes depending on where they are in the tidal zone, and when they are mate-searching in the tidal cycle. This level of complexity in female preferences can partly explain the ‘lek paradox’.

In the second chapter, I examine assortative mating and show that there is a strong correlation between the size of males and females in mated pairs. I show that neither mate availability nor mating constraints can explain the pattern. Since both males and females preferentially mate with larger partners, sexual selection explains the high level of size-assortative mating. In chapter three, I revisit earlier work on this species showing that claw loss and subsequent regeneration strongly disadvantages males in terms of reproductive success. By substantially increasing the sample size, I show that males with regenerated claws are not at a mating disadvantage. This study highlights the importance of large sample sizes in behavioural studies.

In chapter four, I examine the effect of temperature on mating success. Males can live in the sun or shade. There are advantages to living in the shade: males can be active for longer periods and they are less likely to dehydrate or overheat. Females that chose to mate with males living in the shade, however, would incubate their eggs at approximately 3°C lower than
females incubating in sunny territories. I show that this difference in temperature does not affect the timing of crucial reproductive events and does not prevent the females from releasing their fully-formed larvae at the optimal time.

In chapter five, I examine the potential consequence of habitat loss and subsequent overlap between species distributions. We can already observe this: a larger, lower-living fiddler crab is invading the habitat of *Uca mjoebergi*, increasing the competition between these species. I show that the disadvantages experienced by male *U. mjoebergi* from having a heterospecific neighbour is not necessarily as severe as common sense may suggest.
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Introduction

Sexual Selection – a background

Charles Darwin first proposed his theory of sexual selection in 1871 (Darwin, 1871). Sexual selection results in the development of an extreme trait which could include bright or contrasting colouration, exaggerated ornaments, vocalisations, (Andersson, 1994) or a combination of a number of these. The selection of exaggerated traits is a response to an indication of gene quality (Smith, 1991). This is despite such ornaments being energetically costly and can lead to decreased survivorship (Andersson, 1994). However, Darwin did not have the whole story as has been revealed in subsequent research. For example, one of the biggest pieces of information missing from Darwin’s theory was the reasons why females prefer ornamented males (Andersson & Simmons, 2006). This has since been resolved when studies confirmed the Fisherian theory of coevolution of male ornamentation and female preference (Lande, 1981; Kirkpatrick, 1982). Furthermore, Darwin (1871) himself considered broadcast spawning species incapable of sexual selection for a number of reasons (Evans & Sherman, 2013 and papers cited within). However we now know that these animals employ post copulatory sexual selection as a means of increasing fitness (Parker, 1970). Clearly, processes underpinning sexual selection are much more complex than first thought and studies today are still finding new aspects to sexual selection not previously been found (Clark and Backwell, 2015).

Darwin (1871) defined sexual selection as the study of variance in mating success resulting from either female choice or male-male competition. Chapter 1-4 of this thesis looks at aspects surrounding female mate choice while chapter 5 delves into climatic forces influencing male-male competition.
Sexual selection through female choice

A common goal of individuals of all species is to improve their fitness. Fitness is a comparative measure of an individual’s reproductive success relative to other individuals of the same species (Emlen & Oring, 1977). If a female can increase her fitness by selecting the most appropriate partner out of a group of potential suiters, it would be beneficial for her to be choosy. Females can increase fitness through either direct or indirect benefits. Direct benefits for offspring include good incubation sites (low predation, appropriate thermal conditions, good food supply in the area), and indirect benefits include the inheritance of attractive traits, ‘good genes’ or increased ability to attract mates (Anderson, 1994; Møller & Thornhill, 1998). Therefore, a female can improve her reproductive success and output through benefits gained from being choosy (Kokko et al., 2003).

Females of many species are choosy when selecting a mate and go to great lengths to select males with particular traits. However, there are costs associated with being choosy (Booksmythe et al., 2008). Costs incurred can include energy expenditure, increased risk taking and greater opportunity costs (Reynolds & Gross, 1990). The costs that they pay during mate choice must be balanced by the potential benefits their offspring gain, either directly or indirectly.

If females consistently select ‘good quality’ males, and their offspring inherit these good qualities, the variation in the traits promoting reproductive success should decrease over time (Lehtonen et al., 2010). One of the greatest mysteries of sexual selection is why there is
still such high variation for attractive male traits in the face of strong and continued female selection.

The first major theme of this thesis is to examine how variation in female preferences can explain the continued high level of variation in male traits. It is clear that different females can have different preferences and the resultant variation in chosen males can lead to a wide variation in mated male traits (Houde, 1988; Qvarnström et al., 2000; Chaine & Lyon, 2008). Furthermore, female mating preferences can change temporally: females can choose different males at different times as demonstrated by the change in female preference for the white forehead patch in collared flycatchers (Qvarnström et al., 2000) and the temporal variation in female size preferences in a fiddler crab (Reaney & Backwell, 2007; Milner et al., 2010b; Kahn et al., 2013; Clark & Backwell, 2015). Some males would be successful at some times, and others would be successful at different times; leading to a wide variety of males that pass their genes on to the next generation. Additionally, female preferences can vary spatially, with females in some populations, or in some parts of a population, having different preferences to others. In guppies, for example, females in different populations prefer different males: some females prefer larger males, others do not; some prefer more orange colouration, others do not (Houde, 1988).

Another mechanism that can result in variation in female mate choice is size-assortative mating. This is common in many species and can have major evolutionary consequences (Crespi, 1989; de Cara et al., 2008; Taborsky et al., 2014). For example, we know that both male and female U. mjoebergi prefer to mate with larger individuals (Goshima et al., 1996; deRivera, 2005; Reading & Backwell, 2007; Reaney & Backwell, 2007). It is also known that U. mjoebergi mate size assortatively, where large males mate with large females and
smaller males mate with smaller females (Reaney & Backwell, 2007). The mechanisms behind size-assortative mating, however, are rarely examined (Snowberg & Bolnick, 2012; Taborsky et al., 2014). Crespi (1989) discusses three common causes of size assortative mating, i) mate availability; ii) mating constrains; and iii) sexual selection.

Despite variation in female preference being apparent and potentially resulting in greater variability in mate choice and male mating success, if a male loses the sexual cue, i.e. an exaggerated appendage, he may be unable to attract a female at all. The loss and subsequent regrowth of body parts is called autotomy and it occurs in many animal species (Maginnis, 2006 and papers cited within; Fleming et al., 2007). It is costly to regrow the limb which can result in decreased strength and/or delayed growth (Hoso, 2012; Bose & Robinson, 2013). There are often further consequences associated with the loss of an appendage, for example it can result in reduced limb strength, poorer foraging efficiency, reduced running speed or swimming ability (Barrios et al., 2008; Anderson et al., 2012; Marvin, 2013). Autotomy has also been shown to directly influence fitness through decreased mating success (Uetz et al., 1996; Langkilde et al., 2005). While some of these consequences appear to be temporary (Langkilde et al., 2005), sometimes they are permanent (Uetz et al., 1996). For example, wolf spiders can regrow legs that have been lost, however, the decorative tufts used in courtship are absent on the regrown legs resulting in decreased mating success and therefore fitness (Uetz et al., 1996).

While females have an ability to be selective to ensure survival and reproductive success (Kokko et al., 2003), sometimes, animals are faced with challenges outside of their control. One of the major challenges organisms face over the coming decades will come from climate-related environmental change. Tropical animals tend to have narrow thermal tolerances as a result of evolving in relatively constant thermal environments (Dillon et al.,
2010). For tropical ectotherms, the main challenge is to remain cool, and access to shady
refuge sites is one way to do this (Kearney et al., 2009). It has been predicted that most
ectotherms will not survive climate warming without access to shaded micro-habitats (Sunday
et al., 2014).

Not only can individual survival be dependent on thermal constraints but important
biological processes such is incubation of eggs and larval development is also at risk from
exposure to extreme temperatures (Hamasaki, 2003; Brillon et al., 2005; Smith et al., 2015).
Behavioural thermoregulation, such as micro-habitat selection, is one way in which animals
can combat exposure to extreme temperatures (Kearney et al., 2009). Understanding the
complexities associated with micro-habitat selection may allow us to better understand how
species will cope with increasing temperatures associated with climate change.

**Consequences of climate induced interspecific male-male competition**

The development of extreme traits can also occur through male-male competition.
Males can compete either through direct physical fighting (antlers of cervids – Clutton-Brock,
1982), or through indicators of fight ability or dominance such as plumage colouration (golden-
winged warblers plumage – Jones et al., 2017), and vocalisations (natterjack toad choruses –
Arak, 1983). Competition between males can either be over resources that are essential for
successful mating or they will compete for direct access to females (Ligon et al., 1990).

If more than one species require a similar resource or territory for mating success,
interspecific male-male competition can arise. Interspecific competition results in the reduced
fitness of one or both species. Two species of scorpionfly studied by Thornhill (1987), have very similar ecologies (Thornhill, 1980) and compete for access to arthropods as nuptial gifts (Thornhill, 1987). Gobies have been shown to potentially be detrimentally effected by a number of other species including fish, urchins and encrusting tunicates, sponges and cnidarians. These species utilise similar habitats that the gobies use for mating, restricting the available space and therefore mating opportunities (Breitburg, 1987). Competition over space could have profound consequences on one or multiple species experiencing interspecific competition.

Human induced climate change and other anthropogenic disturbance has, and continues, to result in habitat loss for many species worldwide (Fish et al., 2005; Parmesan, 2006). This will result in increased competition both within species and between species (Galbraith et al., 2002). Many studies have demonstrated that when two previously non-overlapping species are forced to compete over a resource, one species is likely to become dominant over the other (Bach et al., 1976; Blank & Figler, 1996; Guiasu & Dunham, 1999). In recent years, an area previously occupied solely by *U. mjoebergi* has been invaded by a larger fiddler crab species, *U. elegans*. This allows us to study consequences, if any, to the smaller resident species from initial invasion through to co-existing with established conspecifics.

**Fiddler crabs as a study system**

Male fiddler crabs have a sexually selected enlarged claw. Both male and female fiddler crabs own their own territory that contains a burrow. All of their feeding and courting is done on the surface while the burrow is used for access to water, escape from predators and
extreme environmental conditions, and mating and incubation of eggs (Fayed et al., 2008). As a result, territory ownership is extremely important for both survival and mating (Milner et al., 2010a). The banana fiddler crab, *Uca mjoebergi*, is predominately a burrow mating species. Once sexually receptive, females leave their burrows and wander through the male population. Males try to attract females to their burrow by waving the enlarged claw. Females choose mates using a two-step process; 1) she will select a male to visit based on phenotypic qualities associated with the enlarged claw (Reaney et al., 2008), and 2) she will decide to stay based on burrow characteristics (Backwell & Passmore, 1996). If she does not like the burrow, she will leave and continue searching. If the burrow meets her requirements, she will stay in the burrow and mate with the male. The male then mate guards the female until she has extruded her eggs onto her pleopods, ensuring that he is the father of the clutch. The male then leaves the burrow and reseals the female in for the subsequent ±21 days while she incubates her eggs. The female releases her eggs at the highest amplitude nocturnal spring tide so that her larvae are swept quickly out to sea and avoid the high levels of inshore predation (Christy, 1982).

Once the male has lost his burrow, either after mating and relinquishing his to a female or after being evicted by another male, he is required to find a new territory to survive and for any future matings. The male can either find an empty burrow, dig a new burrow, or more commonly, fight a resident male for a territory (Jennions & Backwell, 1996). Fighting can be extremely costly and result in injury, loss of the enlarged claw, or even death (Crane, 1975; Milner et al., 2009). Each of these can have significant consequences to individual fitness.
Thesis outline

Chapter 1 – Examines changes in female mate selection, both temporal and spatial, based on in-burrow environmental conditions.

Chapter 2 – Investigates the potential mechanisms behind size assortative mating and explores which of the possible mechanisms drive size assortative mating in *U. mjoebergi*.

Chapter 3 – Assesses the impact of the loss (autotomy) and subsequent regeneration of a male’s large claw; and uses the study to examine the importance of large sample sizes when dealing with small effects.

Chapter 4 – Explores the impact that micro-habitat selection has on the timing of critical reproductive events such as incubation, larval development and larval release.

Chapter 5 – Examines competition between heterospecific fiddler crabs, examining interspecific interactions from first contact through to established mixed species populations.

Conclusion – Discusses the importance of the work highlighted within the chapters and gaps in the knowledge where future research is required.
References


Chapter 1

Temporal and spatial variation in female mating preferences in a fiddler crab

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**Abstract:** Female mating preferences can vary temporally, with females choosing different males at different times; and spatially, with females in different populations preferring different males. This level of complexity is now well established, but I know of no evidence for a mosaic of female preferences within a single population. Here I show that, in the banana fiddler crab, *Uca mjoebergi*, female preferences vary both temporally and spatially. Females living in the high inter-tidal zone changed their mating preference for male size over the duration of the nine day mating period every semi-lunar cycle: early mating females selected larger males with cooler burrows, slowing embryonic development; those mating later, selected smaller males with warmer burrows, accelerating development. Females living lower in the inter-tidal zone, however, did not show this temporal variation: they select the same sized males throughout the mating period. It is only in the high inter-tidal zone, at the start of the fortnightly mating period, that large size confers a mating advantage to males.
INTRODUCTION

When females have a strong preference for an extreme expression of a male trait, the variation in the trait should decrease over time (Lehtonen et al. 2010). Explaining the high level of additive genetic variation in traits that are under sustained female choice remains a core challenge in sexual selection: if females consistently choose attractive males, why are there still unattractive males? One possible explanation is that female preferences are not consistent over time, and there is now growing evidence of temporal variation in preferences (Chaine and Lyon 2008). Qvarnstrom et al. (2000), for example, showed that female collared flycatchers prefer males with large forehead patches late in the breeding season. They used long-term data to show that the number of offspring fledged from a breeding attempt depended on an interaction between the time of the season and the male patch size: most young were fledged from late breeding attempts with large-patched males. The benefit of selecting males with large patches varies with time and females adjust their preferences accordingly.

Another explanation for high levels of additive genetic variation in traits under strong female selection is that female preferences are not consistent over space: females may have different preferences in different populations. Houde (1988), for example, showed that female guppies from two populations differed in their preferences for the extent and intensity of the males’ orange pigment.

While it is now accepted that female preferences can change both temporally and spatially, I know of no study that shows a mosaic of female preferences within a single population. Here I examine a species that has been shown to have temporally varying female mating preferences (Reaney and Backwell 2007; Milner et al. 2010; Kahn et al. 2013), and I show that it has, in addition, spatially varying selection within a single population.
The banana fiddler crab, *Uca mjoebergi*, is an excellent example of a species in which females change their mate preferences over a short time period. In this species, mating occurs over a 5-9 day period each 14-17 day semi-lunar cycle. Previous work has shown that ‘early’ females (those searching for mates at the start of the mating period) choose large males as mates; while ‘late’ females (those that are mate-searching late in the mating period) choose smaller males as mates (Reaney and Backwell 2007). Larger males have larger burrows that have lower temperatures, so by selecting larger males at the start of the mating period, females will slow down the development of their larvae. By selecting smaller males at the end of the mating period, females will incubate in a smaller, warmer burrow and, therefore, speed up the egg development rate (Reaney and Backwell 2007; Kahn et al. 2013). Why do females alter the development rate of their eggs? It is important that the clutch is released during nocturnal spring tides since they rapidly wash the hatchlings out to sea, avoiding the high predation risks inshore (Christy 1978). By slowing down incubation when mating occurs early, and speeding it up when mating occurs late, females ensure that their clutches are ready for release during the optimal tides.

