Investigating the Coexistence of Fiddler Crabs in the Wakatobi Marine National Park, Indonesia

The thesis is submitted in partial fulfilment of the requirements for the award of the degree of Doctor of Philosophy of the University of Portsmouth.

By

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Abstract

The coexistence of ten species of fiddler crab was studied at Ambeua, on the island of Kaledupa, Indonesia. This remarkable level of biodiversity has been used to investigate the species-specific differences that support coexistence. Through studying their morphology, behaviour and the structure of their habitat it can be seen that they occupy distinct but overlapping niches. Each species is described and studied in detail, with notes and imaging based on morphology, phylogenetics, ecology and behaviour. Phylogenetic analysis was conducted using the cytochrome oxidase subunit I and sequences provided strong evidence to support classification.

Five transects were delimited in the area of coexistence as well as four outside of this area. Shore height profiles, shading and substrate type were quantified for all transects. Mouthpart morphology was analysed to determine whether associated substrate type correlated with mouthpart morphology. *Tubuca coarctata, Tubuca demani, Tubuca dussumieri, Paraleptuca crassipes,* and *Austruca triangularis* were all active in shaded areas and on fine muddy substrates. *Austruca cryptica* and *Austruca mjoebergi* were active in unshaded/open areas on sandy substrates, whilst *Gelasimus jocelynae, Gelasimus tetragonon* and *Austruca perplexa* were found in both shaded and unshaded areas on sandy substrates.

Morphological analysis showed that *Tubuca coarctata, Tubuca demani, Tubuca dussumieri, Paraleptuca crassipes, Austruca triangularis, Gelasimus jocelynae* and *Gelasimus tetragonon* all had mouthparts associated with finer sediments whilst *Austruca cryptica, Austruca perplexa* and *Austruca mjoebergi* had mouthparts associated with coarser sediments. Detailed analysis of distribution, individual home ranges and nearest neighbours revealed numerous interspecific overlaps and interactions. The close proximity of the local village increases habitat heterogeneity, with crabs recorded living underneath the stilted houses. These anthropogenic factors are directly altering the ecosystem, increasing niche availability and allowing crabs to dwell in places otherwise uninhabitable.
Dedication

This work is dedicated to Ron, Hilary and Robert Michie and Sarah Pawsey who have always believed in me and encouraged me to follow my dreams.

And to those marvellous little fiddlers that are at the heart of this work

“The future belongs to those who believe in the beauty of their dreams” – Eleanor Roosevelt

“Courage, dear heart” – C. S. Lewis


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The road to achieving and writing a doctorate may be tough and at times can break you, but then there are the people that keep you going and pick you up when you fall, and these are the people I could not have travelled this road without. I quickly learnt that I was not on this journey alone, and it is those traveling companions who deserve praise and thanks and who, in the end, made it worthwhile. Some I may have met for a day, some simply gave me a smile or reassuring words to get me through and some have been here since the beginning, but to all, I am grateful.

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List of contents

Chapter 1 - General Introduction

1.1 Ecological Theory of Coexistence 1
1.2 Fiddler crabs 2
1.3 Study site, the Wakatobi Marine National Park 6
1.4 Overview 9
1.5 Research Aims and Objectives 10

Chapter 2 - Morphological and Phylogenetic Analysis of Ten Species of Sympatric Fiddler Crab (Crustacea: Decapoda: Brachyura: Ocypodidae) in the Indo-West Pacific

2.1 Introduction 11
2.2 Materials and methods 18
2.2.1 Measuring crabs 18
2.2.2 Taxonomy 18
2.2.3 Drawing using camera lucida 18
2.2.4 Confocal Laser Scanning Microscopy (CLSM) 18
2.2.5 Scanning Electron Microscopy (SEM) 20
2.2.6 Phylogenetics 20
2.3 Systematic account 22
2.3.1 Tubuca coarctata (H. Milne Edwards, 1852) 22
2.3.2 Tubuca demani (Ortmann, 1897) 26
2.3.3 Tubuca dussumieri (H. Milne Edwards, 1852) 30
2.3.4 Paraleptuca crassipes (White, 1847) 34
2.3.5 Gelasimus jocelynae (Shih, Naruse and Ng, 2010) 38
2.3.6 Gelasimus tetragonon (Herbst, 1790) 42
2.3.7 Austruca cryptica (Naderloo, Türkay and Chen 2010) 46
2.3.8 Austruca mjoebergi (Rathbun, 1924) 50
2.3.9 Austruca perplexa (H. Milne Edwards, 1837) 54
2.3.10 Austruca triangularis (A. Milne-Edwards, 1873) 58
<table>
<thead>
<tr>
<th>Chapter</th>
<th>Title</th>
<th>Sections</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.4</td>
<td>Genetic sequencing</td>
<td></td>
<td>62</td>
</tr>
<tr>
<td>2.5</td>
<td>Discussion</td>
<td></td>
<td>62</td>
</tr>
<tr>
<td>3</td>
<td>Chapter 3 - The Drivers of Coexistence of Ten Species of Sympatric Fiddler Crab in the Indo-West-Pacific.</td>
<td>3.1 Introduction</td>
<td>65</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3.2 Materials and methods</td>
<td>67</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3.2.1 Crab distribution</td>
<td>67</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3.2.2 Biotic and abiotic factors</td>
<td>70</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3.2.3 Shore height profile</td>
<td>70</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3.2.4 Sediment grain size analysis</td>
<td>70</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3.3 Data analysis</td>
<td>72</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3.3.1 Sediment grain size analysis</td>
<td>72</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3.3.2 Crab distribution</td>
<td>72</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3.4 Results</td>
<td>73</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3.4.1 Shore height</td>
<td>73</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3.4.2 Sediment grain size analysis</td>
<td>73</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3.4.3 Crab distribution</td>
<td>75</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3.5 Discussion</td>
<td>79</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3.5.1 Crab distribution</td>
<td>79</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3.5.2 Sediment grain size analysis</td>
<td>84</td>
</tr>
<tr>
<td>4</td>
<td>Chapter 4 - A Comparative Study of Maxilliped Adaptations of Fiddler Crab Species in Relation to Habitat</td>
<td>4.1 Introduction</td>
<td>87</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4.2 Materials and methods</td>
<td>93</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4.2.1 Sediment grain size analysis</td>
<td>93</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4.2.2 Mouthparts</td>
<td>93</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4.3 Data analysis</td>
<td>94</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4.4 Results</td>
<td>94</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4.4.1 Sediment grain size analysis</td>
<td>94</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4.4.2 Mouthparts</td>
<td>97</td>
</tr>
</tbody>
</table>
Chapter 5 - An Investigation into the Spatial Distribution and Home Ranges of Sympatric Fiddler Crabs

5.1 Introduction
5.1.1 Interindividual spacing
5.2 Methods
5.2.1 Species assemblages
5.2.2 Image analysis
5.3 Data analysis
5.4 Results
5.4.1 Home range size
5.4.2 Nearest neighbour analysis
5.5 Discussion
5.5.1 Quantitative assessment of home ranges
5.5.2 Individual differences in home range

Chapter 6 - General Discussion

6.1 Ecological preferences
6.1.1 