The inter-tidal is well known for pronounced change in ecological conditions across relatively short distances over an elevation gradient. An animal living at 6.6m above the mean low water mark (i.e. the high inter-tidal in northern Australia) is covered by the high tide on only seven days of each 14 day semi-lunar cycle. During the remaining seven days, the mudflat is exposed and reaches temperatures of +50°C. An animal living lower in the inter-tidal zone, say 6.2m, is covered by the high tide on nine days of the semi-lunar cycle, and is covered for a longer time each day. Sea water is ±20-25°C cooler than exposed sediment, so higher inter-tidal zones are warmer, on average, than lower zones because they are submerged by the tide less often and for shorter periods of time.
The banana fiddler crabs that were studied by Reaney and Backwell (2007) and Kahn et al. (2013) lived in the high inter-tidal, at 6.6m above the low water mark, where much of the work on this species has been conducted. The population, however, covers a much wider range of the inter-tidal (from 5.5m-7m above the mean low water mark). Females living low in the inter-tidal would not experience the severe constraints of those living higher; they are covered by high tides on more days and for longer periods of time each day; they do not experience the extremely hot, dry period that occurs on the surface in the higher inter-tidal.

We predict (i) that females living lower in the inter-tidal zone will mate earlier in the mating period than females higher in the inter-tidal (since they have longer incubation durations because they live, on average, at lower temperatures). I also predict that (ii) females in the low zone will mate over a wider range of days than those in the higher zone, and (iii) will not temporally change their mating preferences (they should not choose large males at the start of the mating period and small males at the end of the mating period since they do not need to speed up or slow down their incubation rates) as do the females in the high inter-tidal. This is because their larval release time is less constrained: they are covered by high tides for nine days, whereas the higher females are covered by high tides on only seven days. Lower living females would not have to control the incubation rate of their larvae to the same extent as higher-living females, so they should not need to change their mating preferences over the duration of the mating period.

METHODS

_**Uca mjoebergi**_ is a small fiddler crab that lives in large, high density, mixed-sex populations on inter-tidal mudflats (Reaney and Backwell, 2007). Each individual defends a territory with a central burrow that is used as a heat sink, a water source, a mating and incubation site and to escape from predators (Reaney and Backwell, 2007). The burrow is surrounded by a small area
(±10 cm diameter) of mudflat surface that is used for feeding and courting. Mating occurs over a period of 5–9 days in the 14–17 day tidal cycle (Reaney and Backwell, 2007). A female that is ready to mate will leave her territory and wander through the population of waving males, visiting an average of three males (briefly entering their burrows) before selecting a mate. Mating occurs in the male’s burrow and the male guards the female for a few days until she releases her clutch of eggs onto her pleopods (Reaney and Backwell, 2007). The male then leaves and seals the female into the burrow, where she remains for the following ±20 days to incubate her eggs. She re-emerges at a nocturnal spring tide to release her pelagic larvae into the water (Reaney and Backwell, 2007).

These crabs reproduce all year and they experience only a narrow range of temperatures (annual mean temperature from 1941-2015 taken at Darwin airport: lowest mean maximum = 30.6°C; highest mean maximum = 33.3°C; annual mean maximum = 32.0°C; Australian Bureau of Meteorology http://www.bom.gov.au/climate/averages/tables/cw_014015.shtml). All data were collected during the dry season (May-December) to avoid the monsoonal rains. Data were collected over four tidal cycles (high site) and five tidal cycles (middle and low sites). The maximum tidal range at the study site is 7.8 m. The study population occurs from 5.5 – 7 m above the low water mark. Crabs are surface active only when the surface temperature < 50°C; they remain in their burrows if the surface exceeds 50°C.

We studied a population of *Uca mjoebergi* at East Point Reserve, Darwin, Australia (130°49'51"E, 12°24'32"S). The population covers the higher inter-tidal zone, from ±5.5m–7m above mean low water mark. Previous work on the timing of reproduction was conducted at the higher end of population distribution (6.6m) and was published in Reaney and Backwell (2007). I now add two lower sites (6.4m and 6.2m) where the data were collected using the same protocol as Reaney and Backwell (2007). Data for the low site were collected in 2006, at
the same time as the high site (but unpublished) and data for the middle site were collected in 2012. In order to compare the three sites, I have re-analysed the Reaney and Backwell (2007) data here.

We located a mate-searching female and followed her from a distance of ±1.5m (to avoid disturbing her) and deposited beads at the burrow entrance of each male she visited. The beads were dropped through a long tube after the female had moved sufficiently far enough away to avoid disturbing her. I have used this method of tracking females extensively in this population (Reaney and Backwell, 2007; Fayed et al., 2008; Curran, unpublished thesis). This species is particularly bold and waving and mate-searching often occurs between your feet as you stand in the population. If the female remained in the male’s burrow for 10+ mins, or the male emerged and blocked the burrow entrance with his body (the ‘guarding pose’), I scored it as a mating. Previous work has shown that, if a female stays in the male’s burrow for 5+ mins, she almost always produces a clutch of eggs (Curran, unpublished thesis). Once a mating was noted, I captured the pair as well as the visited but rejected males, and measured them (carapace width for all crabs and major claw length for males).

We followed 21 females at the high (6.6m) site (data from Reaney and Backwell 2007), 46 at the middle site (6.4m) and 30 at the low site (6.2m). I documented the following for each female I tracked:

(i) day of the cycle on which mating occurred (day 0 is the previous spring tide; see Reaney & Backwell 2007)

(ii) The size of the female, mated male and all visited but rejected males

At each of the three sites (high, middle and low), I estimated the population size distribution of males by randomly placing 7-10 plots (35cm x 35cm) in each area, and collecting and
measuring all males in each plot. I measured 82 males from the high site (6.6m); 119 from the middle site (6.4m) and 177 from the low site (6.2m). The population size distribution was collected at each site during the time when the females were tracked (e.g. the data for the high site - both the female tracking and the population size distribution - were collected in 2007).

We measured the temperature at the entrance of males’ burrows in 10 locations across the range of tidal heights (from 5.6 – 6.7m). These data were collected in July 2015 and excludes the very highest area of the mudflat since the crabs no longer occur there. I used a Physitemp Model BAT-12 thermometer and also measured the burrow entrance diameter (mm) and made note of the time of each reading (readings were taken over a three hour period).

The population consists of millions of crabs over a large area. To avoid multiple testing of males, I followed females in different parts of each of the three sites each day. The chance of re-using a male is extremely low. I can be certain that I did not re-use females since I captured them and took them to the laboratory as part of a separate experiment.

All statistical analyses were conducted using IBM SPSS 20. Male carapace width and major claw length are highly correlated (Booksmythe et al. 2010) so I used claw length as the measure of male size in this study. When analysing visited but rejected males, the mean claw length of all rejected males visited per mating was used (Reaney and Backwell, 2007). ANOVA with LSD post-hoc test was used to test if the three sites differed in the timing of mating, whether the size of population males differed between the sites, and whether there was a difference in sizes between mate searching females. General Linear Models were used to test whether there was a change is the size in mate searching females throughout the mating period, whether there was a change in size of the visited but rejected males throughout the mating
period, and whether there was a change in size of the mated males throughout the mating period. General Linear Models were used to test if there was a difference in the length of the mating period at each of the sites, and whether there was a difference in burrow temperatures between the three sites. General Linear models were also used to control for female size when testing whether the size of rejected and mated males changed throughout the mating period.

RESULTS

Did the three sites differ in the timing of mating? Yes, mating occurred later at higher elevations, with peak mating on day 12 at the high site; day 10 at the middle site and day 8 at the low site (Figure 1). There was a significant difference among mean mating time at each of the three locations (ANOVA: $F_{1,2} = 15.43$, $P < 0.001$, Mean ± SD (N) = 12.05 ± 1.07 (21) for high site; 11.00 ± 2.03 (46) for middle site; 9.13 ± 2.22 (30) for low site; LSD Post Hoc tests: all $P < 0.05$). Mating occurred over five days at the high site, eight days at the middle site and nine days at the low site (Figure 1). This is a significant difference (General Linear Model with the number of days over which mating occurred (for four cycles at the high site, five cycles at the middle site and five cycles at the low site) as the dependant variable; cycle number and site as covariates: effect of cycle was non-significant ($F_1 = 0.01$, $P = 0.92$); effect of site was significant ($F_1 = 6.11$, $P = 0.03$).
Did the three sites differ in the population size distribution of males? No, there were no differences in the population size distribution of males at the three sites when looking at major claw length (ANOVA: $F_{2,377} = 0.03, P = 0.971$, Mean ± SD (N) = 14.84 ± 3.21 mm (82) for the high site, 14.95 ± 3.52 mm (119) for the middle site, and 14.94 ± 3.63 mm (177) for the low site).

Did the three sites differ in the temperature of males burrows? Yes, burrows were warmer higher in the inter-tidal zone when controlling for burrow size and time of reading (General Linear Model with burrow temperature as the dependent variable and zone (1 = highest, 10 = lowest), burrow size and time as covariates: $F_{1,100} = 4.09, P = 0.046$). The temperatures are presented in Figure 2.
Figure 2. The entrance temperature of ten burrows at ten plots down the intertidal zone. Plot 1 is the highest elevation and plot 10 the lowest. This was taken from a cross section of the mudflat to attempt to get the greatest representation throughout the temperature gradient and do not necessarily directly align with behavioural sites. However, plots 9 and 10 correspond to the low site and plots 2, 3, and 4 align with the middle site.

Did the three sites differ in the size of mate-searching females? Yes, females at the middle site were smaller than those at the high and low sites (ANOVA: $F_{2,94} = 7.64$, $P = 0.001$, Mean ± SD $(N) = 9.45 ± 1.12$ (21) for the high site, $8.53 ± 0.94$ (44) for the middle site, and $9.13 ± 0.81$ (30) for the low site; LSD Post-hoc tests: $P<0.05$ except for high vs. low site: $P = 0.23$).

Was there a change in female size over the 5-9 day mating period at any of the three sites? No, the size of the mate-searching females did not change over the mating period at any of the sites (Correlation between female size and day of mating: high site: Pearson’s $r = 0.23$, $P = 0.32$, $n = 21$; middle site: Pearson’s $r = 0.11$, $P = 0.48$, $n = 44$; and low site: Pearson’s $r = -0.19$, $P = 0.31$, $n = 30$).
Was there a change in the size of visited but rejected males over the 5-9 day mating period at any of the three sites? No, there was no change in the size of the rejected males throughout the mating period at any of the sites (Correlation between rejected male size and day of mating: high site: Pearson’s r = 0.10, P = 0.77 n = 11, middle site: Pearson’s r = -0.12, P = 0.55, n = 30, low site: Pearson’s r = -0.37, P = 0.15, n = 17). The Mean ± SD (N) of rejected male claw lengths was 14.84±1.63 mm (11 matings; 37 rejected males) at the high site; 16.05±2.29 mm (30 matings, 135 rejected males) at the middle site and 16.60±2.92 mm (17 matings, 57 rejected males at the low site. A General Linear Model was used to control for female size, and it gave the same results: the size of rejected males did not change over the mating period at any site (high site: F_{1,11} = 0.032, P = 0.86; middle site: F_{1,30} = 0.247, P = 0.62; low site F_{1,17} = 1.68, P = 0.22).

Was there a change in size of the mated male over the 5-9 day mating period at any of the three sites? Yes, the three sites differed. There was no change in the size of the mated male over the duration of the mating period at the low site (Pearson’s r = -0.17, P = 0.38, n = 30) but the size of the mated male decreased over the duration of the mating period at the high and middle sites (Pearson’s r = -0.449, P = 0.041, n = 21 at the high site; Pearson’s r = -0.331, P = 0.035, n = 44 at the middle site). A General Linear Model was used to control for female size, and it gave the same results: females at the high and middle sites mated with larger males early in the mating cycle and significantly smaller males later in the mating cycle (Figure 3. linear regression high site: F_{1,19} = 15.44, P = 0.001; middle site: F_{1,42} = 5.71, P = 0.021) while there was no significant difference in mated male size across the mating period at the low site (F_{1,28} = 0.364, P = 0.515).
DISCUSSION

Females that lived in the higher inter-tidal zone mated later than females living lower down. This is likely to be temperature related: burrows were warmer the higher in the inter-tidal that they occurred. Furthermore, higher females would be exposed to air temperature for much longer than lower females. Surface temperatures on the exposed mudflat regularly reach 50°C while sea temperatures maintain a much cooler average of approximately 27°C. Higher females would therefore incubate their eggs at higher average temperatures. This will result in shorter incubation durations (Christy 1982; Yamaguchi 2001; deRivera 2005; Reaney and Backwell 2007; Kerr et al. 2012 and 2014). If females are to release their larvae at the optimal time (a high nocturnal spring tide), higher females would need to mate later than those living further down the inter-tidal zone.
Females that lived higher in the inter-tidal zone mated over fewer days than those living lower down (i.e. their mating was more synchronous). This is probably due to the number of days available for larval release. The higher in the inter-tidal zone, the fewer the days that the area is inundated by the high tides. Larval release is therefore restricted to fewer days the higher the female lives in the inter-tidal. This would result in a narrow window of suitable mating times at higher elevations.

Females that live high in the inter-tidal zone vary their mating preferences temporally (they choose large males at the start of the mating period and smaller males at the end of the mating period) which affects the incubation temperature, and therefore the incubation duration, of their eggs (Reaney and Backwell 2007). Large males have large burrows with lower temperatures, so incubation is slower when mating occurs earlier than the optimal mating day. Small males have small burrows with warmer temperatures, so incubation is faster when mating occurs later than the optimal mating day.

Females that lived low in the inter-tidal did not vary their mating preference for male size, always preferring the same sized males. Although there was a difference in the size of the females at the different elevations (females were smaller at the middle elevation site and equally larger at the high and low site), this difference did not affect the pattern: when controlling for female size, the high and middle zone females temporally varied their preferences for male size and the females that lived lower in the inter-tidal did not vary their preferences temporally.

Why would high, but not low females switch their mating preferences from large males at the start of the mating period to small males at the end of the mating period? Crabs in the three zones studied here form part of a continuous population. There was no difference in the size
distribution of males between the three zones, so females had an equal range of male sizes to select from during the mating interval, indicating observed differences were due to female preference, not male availability. There was no change in the mean size of mate-searching females over the mating period at any of the three sites; and there was no change in the mean claw length of the visited but rejected males over the duration of the mating period at any of the three sites. It is important that the mean claw length of visited but rejected males did not vary temporally at any of the sites since it shows that the larger males in the population did not get ‘used up’, leaving only the smaller males available as mates. Females are visiting larger males at all times, at all sites, so large males are always available.

If Reaney and Backwell (2007), Milner et al. (2010) and Kahn et al. (2013) are correct and females (at high elevation) change their preferences from large to small males in order to control the temperature at which their embryos develop and hence the time at which the larvae are ready for release, then it is possible that females living lower in the inter-tidal do not need to make this adjustment. The high tide covers the lower part of the population on more nights of the semi-lunar tidal cycle, giving females a wider window of opportunity for larval release. If females are able to release their larvae on a greater number of days, then it may not be necessary for them to carefully adjust the incubation duration in order to ensure the larvae are ready for release at the appropriate time. They therefore do not need to speed up or slow down larval development, and so do not change their preferences for burrow temperatures or male size.

The banana fiddler crab appears to be the first species to show both a temporal and spatial pattern of female preferences within a single population. Both the temporal and spatial patterns occur over very short scales: a nine day period and a 0.4m elevation gradient (±100 linear meters). Even though they occur at a small scale, the shifts in female preferences
observed in this species are likely to be evolutionarily important, particularly in partially explaining the variation in male traits that are under strong directional selection (Lehtonen et al. 2010). This study also suggests that extreme care must be taken when extrapolating information from part of a population to the whole population; or between populations of the same species. The sexual selection pressures that operate within a population can be complex and temporally and spatially variable. Studies limited to a single site, or a single point in time, will limit our understanding of selection in the wild (Cornwallis and Uller 2010).

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COMPLIANCE WITH ETHICAL STANDARDS

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References


Chapter 2

Assortative mating in a fiddler crab

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Summary

Non-random mating, particularly positive size-assortative mating, is common and can have major evolutionary consequences. The causes of size-assortative mating, however, are rarely examined. Here I explore the possibility of sexual selection, mate availability and mating constraints causing the strong correlation between male and female sizes in the fiddler crab *Uca mjoebergi*. I show that the full size range of males is available to females throughout the mating period, so mate availability is unlikely to cause size-assortative mating in this species. I also show that mechanical constraints do not prevent females from entering the burrows or mating with the full size range of males. I suggest that the strong size assortative mating that I observed throughout the mating cycle is driven by sexual selection. Both males and females prefer large partners. The benefit to mating with large partners needs further investigation.
Introduction

Non-random mating is ubiquitous and can have profound effects on fitness as well as the genetic structure and demographics of populations (Crespi, 1989). It is also thought to play a role in speciation (de Cara et al., 2008; Taborsky et al., 2014). The most common form of non-random mating is size-assortative mating: large females mate with large males and small females with small males. While studies that detect assortative mating and document its effects are common, understanding the mechanism has received less attention (Snowberg & Bolnick, 2012; Taborsky et al., 2014). There are three common causes of assortative mating (Crespi, 1989): (i) Sexual selection: one or both sexes have a preference for larger mates, and/or there is an intrasexual advantage to large size (e.g. large males are at a competitive advantage over smaller males and can therefore monopolise larger females; Olsson, 1993). (ii) Mate availability: large individuals of both sexes may be active at different times (or different locations) than smaller individuals. (iii) Mating constraints: the size difference between males and females could, for example, prevent copulation.

The fiddler crab, *Uca mjoebergii*, is a particularly interesting species since there is size-assortative mating (Reaney & Backwell, 2007) but it is a more complex situation than in most species. There is the potential for all three potential causes of assortative mating to play a role.

**Sexual selection**: Females are known to preferentially select larger males as mates (deRivera, 2005; Reaney & Backwell, 2007) and larger males preferentially court larger females (Goshima et al., 1996; Reading & Backwell, 2007). ‘Sexual selection’ could therefore potentially cause assortative mating in this species. Previous studies have shown that female preferences for male size change over the duration of the mating period, with larger males preferred earlier in the five day mating period and smaller males preferred later (Reaney & Backwell, 2007; Kahn
et al., 2013; Clark & Backwell, 2015). If females temporally change their preference for male size, how does this affect assortative mating?

Reaney & Backwell (2007), Milner et al. (2010), Kahn et al. (2013), and Clark & Backwell (2015) show that the decrease in female preference for larger males is due to temporal constraints affecting larval development: females select large males at the start of the mating period because their larger burrows are cooler and slow down larval development; they prefer smaller males at the end of the mating period because their smaller burrows are warmer and speed up larval development (Reaney & Backwell, 2007; Milner et al., 2010). The adjustment in female preferences therefore allows females to release their larvae at the optimal time (high nocturnal spring tide).

**Mate availability:** Males and females are not temporally or spatially segregated in this species, so mate-searching females are likely to encounter a large variety of potential mates. A well-established characteristic of fiddler crabs is the highly male-biased operational sex ratio (OSR – Nobbs & McGuiness, 1999), and in this species there are approximately 1000 males to each mate-searching female. At the study site in question, approximately 20-30 males mate on any particular day and these males fall out of the pool of potential mates because they remain underground mate-guarding the female for several days. This still leaves the vast majority of male crabs available to females.

Furthermore, previous studies on the changes in female mating preferences from larger to smaller males over the five day mating period found no evidence of a decrease in the size of males ‘available’ to females (Reaney & Backwell, 2007; Milner et al., 2010; Kahn et al., 2013; Clark & Backwell, 2015). Further evidence, however, is required to establish whether the full size range of males are available to females as potential mates throughout the mating period.
If the larger males are ‘used up’ at the start of the mating period, then females may be constrained by ‘mate availability’. In this species, females visit up to 20 males ($\bar{x} = 3.4$) before selecting a mate. If a full size range of males is available to females throughout the mating period, then the size of visited but rejected males should not change over the mating cycle even though the size of the male selected as a mate decreases. The size range of the visited but rejected males should also not differ from the size range of the male population. If so, then mate availability is unlikely to be the cause of size assortative mating in this species.

**Mating constraints:** There are two potential ‘mating constraints’ that could affect size-assortative mating: (i) disparate sized partners may not be able to copulate due to mechanical constraints; and (ii) females may not be able to fit into the burrows of smaller males, preventing them from selecting them as mates. Previous work has shown that disparate sized pairs are not prevented from successfully copulating (Slatyer et al., 2012). There is an alternative mating tactic in this species: females mate with their male neighbours in return for assistance in fighting off intruders (Milner et al., 2010; Slatyer et al., 2012). In this context, it is possible for very small males to successfully mate with very large females and vice versa (Slatyer et al., 2012), indicating that mechanical constraints due to disparate sized partners do not prevent disparate sized pairs from copulating.

Burrow size, however, may play a role: large females may not be able to fit into the burrows of smaller males. This has been found in other fiddler crab species (Christy, 1983). If some females are too large to fit into a male’s burrow and are therefore unable to mate with him, it may partly or fully explain assortative mating (Christy, 1983). In this scenario, a scatterplot of male and female size should show a triangular pattern in which small females mate with all sized males, and large females mate only with large males.
Here I examine mating in the banana fiddler crab and determine whether (i) there is size assortative mating; (ii) the level of assortment changes over the duration of the mating cycle (along with changes in female preference); (iii) there is the full size range of males available to the females throughout the duration of the mating period, and (iv) burrow size constrains females in their choice of mates.

Methods

_Uca mjoebergi_ is a small inter-tidal fiddler crab that lives in large, high density, mixed-sex populations. Each individual defends a territory with a central burrow that is used as a water source, a mating and incubation site, a heat sink, and to escape from predators. The burrow is surrounded by a small area (~10 cm diameter) of mudflat surface that is used for feeding and courting. Mating occurs over 5-9 days each 14-17 day tidal cycle (Reaney & Backwell, 2007). A female that is ready to mate will leave her territory and wander through the population of waving males, visiting an average of three males before selecting a mate (Reaney & Backwell, 2007). Mating occurs in the male’s burrow and the male guards the female until she releases her clutch of eggs onto her pleopods (Reaney & Backwell, 2007). The male then leaves and seals the female into the burrow, where she remains for the following ± 20 days to incubate her eggs. She re-emerges at a nocturnal spring tide to release her pelagic larvae into the water.

We studied a population of _Uca mjoebergi_ at the Mangrove Conservation Area of East Point Reserve, Darwin, Australia (130°49'51"E, 12°24'32"S) from September – December 2012. This population consists of tens of thousands (in the vicinity of 100 000, or 40 crabs per square meter) of courting males that range in claw length from 8 – 23.8 mm (\( \bar{x} = 14.94, \text{s.d.} = 3.52, n = 119, \) Clark & Backwell, 2015).
We located a mate-searching female and followed her until she selected a mate. I placed glass beads at the entrance of every visited males’ burrow by dropping it down a hollow tube after the female had moved sufficiently far enough away from the burrow to not be disturbed by my actions. These individuals were subsequently captured by placing the end of a ‘catching stick’ at the burrow entrance. Once these individuals exited their burrow to continue courting, the stick was inserted into the burrow entrance leaving the crab stranded on the surface and easily captured by hand. After the measurements were taken (carapace width and major claw length), they were returned to their original burrows.

If the female remained in the male’s burrow for 10+ mins, I scored it as a mating. Previous work had shown that, if a female stays in the male’s burrow for 5+ mins, she almost always produces a clutch of eggs (Curran, unpublished thesis). Once a mating was noted, I captured and measured the pair (carapace width for all crabs and major claw length for males). Carapace width and claw length are highly correlated (Booksmythe et al., 2010) so I use claw length as the measure of male size in this study. I followed 44 females and noted the day of the cycle that mating occurred: day 0 is the previous highest spring tide. This species breeds during the neap tides and the area is uncovered by high tides for 5-9 consecutive days, giving us an eight-hour daily window of activity. I followed 1-2 females each day. The study period was during the dry season, so cloud cover and rain was very rare and I did not collect data on cloudy or rainy days.

We collected population data by demarcated 24 plots (35cm X 35cm) and captured all males within each plot. Measurements (carapace width and major claw length) of 119 individuals were taken during the time of data collection. These data were used in a previous study for a different purpose (Clark & Backwell, 2015).
Data on female sizes and male sizes (mated and rejected), population male sizes and the day of the cycle on which mating occurred were all normally distributed. I ran a General Linear Model with male claw length as the dependent variable, and female size and day of the mating period as covariates. Values for female size and day of mating were ‘centred’ (transformed so the mean value was 0). The interaction between female size and day of mating was included in the model but subsequently removed because it was not significant.

We used Pearson’s correlations to determine if size-assortative mating occurred (correlation between mated male and female sizes, n = 44); whether female size changed over the duration of the mating period (correlation between female size and day of the cycle on which mating occurred, n = 44); whether the size of the visited but rejected males changed over the mating period (correlation between rejected male size and the day of the cycle on which mating occurred, n = 101). I used an independent sample t-test to compare the distribution of visited but rejected male sizes (n = 101) and the size of males in the population (n = 119).

Carapace width and claw length are highly correlated (Booksmythe et al., 2010), so I used claw length as the measure of male size and carapace width as the measure of female size in this study.

Results

There was a strong correlation between the size of the female and the size of the male she selected as a mate (Pearson’s r = 0.41, P < 0.005, n = 44; female carapace width: $\bar{x} = 8.53$, s.d. = 0.94, n = 44; male claw length: $\bar{x} = 16.06$, s.d. = 3.60, n = 44). Larger females chose larger males (Figure 1a). The size of the mate-searching females did not change over the duration of the
mating period (Pearson’s r = 0.11, P = 0.49, n = 44; Figure 1b). The size of the visited but
rejected males did not change over the duration of the mating period (Pearson’s r = 0.0.07, P =
0.48, n = 101; Figure 1c). The visited but rejected males were larger than the males in the
general population (t_{205} = 2.13, P = 0.04; n = 101, 119). The distribution of visited but rejected
and population males is presented in Figure 1d.

The General Linear Model showed that:

(i) The day of mating had a significant effect on the size of the males that were selected
as mates (Table 1): females selected large males at the start of the mating period and
small males at the end (As found previously in Clark & Backwell, 2015).

(ii) Female size had a significant effect on the size of the males she selected as mates
(Table 1): size-assortative mating is occurring.

(iii) The interaction between female size and day of mating was non-significant (Table 1):
the strength of the relationship between male and female size did not change over the
duration of the mating period.

Table 1. Results of the General Linear Model with male claw length as the dependent variable,
and female size and day of the mating period as covariates.

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<tr>
<td>Day of Mating</td>
<td>6.11</td>
<td>1,43</td>
<td>0.02</td>
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<tr>
<td>Female size</td>
<td>11.30</td>
<td>1,43</td>
<td>0.002</td>
</tr>
<tr>
<td>Day of mating * Female size</td>
<td>0.28</td>
<td>1,43</td>
<td>0.60</td>
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I also used the data that my research group had previously collected on male and female sizes
when the female had fully entered the burrow of the male, either for a visit or a mating (n =
I plotted my 44 data points along with these data to look for evidence of a mechanical constraint that may have prevented large females from entering the burrows of smaller males. I found that, in all cases and especially for large females, they were able to fit into much smaller burrows than those they selected: for each burrow that the female selected (solid dots in Figure 2), there were hollow dots below it; suggesting that females fitted into smaller burrows than the one they selected. Although the largest females did not mate with the smallest males and the smallest females did not mate with the largest males, there was no triangular pattern visible in the data that would suggest that large females mated exclusively with large males while small females mated with a full size range of males (Figure 2).
Figure 1. (a) The size of the female and the male she selected as a mate. (b) The size of the females that mated on different days of the mating period. (c) The size of the rejected males over the duration of the mating cycle. (d) The distribution of visited but rejected males (grey lines) and males in the general population (black lines).
Figure 2. The size of mated pairs (solid dots) and the size of pairs in which females were able to fit into the males’ burrows (hollow dots). Note that, for each solid dot, there are hollow dots below it; this indicates that females were able to fit into smaller burrows than the ones they selected.

Discussion

There was strong positive size-assortative mating. The strength of the relationship between male and female size (i.e. the level of size assortment) did not change throughout the mating period, large females preferred larger males while small females preferred smaller males at all times. This is despite the temporal decrease in female preference for large male size over the duration of the mating period as shown by Reaney & Backwell (2007), Kahn et al. (2013) and Clark & Backwell (2015). Out of the three proposed causes of size-assortative mating (mating constraints, mate availability and sexual selection), which play a role in this species?
We found no evidence of mating constraints: all mating females were able to fit into smaller burrows than the ones in which they mated. We also know that large females are capable of copulating with smaller males and vice versa (Slatyer et al., 2012). It may be argued that females select large males because large burrows are better for incubation (as suggested by Christy, 1982). I do not believe this to be the explanation for the pattern observed here since, if larger burrows are beneficial for incubation, they would be beneficial for all females, large and small. Here I find that large females have a stronger preference for big males than do small females.

‘Mate availability’ is also an unlikely explanation for the patterns of size assortment found here. The highly male-biased OSR (Nobbs & McGuiness, 1999) suggests that all male size classes would be equally available to mate searching females throughout the mating period. This is further supported by my finding that the size of the rejected males did not differ over the duration of the mating period, and that the rejected males were larger than males in the general population. This is strong evidence that a full range of male sizes is indeed available throughout the entire mating cycle.

Sexual selection appears to play a major role in causing assortative mating in this species. Females prefer larger males overall (Reaney & Backwell, 2007), but here I show that small females tend to select smaller males while large females tend to select larger males. Goshima et al. (1996) and Reading & Backwell (2007) show that males also prefer larger mates but they will readily mate with a female of any size (Reading & Backwell, 2007).

A preference from both sexes for larger partners is likely to drive the correlation between male and female sizes. The benefit to males of preferentially courting larger females is relatively clear: larger females have more eggs (Hines, 1982; Fukui, 1988; Murata & Wada, 2002). What
are the benefits to larger females selecting larger males as mates? We now know, in this species, that larger females have stronger preferences for big males. What do large females require that small females do not require, and that can be provided by large males but not small males? The most likely candidate is a large quantity of sperm. Large females have larger clutches than small females (Hines, 1982; Fukui, 1988; Murata & Wada, 2002) and therefore require more sperm (Kendall et al., 2002). It has been shown in numerous crab species that larger individuals can have greater sperm counts and ejaculate sizes (Jivoff, 1995; Sasson et al., 2012). Further work needs to be conducted to determine whether fiddler crabs follow this pattern, however, if larger males produce more sperm than smaller males, a strong pattern of size-assortative mating would be a logical result.

Although mating constraints and mate availability do not appear to drive size-assortative mating in this species, sexual selection appears to play a major role. Understanding size assortative mating requires an understanding of all aspects of mate selection driving those choices, and may ultimately assist our understanding of population fitness and population demographics.

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References


Male mating success in a fiddler crab: a lesson in sample sizes

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ABSTRACT

Autotomy and regrowth of a body part occurs in many animal species. It is costly to regrow the limb and there are often additional long-term costs in, for example, limb strength, foraging efficiency and even mating success. In the fiddler crab *Uca mjoebergi*, 7% of males have autotomized and regrown their large claw at some point in their lives. Previous work has shown that there is a great disadvantage to having a regenerated claw. While these males are able to attract mate-searching females to visit them, none of the 84 males observed to have mated in previously collected data had regenerated claws. Since females’ final mate choice is based on burrow structure, it was assumed that males with regenerated claws had poorer burrows. Here I show that, by finding only three cases of a female mating with a regenerated claw male, that there is, in fact, no mating disadvantage to having a regenerated claw. I also show that the burrows of males with regenerated claws are no different to those of original-clawed males. This is a very clear reminder that sample size matters, especially when dealing with rare events.
INTRODUCTION

Many animals can regrow lost body parts (Maginnis 2006 and papers cited within; Fleming et al. 2007); the crown-of-thorns starfish can even regenerate an entire new body when it is cut into quarters (Birkeland & Lucas 1990). The ability to regrow tissues has allowed the evolution of autotomy, also known as appendage loss, which has occurred independently in a broad range of taxa (Fleming et al. 2007). Probably the most widely known vertebrate exhibiting this phenomenon are lizards (Arnold 1988), but autotomy and regrowth also occurs in a multitude of invertebrate species (Maginnis 2006; Fleming 2007) including damselflies (Gleason et al. 2014) and scorpions (Mattoni et al. 2015), and has even been observed in some mammals such as mice (Seifert et al. 2012).

Autotomy is expensive and is usually associated with predator avoidance since the costs of regrowth would be outweighed by the immediate survival benefit. Autotomy can result in costs in two ways. The loss of an appendage can result in reduced feeding efficiency (seastar, Barrios et al. 2008), swimming ability (salamander, Marvin 2013) and running speed (lizard, Anderson et al. 2012), while costs of regrowing the appendage include decreased strength (damselfly, Bose and Robinson 2013) and delayed growth (snail, Hoso 2012). There are also cases where autotomy directly decreases fitness. Males of a skink species have been found to have reduced mating success after dropping their tails (Langkilde et al. 2005), although the effect only lasted until the tail was regrown. The decreased fitness is a longer-term problem in wolf spiders since the regenerated legs do not have the decorative tufts of the originals and are less successful in courting females (Uetz et al. 1996).

In fiddler crabs, males can autotomize their large claw and grow back a new claw that is the same length as the original claw, but the manus (‘palm’) is shorter and the dactyls (‘fingers’)
are longer. It has less muscle, a thinner exoskeleton, and is lighter and less robust than an original claw (Yamaguchi 1973; Backwell & Passmore 1996; Reaney et al. 2008). How does this affect male mating success? We know from previous work that males with regenerated claws are equally likely to be visited by mate-searching females (Reaney et al. 2008), but this does not translate into equal mating success: none of the 33 mated males from previously collected data had regenerated claws (Reaney et al. 2008). In a later study, a further 51 matings were observed and, again, none of the selected males had a regenerated claw (Slatyer et al. 2012). The most likely explanation is that males with regenerated claws have inferior burrows: while regenerated claw males are able to attract females to visit them, their burrows may not be acceptable incubation sites to the females.

Regenerated claws are poorer weapons (Lailvaux et al. 2009) and males with regenerated claws are less likely to win fights and more likely to lose their territories to original clawed males (Reaney et al. 2008). About 7% of the male population have regenerated claws (Reaney et al. 2008) and larger males are more likely to have regenerated claws since there is a cumulative probability of claw loss with age, and the changes in claw structure are permanent.

Here I examine the burrow structure of males with original and regenerated claws and compare them to burrows that were chosen as incubation sites by females and those that they rejected as suitable incubation sites. I predict that males with regenerated claws are less likely to mate than males with original claws; and that this is because they are unable to obtain or defend a burrow that is suitable for incubation.
We also increased the sample size of the previous studies by re-estimating the prevalence of regenerated claws in: (i) the population; (ii) visited but rejected males; and (iii) mated males.

**Study system**

*Uca mjoebergi* is a small, tropical fiddler crab that lives on intertidal mudflats in large, mixed sex colonies. Both sexes defend individual territories that contain a surface area for feeding and courting and a central burrow that is used as a retreat site, water source, heat sink and incubation site. Like all fiddler crabs, male *Uca mjoebergi* have one greatly enlarged claw that is used as a weapon and is waved to attract mate searching females (Crane 1975).

Mate choice is a two-step process. Firstly, when ready to mate, a female will abandon her territory and wander through the population of thousands of males. Males wave vigorously to attract the female to their burrow for mating. Reaney et al. (2008) has shown that females select who to visit based on male traits (e.g. size, wave rate). Females visit up to 20 males ($\bar{x} = 3.4$) before selecting a mate, briefly entering his burrow and either leaving (rejection) or staying to mate (see Reaney et al. 2008 for more detail on the mating system).

Secondly, the female makes her final choice based on the burrow characteristics since the burrow needs to be structurally sound and have sufficient water and appropriate temperature for successful larval development (see Backwell & Passmore 1996). Females mate with the first visited male to meet a threshold criterion of burrow quality (Backwell & Passmore 1996). If she stays, the male seals them into the burrow, mating occurs underground and the male guards the female for several days until she extrudes her eggs onto her pleopods. He then leaves,
resealing the female underground. The female incubates her clutch for ±21 days and releases her larvae into the water at a nocturnal spring tide.

METHODS

We studied a population of *Uca mjoebergi* at East Point Reserve, Darwin, Australia from September – December 2012.

Population

We caught all males with a major claw length of over 8mm (at this size, the males court and have been found to mate) within 38 quadrats (35 x 35 cm), measured them (carapace and major claw) and noted whether they had original or regenerated major claws. I measured a total of 119 males.

Natural mate-searching

We located mate-searching females as they moved through the population of courting males. I followed each female from a distance of ±1.5 m to avoid disturbing her, and used a long, thin tube to deposit a bead next to the burrow of each male she visited as well as the male she selected as a mate. This method has been extensively used and does not scare the female or interrupt the mate choice process in any way (see Reaney & Backwell 2007). A visit was scored if the female entered the male’s burrow but then left and continued to mate-search. A mating was scored if the female entered the male’s burrow and did not leave within 10 mins or if the male sealed the pair underground by plugging the burrow shaft (these are both reliable indicators of mating; see Reaney & Backwell 2007). I captured and measured the visited and mated males (carapace width and claw length) as well as the female (carapace width).
whether the male had an original or a regenerated claw. I collected data for 46 mated males and 168 visited but rejected males.

**Burrow structure**

After removing the occupants, I made casts of 61 burrows consisting of both visited but rejected males, mated males, original clawed males and regenerated claw males. I sprayed builders’ expanding foam (Polyfilla® fast curing polyurethane foam) and let it expand to fill the burrow, with the excess volume spilling out of the burrow entrance (see Backwell & Passmore 1996). After an hour, I dug up the casts, washed off the sand and measured them (see Figure 1 for the measurements I made). Volume was calculated by inserting the casts into a known volume of water and recording the difference from before to after insertion.
Figure 1. Measurements taken from burrow castes. a) Vertical lines = entrance volume. Hashed lines = chamber volume. Horizontal lines = extension volume. Vertical + hashed + horizontal lines = total volume. b) a = entrance angle. b = burrow end angle. c = number of corners. d = widest point. e = narrowest point. c) f = entrance length. g = chamber length. h = extension length. i = total length. j = start of chamber depth. k = end of chamber depth. l = total depth.
Statistical Analysis

Analyses were conducted using IBM SPSS Statistics 20. Carapace and claw length are highly correlated (Reaney & Backwell 2007) and claw length is generally used as the measure of male size. Regenerated claws, however, are very variable in length while they are being regenerated (it takes 4-5 moults for them to reach the original length). In this study I therefore use carapace width as the measure of male size.

We increased the sample size of the previous studies by re-estimating the prevalence of regenerated claws in: (i) the population; (ii) visited but rejected males; and (iii) mated males. Combining the new data with that obtained by Reaney et al. (2008) and Slatyer et al. (2012), allowed me to increase the sample sizes and therefore increase the statistical power of my analyses. The three data sets were all collected in exactly the same way on the same population and at the same time of the year. There was no difference between the three data sets in terms of the proportion of regenerated males to mate (likelihood ratio $\chi^2 = 4.58$, $P = 0.10$) or the proportion of regenerated males to be rejected after having been visited (likelihood ratio $\chi^2 = 0.14$, $P = 0.84$). The males were independent in the three data sets. A table of the three data sets is presented as an electronic appendix (S1).

To compare the proportion of males with regenerated claws in the population, the visited males and the mated males, I used a binary logistic regression (controlling for male size). I included male carapace width as a covariate; original or regenerated claw form as a categorical covariate; and the male type (visited or population in the first regression and mated or population in the second regression) was the dependent variable.
We measured 12 burrow features. To avoid using multiple tests, I divided the measurements into four categories: (i) volume (total burrow volume; volume of the chamber alone; narrowest point of the burrow shaft; widest point of the burrow shaft); (ii) depth (total depth of burrow; the depth at the top of the chamber; the depth at the bottom of the chamber); (iii) length (the total length of the burrow; the length of the chamber); and (iv) geometry (the number of corners; the entrance angle; the end angle). I condensed each group of measurements into a single principle component (see Table 1).
Table 1. The percentage of variance explained by each component of the principle component for the comparison between accepted (mated) and visited but rejected males, and between males with original and regenerated claws.

<table>
<thead>
<tr>
<th>Component</th>
<th>Accepted (20) vs. Rejected (16)</th>
<th>Original (14) vs. Regenerated (11)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Volume</td>
<td>Total: 65%</td>
<td>66%</td>
</tr>
<tr>
<td></td>
<td>Chamber volume: 21%</td>
<td>23%</td>
</tr>
<tr>
<td></td>
<td>Narrowest point: 13%</td>
<td>8%</td>
</tr>
<tr>
<td></td>
<td>Widest point: 2%</td>
<td>2%</td>
</tr>
<tr>
<td>Depth</td>
<td>Total: 84%</td>
<td>73%</td>
</tr>
<tr>
<td></td>
<td>Chamber top: 12%</td>
<td>19%</td>
</tr>
<tr>
<td></td>
<td>Chamber bottom: 4%</td>
<td>8%</td>
</tr>
<tr>
<td>Length</td>
<td>Total: 84%</td>
<td>83%</td>
</tr>
<tr>
<td></td>
<td>Chamber: 16%</td>
<td>17%</td>
</tr>
<tr>
<td>Geometry</td>
<td># corners: 50%</td>
<td>41%</td>
</tr>
<tr>
<td></td>
<td>Entry angle: 28%</td>
<td>40%</td>
</tr>
<tr>
<td></td>
<td>End angle: 22%</td>
<td>18%</td>
</tr>
</tbody>
</table>

We used General Linear Models to compare each of the four PCs between accepted and rejected burrows (dependent variable = PC score; covariate = male carapace width; and fixed factor = accepted/rejected status). I analysed the burrow cast measurements in two steps: (i)
first I compared the burrows of males that were visited but rejected by a female (n = 16) with those that were visited and accepted as mates (n = 20). I only used burrows of males that had original claws. I used this comparison to determine the burrow characteristics that were predictors of mating success. (ii) I then compared the burrows from a random sample of males in the population, 14 of them with original claws and 11 with regenerated claws. This allowed us to determine whether males with regenerated claws were less likely to have the preferred burrow characteristics than males with original claws.

RESULTS

Population

Of the 119 males I captured, 113 had original claws and six had regenerated claws (5%). When I combined this with the data from Reaney et al. (2008) and Slatyer et al. (2012), there were 384 males with original claws and 28 with regenerated claws (6.8%). Larger males were more likely to have regenerated claws (Binary Logistic Regression, Wald test: $\chi^2_1 = 11.84, P = 0.01, n = 384, 28$).

Natural mate-searching

Of the 46 females I successfully followed until they selected a mate, 43 of them chose a male with an original claw and three mated with a male that had a regenerated claw (6.5%). This is the first account of males with regenerated claws successfully mating. When combined with the data from Reaney et al. (2008) and Slatyer et al. (2012), only 3.1% of mated males had regenerated claws: 94 mated males had original claws and three had regenerated claws.

There were 168 males that were visited but rejected by females. Of these, 22 had regenerated claws (12%). When combined with the Reaney et al. (2008) and Slatyer et al. (2012) data, there
were 308 visited males, 270 had original claws and 38 had regenerated claws (12.3%). This is a significantly higher proportion of regenerated claws than that found in the population (population: 384 original, 28 regenerated, Fisher’s Exact Test: P = 0.01). However, larger males are more likely to have regenerated claws, and male size may affect female choice, I used a binary logistic regression to control for male size when comparing the proportions of males with regenerated claws in the population (6.8%), in the visited males (12.3%) and in the mated males (3.1%). There was no clear advantage or disadvantage to having a regenerated claw in terms of attracting a female to visit the male’s burrow (12.3% of visited males had regenerated claws; 6.8% of males in population had regenerated claws: Binary Logistic Regression, Wald test: $\chi^2_{1} = 3.36, P = 0.07$).

There is also no clear disadvantage to having a regenerated claw in terms of mating (3.1% of mated males had regenerated claws, 6.8% of males in the population had regenerated claws; Binary Logistic Regression, Wald test: $\chi^2_{1} = 2.88, P = 0.09$). Mated males were only marginally less likely to have a regenerated claw than were males in the population.

Mated males, however, were less likely to have a regenerated claw than were visited but rejected males (3.1% of mated males and 12.3% of visited but rejected males had regenerated claws: Binary Logistic Regression, Wald test: $\chi^2_{1} = 6.44, P = 0.01$; controlled for male size).

What makes a ‘good’ burrow?

The only significant difference between accepted and rejected burrows was their volume: burrows that were rejected had a mean volume of 77.44 ± 33.04 ml while those that were accepted were far smaller: 51.15 ± 23.83 ml. Females selected burrows with smaller volumes (Table 2).
Table 2. GLM results for the comparison between burrow types (accepted /rejected burrows; or original/ regenerated male burrows). All df =1. Asterisks indicate significant P values.

<table>
<thead>
<tr>
<th>Principal Component of Burrow Structure</th>
<th>Accepted (20) vs. Original (14) vs. Rejected (16) Regenerated (11)</th>
<th>F (p)</th>
<th>F (p)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Volume</td>
<td>Male size</td>
<td>3.69 (0.06)</td>
<td>1.75 (0.20)</td>
</tr>
<tr>
<td></td>
<td>Burrow type</td>
<td>4.47 (0.04)*</td>
<td>0.92 (0.35)</td>
</tr>
<tr>
<td>Depth</td>
<td>Male size</td>
<td>0.39 (0.54)</td>
<td>0.22 (0.64)</td>
</tr>
<tr>
<td></td>
<td>Burrow type</td>
<td>0.66 (0.42)</td>
<td>3.22 (0.09)</td>
</tr>
<tr>
<td>Length</td>
<td>Male size</td>
<td>0.23 (0.64)</td>
<td>0.35 (0.56)</td>
</tr>
<tr>
<td></td>
<td>Burrow type</td>
<td>1.65 (0.21)</td>
<td>5.62 (0.03)*</td>
</tr>
<tr>
<td>Geometry</td>
<td>Male size</td>
<td>4.12 (0.05)*</td>
<td>0.81 (0.38)</td>
</tr>
<tr>
<td></td>
<td>Burrow type</td>
<td>0.13 (0.73)</td>
<td>0.72 (0.40)</td>
</tr>
</tbody>
</table>

We found that male size affected burrow geometry: larger males had significantly larger entrance angles to their burrows (Pearson’s correlation: r = 0.40, n = 36, P = 0.02), but since this is not a feature that differentiated accepted and rejected burrows, it appears that it is not used by females in mate selection.
**Do males with regenerated claws have ‘poor’ burrows?**

In the comparison between the burrows of males with original or regenerated claws, I found no effect of male type on any of the ‘important’ burrow characteristics (i.e. volume). They did differ, however, in burrow length: males with original claws had longer burrows ($23.09 \pm 3.87$ cm) than those with regenerated claws ($19.32 \pm 4.04$ cm). Burrow length, however, is not a feature that females use when selecting mates, so this difference between original and regenerated males’ burrows is not important to females.

The non-significant difference between the burrow volume of males with original and regenerated claws is of interest since it is this burrow feature that is used by females in mate selection. Males with regenerated claws do not differ from those with original claws in terms of the burrow feature that females use in burrow selection (i.e. volume).

**DISCUSSION**

Claw loss is rare in this population, only 6.8% of males have lost a claw at some stage of their lives. Larger males were more likely to have regenerated claws, but this is expected since there is a cumulative probability of losing a claw with age (Reaney et al. 2008). Mate-searching females visit an average of 3 males before selecting a mate. Of the visited males, 12.3% had regenerated claws. Is this significantly higher than the 6.8% of regenerated claws in the population? It is necessary to control for male size when making this comparison since large males are more likely to have regenerated claws (Backwell et al. 2000), and larger males are preferred by females as mates (Backwell & Passmore 1996; Reaney & Backwell 2007), although this relationship is complex and temporally variable (Reaney & Backwell 2007; Milner et al. 2010; Kahn et al. 2013; Clark & Backwell 2015). When controlling for male size, there was no
difference between the proportion of males with regenerated claws that were visited by females and that which occurred in the general population. Although regenerated claws are lighter, cheaper to produce and not as energetically expensive to carry, there is no clear advantage to having a regenerated claw in terms of attracting a female to a male’s burrow.

The proportion of mated males that had regenerated claws was 3.1%. Only three of the 97 females I followed selected a male with a regenerated claw as a mate. This low proportion, however, is no different from the 6.8% of males in the general population that have regenerated claws when you control for male size. Males with regenerated claws are as capable of meeting the threshold criterion for burrow quality as are males with original claws. This result is surprising and unexpected. Before this study, I had followed 84 females and none of them had mated with a regenerated claw male, convincing us that these males must have inferior burrows. By increasing the sample size, and finding only three females that mated with a regenerated claw male, a significant difference could no longer be found. This is a very clear reminder that sample size matters, especially when dealing with rare events. The switch from a significant difference to non-significant difference between mated and population males is based on finding only three cases (out of 97) of females selecting mates with regenerated claws.

If females are just as likely to stay and mate in a burrow owned by an original claw and a regenerated claw male, it now seems unlikely that the burrow quality differs between them. What makes a ‘good’ burrow? I compared accepted (i.e. mated) and rejected burrows and found that there was only one predictor of whether a burrow was deemed suitable or unsuitable for incubation: volume. Females preferentially mated with males with low volume burrows and rejected high volume burrows. This is very similar to the result found in another
fiddler crab species (Backwell & Passmore 1996) where burrow volume was the strongest predictor of whether a female would accept or reject a male as a mate. In both species, females have a clear and strong preference for burrows with smaller volumes.

Do males with regenerated claws have burrows of similar volume to males with original claws? Yes, they do. There is no difference in burrow volume, suggesting that females would be just as likely to accept the burrow of an original claw and a regenerated claw male.

One caveat is that there is a significant difference in the proportion of males with regenerated claws that were visited and rejected and those that were mated (12.3% vs. 3.1%). We know that females select mates in a two-step process: first they select a male to visit based on his phenotypic traits, and then they select whether to leave or stay and mate based on the burrow characteristics (Backwell & Passmore 1996; Reaney & Backwell 2007). Here I show that the subset of males in the population that were visited by females were the larger males and, since larger males are more likely to have regenerated claws due to the cumulative probability of claw loss with time, they were more likely to have regenerated claws. Of these visited males, the subset that was selected as mates were less likely to have regenerated claws. This is not due to female choice based on male characteristics but rather to female selection for burrow size (Reaney & Backwell 2007). Since females base the decision to leave or stay on the characteristics of the burrow, this suggests that there may be a difference in burrow structure between rejected and accepted males, possibly an unmeasured variable such as temperature or sediment structure. A possible burrow feature that was not measured but may affect female behaviour is the acoustic property of the sediment around the burrow. Fiddler crabs are known to drum on the sediment during and after courtship (Aicher & Tautz 1990; von Hagen 2000) and the acoustic properties of the sediment may differ between burrows.
occupied by males with original and regenerated claws. Another possible explanation for the lack of a difference in burrow structure between males with original and regenerated claws is that regenerated claw males are able to ‘bluff’ their opponents during fights, allowing them to defend their burrows. In a closely related fiddler crab, *Uca annulipes*, Callander et al. (2012) showed that males with regenerated claws were able to defend their territories during the mating period by investing in more costly fighting tactics and escalated fights. During the non-mating periods, they did not pay this cost and were significantly more likely to be evicted from their burrows. It is possible that this also occurs in *Uca mjoebergi*. Future work will examine each of these possibilities.

Previous work has shown that regenerated claw males are equally likely to be visited by mate searching females (Reaney et al. 2008); however, this is not transferred through to mating success, since regenerated males are less likely to be selected as mates. While regenerated claw males are more likely to be rejected, there is no difference in the prevalence of regenerated males when comparing the population versus mated males.

The loss and subsequent regeneration of a body part is common and costly (Seifert et al. 2012; Gleason et al. 2014; Mattoni et al. 2015). In some cases, autotomy has been found to decrease mating success (Uetz et al. 1996; Langkilde et al. 2005; Reaney et al. 2008). In those species of fiddler crabs that do not use their major claw in combat or mate attraction (Crane 1975), there is unlikely to be an effect of claw regeneration on fitness. Here I show that, even in species where the claw is used extensively in combat and courtship, and where a fitness cost is expected, limb regeneration does not necessarily decrease a male’s mating success. Some fiddler crab species mate both in males’ burrows and on the surface. Very little is known about surface mating but it has been shown that surface mated males gain very little paternity.
(Reaney et al. 2012). The effect of claw regeneration on surface mating in fiddler crabs needs further study.

**Broader implications of this study**

Many studies have found that males with regenerated body parts suffer on-going consequences (Bose & Robinson 2013; Barrios et al. 2008; Marvin 2013; Anderson et al. 2012; Hoso 2012; Langkilde et al. 2005; Uetz et al. 1996). However, there are also cases where the consequences of regeneration are transient. For example, in some lizards, the detrimental impacts of tail autotomy only occurred during the regrowing phase (Langkilde et al. 2005). I started this study with two clear predictions: (i) males with regenerated claws would be less likely to mate, and (ii) this would be because they had poor quality burrows. Neither is correct. Males with regenerated claws are not at a mating disadvantage and they do not have inferior burrows. The detrimental effects of claw loss appear to be transient in this species.

There is another strong message to be taken from this study: sample size matters. When an event is rare, a small number of cases can alter the outcome in a comparison of two proportions. The number of observations of the rare event constitutes the information content of the data set; it is easy to underestimate the probability of a rare event when you have small sample sizes (King & Langche 2001). For binary variables and very rare events (<1/100), a sample of <200 is considered too small (King & Langche 2001). The problem is not the rarity of events, but rather the effect a small number of cases can have on the outcome: 2 events per 100 observations is a lot less reliable than 200 events in 10000 cases.
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COMPLIANCE WITH ETHICAL STANDARDS

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Conflict of Interest: Both Authors declare that no conflict of interest exists for this study.

Ethical approval: All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. This article does not contain any studies with human participants performed by any of the authors.
REFERENCES


Callander S, Bolton J, Jennions MD, Backwell PRY (2012) A farewell to arms: males with regenerated claws fight harder over resources, Anim Behav 84:619-622


Yamaguchi Y (1973) Asymmetry and dimorphism of chelipeds in the fiddler crab Uca lactea De Haan. Zoological Magazine 82:154-158
Chapter 4

Micro-climate and incubation in a fiddler crab species

Huon L. Clark & Patricia R.Y. Backwell

Abstract:

Many tropical ectotherms will probably not survive climate warming without having access to thermal refugia. Temperature affects adult survival, but has just as strong an effect on larval stages, often due to its effect on reproductive timing and incubation duration. Intertidal animals usually have tight constraints on the timing of larval release since the planktonic larvae must be washed out to sea on the highest nocturnal spring tides. Can females behaviourally compensate for the effects of temperature over the naturally occurring temperature range by shifting the timing of mating and/or fertilisation or by adjusting the incubation duration? This experiment compares the timing of key reproductive events, as well as the size of eggs and larvae, in fiddler crabs that incubate over a 6°C range of temperatures. There was no effect of temperature on the timing of reproductive events or the size of eggs or larvae produced. In other crab species, the effect of temperature on incubation duration is exponential, with smaller decreases in incubation duration at higher temperatures. Since the natural incubation temperatures are unusually high for this species, the effect of further increases in temperature may not have had as marked an effect as it does for species breeding in slightly cooler temperatures. Future climate warming may not have as strong an effect as may be expected for this species.
1. INTRODUCTION

Tropical animals evolved in relatively constant thermal environments and tend to have narrow thermal tolerance ranges (Dillon et al., 2010). For tropical ectotherms, the primary challenge is to remain cool, and behavioural thermoregulation is one way to achieve this (Kearney et al., 2009). Behavioural thermoregulation includes body orientation (tropical butterflies, Bonebrake et al., 2014), increased heart and circulation rate (mushrooming in limpets, Williams et al., 2005), and- most importantly- retreating into the shade (Kearney et al., 2009). The ability of tropical ectotherms to survive climate warming will depend largely on the availability of shade (Kearney et al., 2009) and it has been predicted that most will not survive without having access to thermal refugia (Sunday et al., 2014).

Many studies document the effect of temperature on adults, but the effect is even more pronounced on larval stages. Temperature influences larval incubation duration (Hamasaki, 2003; Smith et al., 2015), and survival (Brillon et al., 2005). In a mosquito, for example, development rate and survival of the larvae were significantly reduced with a relatively small increase in temperature from 30 - 32°C (Paaijmans et al., 2013). Females can control the temperature at which their eggs develop by selecting cooler micro-habitats. In the lark bunting, for example, nests that were exposed to solar radiation fledged fewer young than nests that were protected from solar radiation (Pleszczynska and Hansell, 1980); and females were more likely to mate with a male that has a nest site that was protected from solar radiation (Pleszczynska, 1978; Pleszczynska and Hansell, 1980).
Tropical intertidal ectotherms can experience extremely high temperatures. The banana fiddler crab, *Uca mjoebergi* (Rathbun, 1924), for example, lives on intertidal mudflats that reach 50°C even though their critical thermal limit is 40°C (Munguia et al., In Prep). They are able to reduce their body temperature by retreating into their burrows (Smith and Miller, 1973) or by selecting cooler micro-habitats: they can live in the shade of the mangrove trees (Darnell et al., 2013; Powers and Cole, 1976; Smith and Miller, 1973; Thurman, 1998).

Thermoregulatory micro-habitat selection may also affect the larval stages, since females select the burrow in which she will incubate her eggs: if a female incubates in a burrow in the shade, her eggs would develop at lower temperatures than if she incubates in a burrow exposed to the full sun. Since the temperature in the burrow is much lower than on the surface, the incubation temperature is unlikely to affect larval survival, but it will very likely have an effect on incubation duration. In other fiddler crab species, even small increases in temperature decrease incubation durations (an increase of 2°C can decrease incubation by ~1.5 days; Christy, 1982; Kerr et al, 2012). Faster incubation may not be problematic for many species, but it can have adverse consequences for intertidal species that have planktonic larvae (barnacles, anemones, starfish, crustaceans, sponges etc.). The most critical temporal constraint these species face is the timing of larval release: it is generally confined to ~3 nights of peak amplitude spring tides (Christy, 1982) so that the larvae are quickly washed out to sea and avoid the high levels of predation inshore.

There are very few studies that examine the effects of incubation temperature on the timing of larval release in tropical intertidal ectotherms. By examining how the naturally occurring range of temperatures affect the timing of larval release, and determining whether females can behaviourally compensate for these thermal effects, may allow us to predict the effects that climate warming may have on these species.
1.1. Study species

*Uca mjoebergi* is a small fiddler crab that lives in mixed-sex, high density populations on intertidal mudflats along the northern coast of Australia (Backwell and Jennions, 2004). Males have a single enlarged claw that is used as a weapon during territorial disputes and to attract females during courtship displays (Crane, 1975). Males and females each defend a territory that consists of a small area of mudflat surface where feeding and courting occur, and a central burrow used to access water, avoid extreme weather and predators, and where mating and incubation occur (Crane, 1975). The species live high in the intertidal zone and their habitat is covered by high tides of >6 m. For 5-10 days each semi-lunar tidal cycle (during the neap tides), the population is not inundated at all (Clark and Backwell, 2015). It is in this period that females mate-search and males court them. Temperatures are high, with the sediment surface reaching >50°C (Munguia et al., In Prep). The population covers areas of mudflat that are open and in the full sun as well as areas that are partially or fully under mangrove trees and are shaded for part or all of the day. Males court in both the sun and shade areas, and mate-searching females freely move between the sunny and shaded areas.

When ready to mate, females leave their territories and wander through the population of courting males searching for a mate (Clark and Backwell, 2015). A female visits multiple males by approaching them, briefly entering their burrows and then leaving (if the male or his burrow are rejected) or staying (if the male and burrow surpass a quality threshold; Reaney and Backwell, 2007). If the female accepts the male, she stays underground with him in his burrow. He seals the burrow entrance with a sand plug, and mating occurs within the next few hours. The female stores the male’s sperm in a spermatheca and, 1-5 days later, she extrudes her eggs which are fertilised as they pass the spermathecal opening. The male remains underground, guarding the female until she fertilises and extrudes her eggs. He then
leaves the burrow, resealing the female underground to incubate her eggs. The male wanders through the population, fighting with resident males to win a new territory. The female incubates her clutch for ~ 21 days before releasing her larvae into the water column at the highest amplitude nocturnal spring tide. The timing of larval release is critical if the larvae are to avoid the high levels of predation in the mangroves. By releasing the larvae at the ebbing, nocturnal highest spring tide, the female ensures the larvae get washes out to sea as quickly as possible (Morgan and Christy, 1995).

If a female selects a male with a territory in the sun, she will incubate her eggs at a higher temperature than females that select males with territories in the shade. Increased temperature decreases incubation duration (Sanford et al., 2006; Yamaguchi, 2001). This may affect the tight temporal constraint on the timing of larval release. In order to release her larvae during the highest nocturnal spring tide, females would need to compensate for a shorter incubation when selecting a male with a territory in the sun and the longer incubation duration when in the shade. Five non-mutually exclusive mechanisms that females could potentially use to compensate for this affect are: (i) they could select sun or shade males based on the timing of mate selection: early mating females could select males in the shade since this would slow down the development rate, and later mating females could select males in the sun since this would speed up larval development; (ii) they could select different size burrows when mating in the sun or shade: smaller burrows are warmer than larger burrows (Reaney and Backwell, 2007) so females that mate in the shaded areas could select smaller burrows to compensate for the lower incubation temperatures and vice versa; (iii) females could adjust incubation temperatures by moving up or down the burrow shaft (Kerr et al., 2012): females that mate in the sun could move to the lower reaches of the burrow shaft while females that mated in the shade could sit higher up in the burrow shaft; (iv) they could alter
the time between mating and fertilisation. The female could delay fertilising her eggs if she is in a warmer burrow, and fertilise them earlier if she is in a cooler burrow; (v) females could slow the rate of egg development by adjusting the size of eggs they produce. There is a strong negative relationship between egg size and temperature in many crustaceans (Kobayashi and Matsuura, 1996; Sheader, 1996) including fiddler crabs (Christy, 1982; Kerr et al., 2012). Egg size varies according to the amount of yolk laid down (Sheader, 1996). Crustaceans are unusual in that they can rapidly produce yolk using several sites for its production: hepatopancreas, ovaries and hemolymph (Quackenbush and Keeley, 1988). This allows females to alter yolk content shortly before fertilisation. The incubation period of *U. mjoebergi* females could potentially be extended or decreased by producing larger/smaller eggs.

The following terminology is used to describe the stages of the mating process:

‘Mating day’ is the day on which the female approaches a male, enters his burrow and the male seals the pair underground (mating occurs within a few hours of burrow sealing).

‘Fertilisation day’ is the day on which the male leaves the burrow, resealing the female underground (the male leaves as soon as the female has extruded her eggs – fertilising them as they move past the spermatheca). ‘Mate guarding period’ is the time between the mating day and the day of fertilisation (the male remains underground with the female, preventing her from re-mating until her eggs have been fertilised). ‘Larval release day’ is the day on which the eggs hatch into the water. ‘Incubation period’ is the time between fertilisation and larval release day.

### 1.2. Aims

I set out to explore the following hypotheses; (1) That burrows in the full sun would be warmer than those in the shade, (2) that females alter the time between mating and egg
release to ensure the appropriate time for incubation is available, (3) Females alter egg sizes to ensure larvae are released at the appropriate time, and (4) females can adjust the timing of mating events according to the natural temperatures they experience during mating and incubation. Natural incubation temperatures that females would experience when selecting a male that has a territory in the sun or shade was determined. The fourth and fifth mechanism above (the first three mechanisms are the topic of an earlier study: Kerr and Backwell, In Prep) were then explored to ascertain their role in managing micro-habitat induced temperature variation. It was then determined whether the naturally occurring range of incubation temperatures affect the timing of mating, fertilisation and/or larval release. An examination of eggs produced by females at different temperatures was conducted to determine whether females adjust yolk size in order to control incubation duration; and whether larvae produced at different temperatures differ in size.

2. METHODS

Field work was conducted at East Point Reserve, Darwin, Australia (12°24'31.89"S 130°49'49.12"E) and laboratory work was conducted at the North Australian Research Unit, Darwin (12°22'11.81"S 130°52'17.31"E) between September and December 2012.

Incubation temperatures that females would experience if they selected a male with a territory in the sun or in the shade were ascertained. Four iButtons were buried at a depth of 20 cm (which is the average depth of the incubation chamber in a male’s burrow: Clark and Backwell, 2016), two in the open area of mudflat that gets full sun during the day; and two in the shaded part of the mudflat where the mangrove trees protect the surface sediment from the full impact of the sun. The iButtons logged temperatures at hourly intervals for 44 days (22 October – 4 December 2012).
Mate-searching females were located and watched as they moved through the population of courting males and were followed until they selected a male, remained in his burrow, and he sealed the burrow entrance. The burrow entrance was then isolated by surrounding it with a plastic collar (10cm diameter) to prevent crabs from entering or leaving the area. After three hours, the pair of crabs was removed with a trowel and measured, then the male was released back onto the mudflat and the female was transported to the laboratory in a cup with a small amount of sea water. These females are likely to have already mated but had not yet fertilised their eggs. The females were randomly assigned to one of four temperature regimes: 28°C, 30°C, 32°C or 34°C (corresponding to the range of natural incubation temperatures). Each female was housed individually in a small container (5x5 cm) with a 1 cm depth of seawater and a stone to allow her to emerge from the water. The containers were floated in tubs of water that were heated to the appropriate temperature using AquaOne 100W submersible aquarium heaters. Temperatures were continuously monitored using a BAT-12 microprobe thermometer to ensure that they did not fluctuate more than 1°C from their assigned temperature. Since females naturally incubate in darkness, each tub was covered with thick fabric. The water in the female compartments was changed every second day (suctioning out the old water and replacing it with freshly collected sea water).

It was noted when the females extruded a clutch of eggs and 10-20 eggs were removed with a pair of tweezers (a clutch consists of 2000-5000 eggs) and preserved in 10% formalin (4% formaldehyde) to prevent egg shrinkage or expansion. Females were inspected once a day until her eggs hatched, and the day of release was noted. Approximately 10 larvae were collected and preserved in 10% formalin. Once the females had released their larvae, they were returned to the mudflat and placed in artificially created burrows. Larvae were
released into the sea during the high spring tide. Data was collected on 41 females. Although almost all these females successfully hatched their clutches, power outages caused unacceptable fluctuations in temperature, limiting usable incubation data to 16 clutches. Information on mating day, mate guarding duration, and fertilisation day (10 females at 28°C; 11 females at 30°C; 9 females at 32°C; 11 females at 34°C) was recorded for 41 females. For the 16 females that released their larvae after experiencing stable incubation temperatures, incubation duration and larval release day was documented (2 females at 28°C; 6 females at 30°C; 4 females at 32°C; 4 females at 34°C).

The egg and larvae samples were photographed using a Leica WildMZ8 microscope and Nikon Coolpix 5700 camera. A 1mm scale with 1/100th mm gradations was placed under the specimens. Measurements were taken using ImageJ. Egg height and width and yolk height and width were measured. Eggs were collected and preserved on the morning of extrusion so all egg measurements were taken at the same developmental stage. Larval measurements included body height, width and length, eye height and width, tail width and length, and dorsal spine height. Figure 1 shows the larval measurements taken.
Figure 1: Larval measurements: a) body width, b) eye width, c) eye height, d) body length, e) tail width, f) body height, g) dorsal spine height, and h) tail length.

All analyses were conducted using IBM SPSS 20. Claw length was used as a measure of size since carapace and claw are highly correlated (Reaney and Backwell, 2007). Principal component analysis was conducted on the egg and larval sizes. All data were normally distributed, the residuals were normally distributed and the plots of the residuals against the predicted values produced a shotgun pattern.
3. RESULTS

*Was there a difference in incubation temperatures between open and shaded areas?*

Yes, at 20 cm depth, shaded sites were significantly cooler than sites in the full sun (paired *t* test: \( t_{3326} = 67.685, P = 0.000 \), Figure 2). The mean temperature of the shaded sites was 30.6 ± 1.10°C (range = 28-37.5°C; \( n = 2072 \)) and in the sunny areas it was 33.8 ± 1.88°C (range = 30-40°C; \( n = 2069 \)). Females that select mates with territories in the shaded areas of the population, incubate their eggs at an average of 3.3 °C cooler.

![Graph](image)

**Figure 2:** Sediment temperatures at 20 cm depth in the sun (white bars) and the shade (black bars). The data are the mean from two iButtons in the sun and two in the shade.

*Did temperature affect incubation duration?* No, there was no difference in the duration between fertilisation and larval release for females that incubated at 28°C, 30°C, 32°C and 34°C (General Linear Model \( F = 0.56, \) d.f. = 3; \( P = 0.37; n = 16 \); dependent variable =
incubation duration, fixed factor = incubation temperature, covariate = female size; female size did not have a significant effect). Incubation took 16.4 ± 0.8 days (n=16, see Fig 3).

Figure 3: Timing (relative to spring tide) of mating, fertilisation and larval release at four incubation temperatures. The white section of the bar represents mate guarding (period between mating and fertilisation) and the number within it is the guarding duration. The shaded section of the bar represents incubation and the number within it is the incubation duration. The horizontal hashed boxes indicate the spring tides.

*Did temperature affect the timing of fertilisation?* No, there was no effect of temperature on the day (relative to the spring tide) on which females fertilised their eggs (General Linear Model F = 0.54, d.f. = 3; P = 0.66; n = 41; dependent variable = fertilisation day relative to spring tide, fixed factor = incubation temperature, covariate = female size; female size did not have a significant effect). On average, females fertilised their eggs 14.7 ± 1.06 days after the last spring tide (n = 41).
Males guarded females for an average of 5.1 ±1.3 days before they fertilised their eggs. There was no effect of temperature on the time between mating and fertilisation (General Linear Model $F = 0.13$, d.f. = 3; $P = 0.94$; $n = 41$; dependent variable = male guarding duration, fixed factor = incubation temperature, covariates = female size and day of mating relative to spring tide; see Fig 4) Female size did not have a significant effect on the duration of mate guarding ($F = 0.37$, d.f. = 1, $P = 0.55$), but the day of mating had a strong effect ($F = 52.89$, d.f. = 1, $P < 0.001$): females that mate early in the tidal cycle have longer mate guarding durations (see Fig. 5, allowing all females to start incubation at approximately the same time (14.7 ± 1.06 days after the last spring tide).

Figure 4: The timing of mating and fertilisation (mate guarding duration) at four incubation temperatures. Numbers at the top of the bars indicate the mate guarding durations.
Figure 5: The duration of mate guarding with respect to timing of mating.

*Did temperature affect the larval release day?* No, all females released their larvae very close to spring tide: from one day before to two days after spring tide; and this was not affected by incubation temperature (General Linear Model $F = 0.77$, d.f. = 3; $P = 0.53$; $n = 16$; dependent variable = hatching day relative to spring tide, fixed factor = incubation temperature, covariate = female size; female size did not have a significant effect).

*Did incubation temperature affect the size of the eggs, the amount of yolk within the eggs, or larval size?* No, incubation temperature did not affect egg size or the amount of yolk laid down in the egg (Table 1). It also did not affect the size of the hatched larvae (Table 1). Female size did not affect egg size or larval size, but larger females laid down more yolk, using more of the available space within the egg capsule, in their eggs (Table 1).
Table 1: The proportion of variance explained by each component of the principle components for egg and larval measurements with respect to temperature and female size.

<table>
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<th>Factor</th>
<th>Eggs</th>
<th>Eggs</th>
<th>Larvae</th>
<th>Larvae</th>
<th>Larvae</th>
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<tr>
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<td></td>
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<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td>Body length</td>
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<td>0.56</td>
</tr>
</tbody>
</table>

Temperature:

| F (d.f.) | 0.27 (1) | 1.29 (1) | 0.004 (1) | 0.79 (1) | 2.80 (1) |
| P        | 0.60 | 0.26 | 0.95 | 0.39 | 0.12 |

Female size:

| F (d.f.) | 0.29 (1) | 5.39 (1) | 0.66 (1) | 0.80 (1) | 0.28 (1) |
| P        | 0.60 | 0.03 | 0.43 | 0.39 | 0.87 |
**Did temperature effect the survival of egg clutches?** The incubation temperature did not affect hatching success (Likelihood Ratio Test: $G = 2.89$, d.f. = 3, $P = 0.41$). At 28°C, 2/10 clutches hatched (20%); at 30°C, 6/11 clutches hatched (55%); at 32°C, 4/9 clutches hatched (44%); at 34°C, 4/11 clutches hatched (36%).

4. **DISCUSSION**

Females that select to mate with males whose territories are in the shade will incubate their clutches at ~ 3°C lower than females that select males with territories in the full sun. Such a large difference in incubation temperature is known to affect incubation durations in other fiddler crab species: a 1°C drop in temperature results in an increase in incubation duration of 0.7 – 1.88 days (Christy, 1989; Kerr et al., 2012; Sanford et al., 2006), so a 3°C drop in temperature should increase incubation duration by 2-5 days. This could prevent females from releasing their larvae at the optimal peak amplitude spring tide (Christy, 1982).

When incubation temperatures were controlled in the laboratory, no effect of temperature (over a 6°C range of 28-34°C) on the incubation duration or the timing of larval release was found. Although females mated over a wide range of days (relative to spring tide), they extruded their eggs over a narrow range of days. This indicates that they adjust the mate guarding duration (the time between mating and egg extrusion) in order to control the timing of egg extrusion. This occurred irrespective of the incubation temperature. How can a 6°C change in temperature have no effect on incubation duration or larval release timing? It is not believed that this is due to the relatively small sample size since the adjustments females made to the mate guarding durations throughout the mating period were detected, so
changes in incubation durations should be detectable unless the effect was very small. It is possible that incubation temperature did not affect incubation duration because of the high temperatures at which this species incubates. In the previous studies on fiddler crabs, where temperature was found to affect incubation duration, the temperatures were lower than those in the present study: 18 - 26 °C (Sanford et al., 2006); 22-28°C (Christy, 1989); 23-29°C (Kerr et al., 2012), and the effect of temperature was exponential, with a smaller decrease in incubation duration at higher temperatures (Kerr et al., 2012; Sanford et al., 2006; also for other decapod species: Anger et al., 2003). In the current study, the natural incubation temperatures ranged from 31°C in the shade to 34°C in the sun. Because they naturally incubate at higher temperatures, the effect of temperature on incubation duration may fall in the asymptote region of the exponential relationship. This suggests that the effects of future climate warming may not be as detrimental to this species (or other species breeding at such high temperatures) as it would in species that breed at lower temperatures.

Given that the incubation duration is constant irrespective of temperature, do females release larvae at different stages of development? No evidence of this was found: there was no difference in the multiple size measures that were made on the zoeae raised at different temperatures. There was also no evidence found that females adjust the size of the eggs they produce or the amount of yolk they lay down depending on the incubation temperature. In many crustaceans, there is a strong negative relationship between egg size and temperature (Kobayashi and Matsuura, 1996; Sheader, 1996) including fiddler crabs (Christy, 1982; Kerr et al., 2012): females produce larger eggs with more yolk when the incubation temperatures are low. In the current study, although females could potentially adjust the egg or yolk size to speed up or slow down the incubation rate, no evidence for this was found. Again, this should have been detected, even with the relatively small sample size, since an effect of female size
was found: larger females laid down more yolk in their eggs than did smaller females. Either there is no effect of incubation temperature on egg or yolk size, or the effect is too small to detect.

4.1. Conclusions

If a female selects a male with a territory in the sun, she will incubate her eggs at a higher temperature than females that select males with territories in the shade. This, however, does not affect the timing of crucial reproductive events: females begin and end incubation at the most appropriate times irrespective of incubation temperature. Selecting a mate in the sun or shade does not disrupt the critical timing of larval release. In other crab species, the effect of temperature on incubation duration (and other key reproductive events) is exponential, with smaller decreases in incubation duration at higher temperatures (Anger et al., 2003; Kerr et al., 2012; Sanford et al., 2006). Since the natural incubation temperatures are high for this species, the effect of further increases in temperature would not have as marked an effect as it does for species breeding in slightly cooler temperatures. Tropical ectotherms may be better adapted to dealing with increases in temperature than their more temperate relatives.

Under natural conditions, females may be able to control incubation temperatures much more than they could under the laboratory conditions for this study: they could select different size burrows, move up or down the burrow shaft, or mate at different times of the cycle or season. None of these behavioural modifications appear to be necessary since the females do not need to adjust incubation durations at different temperatures. In terms of incubation duration and larval release time, there is no disadvantage to females in selecting a mate with a territory in the sun or in the shade.
Why then do females prefer males that are located in the shade (Kerr and Backwell, In Prep)? There may be other advantages to living in the shade: males can spend longer periods of the day on the surface, giving them more time for feeding, courting, mate-searching etc. (Darnell et al., 2013; Levinton et al., 2015). Since males that have shaded territories are able to feed for longer periods of time, they may be able to court more (or more vigorously). Females that select to mate with a male in a shaded territory will not disadvantage her eggs or larvae (e.g. by releasing them at a sub-optimal time), but they may benefit from having the shaded territory once the larvae have been released: these females could feed for longer periods each day, possibly allowing them to grow faster. Larger females produce more eggs, so this may have a direct impact on female fitness. This requires further study.

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References


Chapter 5

Territorial battles between fiddler crab species

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ABSTRACT

Many species worldwide are impacted by habitat loss. This may result in increased competition both within species and between species. Many studies have demonstrated that when two previously non-overlapping species are forced to compete over a resource, one species is likely to become dominant over the other. This study explores the impact a larger species of fiddler crab (*Uca elegans*) has when invading an area previously used solely by a smaller species (*Uca mjoebergi*). It was found that while some impacts do exist for *U. mjoebergi*, they are not necessarily as severe as those found in studies on similar organisms. This information may aid us in determining potential consequences for individuals facing competition from newly arriving heterospecifics as a result of a rise in sea-level.
Habitat loss is a factor limiting the distribution of many species world-wide (1, 2), and has often been attributed to anthropogenic causes such as climate change (3) and/or development such as the construction of buildings and infrastructure (1). Reductions in available habitat may place an upper limit on the total numbers of individuals reliant on a given space (4). Studies on shore birds demonstrated a decline in numbers after events resulting in local habitat loss as a result of increased intraspecific and interspecific competition (5, 6). Increased competition is costly as it results in reduced fitness or even survivorship for individuals (7) or even entire species (8).

Studies on interspecific competition have revealed that one species usually emerges as the dominant at the cost of the subordinate species (8-10). Consequences resulting from competition include loss of valuable resources (e.g. food or shelter – 7, 9), increased exposure to predators or environmental conditions (11), or if the competitive encounters are of an aggressive nature, injury or death (12-14). Understanding competitive processes may aid in predicting biodiversity losses associated with habitat losses and/or restrictions.

Interspecific competition occurs when two species occupy the same ecological niche and require the same resource (8, 9). It is most easily studied when competitive behaviours are easily decipherable and observable (15). Fiddler crabs are the perfect species for studying interspecific competition since space is usually limited but is extremely valuable to individuals (12, 16).
Fiddler crabs live in mid sexed, high density populations where each individual occupies a territory containing an area of sediment surface for feeding and courtship display as well as a central burrow that is used for avoidance of predators and extreme weather, access to water, mating and incubation (17). As a result, territories are essential for reproduction and survival of fiddler crabs (16). Males have one greatly enlarged claw that they use to attract mates and in territorial disputes with other males (12, 18). Male crabs can either give their burrow to a female after reproduction, or may lose it through an aggressive interaction with a ‘floating’ male (a territory-less individual who wanders through the population looking for a new territory). Males that lose their territories must then either dig a new burrow (usually on the outer edges of the population since that is often the only place where undisputed space can be found), find an empty burrow or fight a resident male for a burrow (19).

Multiple fiddler crab species often use the same mudflat and it is common for species to occur in specific intertidal zones due to preferences for sediment type or vegetation structure (20). Some species are more adapted to accessing aquatic oxygen and are therefore more capable at withstanding prolonged submersion by the tide (21). An increasing tidal height could cause changes to all of these factors (22, 23) and may result in a concertinaing of species.

The banana fiddler crab, *Uca mjoebergi*, is one of the smaller species of fiddler crab (average carapace width = 11 mm, average claw length = 16 mm) located along the coast of Northern Australia and it inhabits the highest intertidal zone. In recent years, a larger species, *U. elegans* (average carapace width = 15 mm, average claw length = 23 mm), has begun to invade that area, providing a unique opportunity to study the effects of interspecific competition, since two previously spatially exclusive species are now co-occurring. The aim of
this study was to explore the consequences of interspecific competition from initial settlement through to ongoing interactions of established mixed species populations.

When an individual of the larger invading species arrives in the population of *U. mjoebergi*, it will need to locate a burrow. Does it fight and evict a *U. mjoebergi*? If so, does the size of resident males influence an invaders decision of who to fight, or does a ‘residency effect’ (the greater probability of the territory-owner to win the fight) help resident *U. mjoebergi* to keep their territories? Fight outcome is dependent on male size in most fiddler crab species including the study species, *U. mjoebergi* (17, 19, 24-26). This suggests invading *U. elegans* may target smaller individuals (both smaller conspecifics and the smaller heterospecific *U. mjoebergi* males) to increase their chances of winning a territory. However, access to a burrow provides leverage during fights and retreat sites for residents resulting in a resident effect in fight outcome, which may aid residents in the defence of their territory (17, 19).

Resource quality may also influence a *U. elegans* male’s decision on who to fight for a new territory (7). We know that resource quality is an important factor in conspecific fights (19, 26). If a male wins a fight with a smaller male, the burrow will need to be enlarged, adding to the cost of fighting a smaller rival (19, 27), particularly if there is a large discrepancy between the size of the invading crab and the newly acquired burrow. This may be compounded if the burrows of different species have a different structure as is probably the case between *U. mjoebergi* and *U. elegans* burrows (Clark, pers. obs.)
Once territories have been won and territorial boundaries between neighbouring residents have been established, there may be both benefits and costs for resident *U. mjoebergi* to having a *U. elegans* neighbour. Over time, aggression between neighbouring individuals should decrease (17, 25, 28). One benefit of having a heterospecific *U. elegans* neighbour is that they do not compete in female attraction. Another benefit is that *U. elegans* has been observed assisting smaller, neighbouring *U. mjoebergi* in territorial defence against another *U. elegans* (29).

However, heterospecifics can also have detrimental impacts (30). For example, resident *U. mjoebergi* may suffer a reduction in territory size since new neighbours renegotiate territory boundaries through aggressive interactions (17, 24, 25, 31) with the winner usually obtaining a small amount of extra territory (24, 32).

This study aims to assess whether the smaller *U. mjoebergi* will experience any detrimental impacts as a result of the invasion by *U. elegans* by addressing the following hypotheses. i) That *U. mjoebergi* residents will be evicted by larger *U. elegans* invaders? ii) That *U. elegans* preferentially evict heterospecific *U. mjoebergi* over conspecific *U. elegans*? iii) That resident *U. mjoebergi* suffer greater costs associated with increased fighting behaviours when residing next to a new *U. elegans* neighbour when compared to a new *U. mjoebergi* neighbour, and iv) That resident *U. mjoebergi* will suffer greater costs associated with reduced surface behaviours when residing next to an established *U. elegans* neighbour compared to an established *U. mjoebergi* neighbour?
METHODS

Research was conducted at East Point Coastal Reserve, Darwin, Australia (12°24'31.89"S 130°49'49.12"E) between the months of September and December of both 2013 and 2014. All carapace and claw measurements were made with the use of callipers to the nearest 0.1mm. If a crab had to be held out of the burrow for any length of time, it was placed in a small cup of water in a shaded area to prevent it from overheating.

Burrow acquisition

Are U. mjoebergi evicted by invading U. elegans? Twenty arenas (20 cm high and 40 cm diameter) were set up around three naturally occurring male U. mjoebergi individuals that were specifically chosen for their relative sizes: one was large, one medium and one small male. In each of the arenas, I individually added a single U. elegans male by capturing him in an area of the mudflat away from the experimental arenas, measuring him and placing him in an upturned clear plastic container within the arena. When all the natural arena occupants were surface active (approximately 2 minutes), the ‘intruding’ U. elegans was remotely released by pulling a cable connected to the release container. After one hour, it was noted which individual (if any) the invader had evicted from their burrow. Burrow sizes of the naturally occurring residents were noted as burrow entrance diameter is closely correlated with resident size (33).

Do U. elegans preferentially evict U. mjoebergi over another U. elegans? Twenty arenas (20 cm high and 40 cm diameter) were placed around two naturally occurring males,
one *U. mjoebergi* and one *U. elegans* male. Once again, an individual *U. elegans* was placed in the arena following the same methodology as depicted above?

*Neighbour interactions: new neighbours*

*Do U. mjoebergi engage in more fighting behaviour when residing next to a new U. elegans neighbour as opposed to a new U. mjoebergi neighbour?* We selected 25 naturally occurring pairs of *U. mjoebergi* male neighbours (abutting territories), and captured one of the pair. In 13 trials, I replaced the neighbour with a *U. elegans* male, and in 12 trials, I replaced him with a *U. mjoebergi* male. I video recorded each pair for 30 mins after they both re-emerged from their burrows and were surface active. Trials ended early if either the resident or intruding neighbour left voluntarily, were evicted, or sealed themselves into their burrow. From the videos, I counted the number of fights as well as the fight escalation level: display, push, or grapple. The ‘display’ phase is the first stage and represents the lowest escalation level: males face each other and wave. The ‘push’ is the second stage and is medium escalation: males alight their claws face-on and push each other. The final stage, high escalation, is grappling: males interlock their claws and grapple with each other. Each fight was assigned a score based on the maximum escalation level of the fight (display = 1; push = 2 and grapple = 3). The total duration of each fight was recorded as well as durations of each escalation level within each fight. Claw and carapace sizes were recorded for each of the crabs. Distance between the two burrows was also noted.
Neighbour interactions: established neighbours

Do *U. mjoebergi* experience a reduction in surface behaviours associated with residing next to an established *U. elegans* neighbour as opposed to an established *U. mjoebergi* neighbour? Naturally occurring, established male neighbours were assessed by observing 44 naturally occurring pairs of crabs; half of them were pairs of *U. mjoebergi* neighbouring males and half were pairs in which one male was a *U. mjoebergi* and the other was a *U. elegans* male. In the pairs of *U. mjoebergi* males, the smaller of the males was assigned to be the focal male and the other to be the neighbour (since *U. elegans* is larger than *U. mjoebergi*, this meant that the focal male was always the smallest male in conspecific and heterospecific trials). For each male in each pair, I documented the total amount of time the male spent surface-active and the amount of time spent in the burrow over a 30 minute observation period. I measured the size of each crab and the distance between their burrows.

**Statistical analysis**

All stats were conducted using IBM SPSS 22. I used carapace width as a measure of size since carapace width and claw length are highly correlated (33).

Arena data were assessed with correlations. Neighbour interaction data were assessed with Mann-Whitney U non-parametric tests as the data distribution could not be normalised. Where multiple correlations were run on the same data, the raw $P$ values were used in a Benjimini-Hochberg False Discovery Rate test (FDR) with a false discovery rate of 15%. Raw $P$ values are presented with their significance against the FDR-adjusted alpha levels brackets.
Due to unpredictable crab behaviour, trial lengths varied during the neighbour interaction trials and the data were weighted to account for these differences. Some trials were too short to be included (under 5 minutes) and thus, were removed from the analysis. Information unaffected by trial length (i.e. crab size and distance) were left in the analysis resulting in differing N values within the same set of analysis on some tests.

RESULTS

Burrow acquisition

In the 20 experiments where a *U. elegans* male was placed in an arena with three naturally occurring *U. mjoebergi* males (one large, one medium and one small); the intruding *U. elegans* male evicted one of the residents in 15 cases (75%). In eight cases, the large *U. mjoebergi* male was evicted; in one case, the medium male was evicted and in six cases, it was the smallest *U. mjoebergi* male that was evicted. There was no correlation between the size of the introduced *U. elegans* male and the burrow diameter of the evicted *U. mjoebergi* male (Pearson’s $r = 0.186$, $P = 0.508$, $N = 15$).

In the 20 experiments where a *U. elegans* male was introduced into an arena with a naturally occurring pair of males; one *U. mjoebergi* and one *U. elegans*; there were 13 evictions: in five cases, the introduced *U. elegans* male evicted a *U. mjoebergi* male, and in eight cases, he evicted a *U. elegans* male. This is a non-significant difference (binomial $P = 0.58$; $n = 5, 8$): an intruding *U. elegans* male was just as likely to evict a conspecific as a heterospecific male. There was no correlation between invader size and the size of the evicted
individual’s burrow (Pearson’s $r = -0.521$, $P = 0.068$, $N = 13$). Naturally occurring *U. elegans* were significantly larger than their *U. mjoebergi* neighbours (Mann-Whitney U test: $Z = -2.963$, $P = 0.003$, $N = 20$)

*Neighbour interactions: new neighbours*

When a resident *U. mjoebergi* male had his naturally occurring conspecific neighbour replaced by a *U. elegans* male, they engaged in significantly more fights (Mann-Whitney U test: $Z = -2.268$, $P = 0.022$, $N = 12; 13$) and spent more time in low escalation level (display) fights (Mann-Whitney U test: $Z = -2.184$, $P = 0.03$, $N = 12; 13$) than when his neighbour was replaced with another *U. mjoebergi* male. There was no significant difference between the total fight time (Mann-Whitney U test: $Z = -1.775$, $P = 0.077$, $N = 12; 13$), number of fights reaching any of the escalation levels (Mann-Whitney U test: $Z = -1.994$, $P = 0.06$, $N = 12; 13$), total time spent push fighting (Mann-Whitney U test: $Z = -1.671$, $P = 0.11$, $N = 12; 13$), or total time spent grapple fighting (Mann-Whitney U test: $Z = -1.321$, $P = 0.247$, $N = 12; 13$) when the naturally occurring neighbour was replaced with a conspecific or a heterospecific male.

*U. elegans* males are usually larger than *U. mjoebergi* males, and since the relative size of rivals affects fight escalation, I found a positive correlation between the size of the new neighbour and the escalation level of fights (Pearson’s $r = 0.621$, $P = 0.001$, $N = 25$, significant using the FDR adjusted alpha value of 0.006; Figure 1). As focal size increases, so too does the time spent push fighting (Pearson’s $r = 0.506$, $P = 0.010$, $N = 25$, significant using the FDR adjusted alpha value of 0.0125). There was no correlation found between any of the other tested variables (all $P > 0.031$, all non-significant using the FDR adjusted alpha values).
Figure 1: Fight escalation level when compared to intruding neighbour size (black dots = *U. mjoebergi* intruding neighbour; white dots = *U. elegans* intruding neighbour)

*Neighbour interactions: established neighbours*

When examining naturally occurring neighbouring males, I found that the established neighbour was larger when it was a *U. elegans* male than when it was a *U. mjoebergi* male (Mann-Whitney *U* test: $Z = -5.401$, $P = 0.000$, $N = 22$; 22). The distance between the burrows of neighbours was significantly larger when the neighbour was a heterospecific (rather than a conspecific) male (Mann-Whitney *U* test: $Z = -2.136$, $P = 0.033$, $N =22$; 22). In heterospecific pairs, the *U. elegans* neighbours spent significantly more time in their burrows than did the *U. mjoebergi* neighbours (Mann-Whitney *U* test: $Z = -2.569$, $P = 0.01$, $N =21$; 21). There was no significant difference between the time the focal males spent in their burrows (Mann-Whitney *U* test: $Z = -0.239$, $P = 0.811$, $N =20$), or the total time spent interacting (Mann-Whitney *U* test: $Z = -0.383$, $P = 0.701$, $N =12$) when they had heterospecific or conspecific neighbours.
As the size of the focal *U. mjoebergi* males increased, so too did the distance between the burrows (Pearson’s $r = 0.454$, $P = 0.002$, $N = 44$, significant using the FDR adjusted alpha value of 0.025; Figure 2). Similarly, as the size of the established neighbour increased, so too did the distance between the burrows (Pearson’s $r = 0.315$, $P = 0.037$, $N = 44$, significant using the FDR adjusted alpha value of 0.075; Figure 3). As the distance between the burrows increased, the smaller resident spent less time in the burrow (Pearson’s $r = -0.353$, $P = 0.022$, $N = 42$, significant using the FDR adjusted alpha value of 0.05; Figure 4). No other correlations were observed between the other tested variables (all $P > 0.26$, all non-significant using the FDR adjusted alpha values).

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Figure 2: Distance between burrows of resident *U. mjoebergi* and established neighbours when compared to the size of the small resident *U. mjoebergi* (black dots = *U. mjoebergi* living next to an established *U. mjoebergi* conspecific, white dots = *U. mjoebergi* living next to an established *U. elegans* heterospecific).
Figure 3: Distance between burrows of resident *U. mjoebergi* and established neighbours when compared to the size of the established neighbour (black dots = *U. mjoebergi* established neighbour, white dots = *U. elegans* established neighbour).

Figure 4: Effects of the distance between resident *U. mjoebergi* burrow and established neighbour burrow on the time the smaller resident *U. mjoebergi* spent in the burrow (black dots = *U. mjoebergi* established neighbour, white dots = *U. elegans* established neighbour).
DISCUSSION

Our study population of *Uca mjoebergi* has increasingly been ‘invaded’ by a larger fiddler crab species, *U. elegans*, over the past ten years. Fiddler crab species seldom overlap in distribution since each species is adapted to very particular sediments, micro-climates and intertidal zones (20, 21). The overlap we currently observe therefore provides an ideal opportunity to study inter-specific interactions, especially since *U. elegans* are much larger than *U. mjoebergi* and are likely to out-compete them.

Here I show that *U. elegans* males will fight the smaller *U. mjoebergi* males for their territories, and are able to evict the resident male in 75% of cases. They do not particularly target specific sizes of *U. mjoebergi* males; all size classes were attacked and evicted. Fiddler crabs usually target smaller individuals in territorial fights in order to improve their chances of winning the fight (17, 19, 24-26). However, invading individuals may also target individuals of a similar size to themselves, to avoid excessive burrow modifications after a territory has been won (19, 27). Neither of these factors appear to play a role when invading *U. elegans* evict *U. mjoebergi*, since all size classes were evicted.

We also show that, when given a choice of fighting with a conspecific or a heterospecific male, *U. elegans* are non-selective: they are just as likely to attack a *U. mjoebergi* as a *U. elegans* resident. This is interesting since the two species potentially have different burrow structures (Clark, pers. obs.) which would represent different resource qualities and should, therefore, affect the male’s decision on who to fight (7). However, *U.
*elegans* evicted both conspecifics and heterospecifics. While *U. mjoeberti* were not specifically targeted by *U. elegans* intruders, they were also not avoided.

Once an invader has acquired a burrow, negotiations with the surrounding new neighbours over territorial boundary lines begins (17, 24, 25, 31). Here I show that *U. mjoeberti* residents engage in a greater number of low escalation aggressive displays when they gain a new, heterospecific territorial neighbour than when their new neighbour is a conspecific male. There was no difference in any of the other aggressive indicators examined: When *U. mjoeberti* males were given a new conspecific or a new heterospecific neighbour, the time they spent in fighting did not differ; the fight durations did not differ; the escalation level of the fights were no different; and the number of escalated (grapple) fights did not differ.

After territorial boundaries have been set, aggression between neighbours dissipates (17, 25, 28). Here I found that there was a greater distance between burrows of established heterospecific neighbours compared to established conspecific neighbours (two *U. mjoeberti*) and this distance was related to the sizes of both of the neighbouring individuals. This indicates that *U. elegans* occupy larger territories than *U. mjoeberti* either due to their larger size (hence their increased requirement for feeding space) or due to their increased aggression, reducing the available space for the original, resident species, however, territory sizes and resultant impacts need further investigation and this is the focus of another study. Resident *U. mjoeberti* also spent more time in the burrow, the closer the two burrows were to each other.

Established *U. elegans* neighbours also spent more time in the burrow than the established *U. mjoeberti* neighbours. It would seem that this would translate into more
available time on the surface feeding and courting for those individuals residing next to an established *U. elegans* neighbour, however, there was no difference in time spent down between resident *U. mjoebergi* living next to established heterospecifics when compared to individuals living next to established conspecifics. This suggests that, once territorial boundaries have been set, *U. mjoebergi* males do not appear to be at a disadvantage when having a conspecific or a heterospecific neighbour.

**Conclusions**

Based on our knowledge of fiddler crab fight behaviours (17, 19, 24-26) and studies on similar species (8-10), it could be expected that individuals of a smaller species will be disadvantaged when their population is invaded by a larger species that uses the same resources. Here I show that common sense can be wrong. There was very little effect on the small *U. mjoebergi* males when they live among conspecifics and when their neighbours are the larger, heterospecific *U. elegans* males. Male *U. elegans* will fight the smaller species for their territories and, in fact, are equally likely to fight them as they are to fight males of their own species. When they gain a new heterospecific neighbour, *U. mjoebergi* males produce more aggressive displays than they would with a new conspecific neighbour; but this is likely to be because *U. elegans* males are larger and therefore pose a greater potential threat. There is no disadvantage to the *U. mjoebergi* residents in terms of fight duration, escalation, or the total amount of timer spent fighting when they have a new heterospecific or a conspecific neighbour. There is also no disadvantage to having a heterospecific neighbour once the boundaries have been settled: *U. mjoebergi* males spend equal amounts of time on the surface when they have heterospecific or conspecific neighbours. The larger heterospecifics do not scare them into their burrows or prevent them from engaging in their normal surface...
activities. In fact, once territorial boundaries have been established, studies have revealed that *U. mjoeb ergi* may benefit from having a heterospecific neighbour (i.e. reduced competition for mates and assistance in heterospecific fights - 29).

This study has demonstrated that interspecific competition need not have the negative impacts on individuals as shown by Guiasu and Dunham (10) and may not necessarily result in local extinction of species. This is true even when the species live in high density, mixed species populations that are forced to compete for limited resources that are required for survival (4, 5, 34). When two sympatric species occupying the same or similar niches and are forced to interact, one often emerges as the dominant species at the expense of the other (8, 9, 10); but not always. In a world with a changing climate that will result in a reduction of available habitat for many species (2), it is imperative we understand the consequences of species overlap so as to understand the true impact on biodiversity and species survival.

ETHICS

All applicable international, national and/or institutional guidelines for the care and use of animals were followed. This article does not contain any studies with human participants performed by any of the authors.

DATA

Data can be located at Dryad doi:10.5061/dryad.k3c3v
COMPETING INTERESTS

We have no competing interests

AUTHORS CONTRIBUTION

HC primarily conceived and designed the project, carried out the field work and video analysis, performed statistical analysis and drafted the manuscript. PRYB assisted in project conception and design, statistical analysis and helped draft the manuscript. Both authors gave final approval for publication.

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REFERENCES


Conclusion

Consequences of climate induced interspecific male-male competition

In order to maximise reproductive success, territorial individuals must maintain ownership of good quality territories. Territory ownership is essential to survival and reproduction in fiddler crab species (Milner et al., 2010a). In chapter 5, I demonstrated that *Uca mjoebergi* were at risk of losing territories and suffering continued costs associated with the invasion of their mudflat from the larger *U. elegans*.

It is difficult to know exactly why *U. elegans* have begun to invade this particular mudflat. It could simply be a natural invasion. *Uca sp.* larvae drop out of the water column upon detecting adult individuals on a mudflat, however can settle in the absence of an adult if the sediment is correct (Christy, 1989; O’Connor, 1990; O’Connoe 1991). This is not uncommon with many *Uca* species co-habitating on a single mudflat (Teal, 1958). The size discrepancy between the two species in question may help explain how the invading *U. elegans* were able to establish a viable population in such a high density population of *U. mjoebergi*.

*U. elegans* are larger (Clark and Backwell, 2017) and more aggressive (How and Hemmi, 2008) than *U. mjoebergi*. Since fight outcome in fiddler crabs is determined by size (Hyatt and Salmon, 1978; Jennions and Backwell, 1996; Backwell and Jennions, 2004; Fayed et al., 2008; Detto et al., 2010), *U. elegans* can use this to an advantage in evicting smaller *U. mjoebergi* and establishing a population within an area once solely occupied by *U. mjoebergi* (Clark and Backwell, 2017).
When two species require a similar resource or territory for mating success, interspecific male-male competition can arise. Interspecific competition results in the reduced fitness of one or both species as shown by Thornhill (1987) and Breitburg (1987). It stands to reason that *U. mjoebergi* must suffer a fitness cost associated with competition with *U. elegans*, however the effect of this is not necessarily as large as expected.

Once territorial boundaries were established, *U. mjoebergi* suffered no reduction in activity when living next to a conspecific as opposed to a heterospecific (Clark and Backwell, 2017). In fact, Booksmythe et al. (2010a) showed that *U. elegans* assist smaller *U. mjoebergi* in territory defence against other aggressive *U. elegans*. Furthermore, males of differing species do not compete for the same females for mating. There are other factors that may result in reduced fitness when residing next to a conspecific (see Gröning and Hochkirch, 2008). For example, it is possible that fights result in a realigning of territorial lines (Stamps & Krishnan, 2001; Backwell & Jennions, 2004; Fayed et al., 2008; Detto et al., 2010) with the winner usually obtaining a small amount of extra territory (Backwell & Jennions, 2004; Booksmythe et al., 2010b). Given the size advantage of *U. elegans*, it is safe to assume that resident *U. mjoebergi* may suffer a loss in territory size. The costs associated with residing next to a conspecific may be diminished or nullified by the benefits gained. Further research is required to determine the true cost/benefit ratios of having a conspecific neighbour over a heterospecific neighbour.

Similarly, I demonstrated that *U. mjoebergi* were evicted by larger *U. elegans* however this rate was halved when there was a choice given between a *U. elegans* evicting a conspecific or heterospecific (Clark and Backwell, 2017). This suggests that while there is an initial eviction rate of *U. mjoebergi*, this slows down as *U. elegans* numbers increase and it is possible that *U.
*mjoeb ergi* maintain a strong presence from the protection they gain in coalitions described above (Booksmythe et al., 2010a).

The negative effects of interspecific competition have been explored (Breitburg, 1987; Thornhill, 1987; Gröning and Hochkirch, 2008), and this may be compounded with the loss of habitat resulting from the impacts of human induced climate change (Galbraith et al., 2002). However, we need to understand all of the complexities of interspecific interactions before we can assert with certainty that this will result in the loss of all subordinate species, since it has been shown that harmonious mixed species populations are possible (Teal, 1958; Booksmythe et al., 2010a; Clark and Backwell, 2017).

**Sexual selection through female choice**

Once a male has won a territory, he can then set about making it attractive to prospective suiters. Females fiddler crabs select males with burrows of an appropriate temperature (Reaney & Backwell, 2007; Milner et al., 2010b; Kahn et al., 2013; Clark & Backwell, 2015). However, I have demonstrated that micro-habitat selection does not impact egg and larval development in *U. mjoeb ergi* (Clark and Backwell, 2016). This is despite a female preference for males with a territory in the shade (Kerr & Backwell, In Prep). Why do females prefer shaded habitats? Do females gain fitness advantages through increased feeding after acquiring a burrow in a shaded area? The temperatures we analysed were within the micro-habitat ranges of the natural population. What effect will temperature increases associated with climate change be?
Other studies have shown that temperature can impact incubation duration in fiddler crabs but these studies were conducted on species adapted to live in lower temperatures than my study species (Christy, 1989; Sanford et al., 2006; Kerr et al., 2012). It is possible that due to the naturally high incubation temperatures, the detrimental impacts associated with temperature increases due to climate warming will not be as severe in this species. However, further studies should be conducted on incubation and larval development at higher temperatures to assess the true impacts of climate change on these processes.

In order to attract females to their burrow, male fiddler crabs need to be able to demonstrate a phenotypic quality first and they do this by waving their enlarged claw. However, males can autotomise their enlarged claw during fights or difficult moults. Autotomy in other species has been shown to have both short term (Langkilde et al., 2005) and long term effects on the fitness of individuals (Uetz et al., 1996).

Fiddler crabs have also been shown to suffer consequences to autotomy such as a lighter claw that has less muscle, a thinner exoskeleton and is less robust (Yamaguchi, 1973; Backwell and Passmore, 1996; Reaney et al., 2008). As a result, regenerated claws are poorer weapons (Lailvaux et al., 2009). Despite this, previous studies have shown that regenerated claw males are equally likely to attract a female to the burrow (Reaney et al., 2008), but this does not translate into mating success (Reaney et al., 2008; Slatyer et al., 2012). It stands to reason then that females are rejecting these males after inspecting the burrows.

I compared original and regenerated claw male burrows and found no difference between them for characteristics important in female selection. Furthermore, after finding
only three regenerated claw males that mated and combining my data with that collected by both Reaney et al. (2008) and Slatyer et al. (2012), there was no longer a significant difference between number of regenerated claw males in the general population and those that successfully mated. Callander et al. (2012) showed that regenerated claw males of a closely related species could bluff their opponents during the mating period by escalating fights and undertaking more risky behaviours. This allowed them to maintain access to good quality territories during the mating period. This could be true for *U. mjoebergi* but further study is required to assess this.

One significant result I did find was that there was a difference between the proportion of regenerated claw males in the visited but rejected category and the mated male category. This implies that there is something about the regenerated claw males or their burrows that hinders their mating success to some degree. Maybe that is an unmeasured burrow characteristic such as temperature or sediment structure but this needs examination. Additionally, fiddler crabs are known to drum on the sediment during and after courtship (Aicher & Tautz, 1990; von Hagen, 2000). The altered physical characteristics of the regenerated claw may impact the drumming aspect of courting but again, this requires further research.

Some of the phenotypic characteristics female fiddler crabs seek during mating include claw colour, wave rate and height and claw size. Females tend to select larger males overall (Reaney & Backwell, 2007) however we show that small females prefer smaller males and large females prefer larger males. We propose that since larger females produce more eggs (Hines, 1982; Fukui, 1988; Murata & Wada, 2002), they would require more sperm. Larger
males of other species have been shown to have larger ejaculates (Jivoff, 1995; Sasson et al.,
2012) but further research is required to determine if this is the case for *U. mjoebergi*.

Why do smaller females prefer smaller males? I was able to reject the possibility that
large females cannot fit into smaller burrows, thus a mechanical constraint was not the cause
of size assortative mating. However, de Rivera (2005) demonstrated that females would
ultimately select a male with a burrow aperture that closely matches her body size. When a
female was moved after mating to a previously rejected, larger aperture burrow, the
incubation rate was altered having negative fitness consequences. De Rivera (2005) argues
that different females may have different ideals in burrow characteristics such as aperture.
Reaney and Backwell (2007) also found size assortative mating in *U. mjoebergi* and also found
that male size was closely related to burrow aperture. Female *U. mjoebergi* may also require
unique burrow characteristics for egg incubation however this would require further research.
While this was not discussed in the original article, it does not change the conclusions that size
assortative mating is driven by sexual selection in *U. mjoebergi*.

Burrow selection is very important for female *U. mjoebergi* as demonstrated by the
finding that females change their preference for male size both spatially and temporally (Clark
and Backwell, 2015). This result was put down to temperature requirements for incubation.
This seems contradictory to the findings in chapter 4, where I demonstrated that temperatures
associated with micro-habitat selection had no impact on egg and larval development (Clark
and Backwell, 2016). However, the prolonged heat exposure at the high site compared to that
at the low site may still have an influence on development rates. The low site is subjected to
cooler sea temperatures for more hours per day, and more consecutive days. These
exaggerated temperature differences may be significant compared to the much smaller
variation found through micro-habitat choice. This variation in female preference may result in a wider range of variation in sexually selected male traits (Houde, 1988; Qvarnström et al., 2000; Chaine & Lyon, 2008).

**Impacts on Sexual Selection and Fitness**

Throughout this dissertation, I have demonstrated a number of ways in which both male and female fiddler crabs have increased their success at reproduction and therefore increased their fitness. Females are choosy in order to improve their fitness (Kokko et al., 2003), however, clearly mate choice is influenced by more than just phenotypic characteristics. This provides mechanisms by which phenotypically less attractive males can improve their fitness.

Female fiddler crabs, generally speaking prefer larger males (Reaney & Backwell, 2007), and those with an original claw (Reaney et al., 2008; Slatyer et al., 2012), however I have demonstrated that both smaller males and males with regenerated claws can successfully mate given the right circumstances. Even when competing against a much larger and more dominant species, these small males have found ways in which to not only survive, but decrease competition for mates (Booksmythe et al., 2010a) and therefore potentially improve their fitness.

This research helps solve one of the biggest mysteries of sexual selection; why is there still such high variation for attractive male traits in the face of strong and continued female selection?
References


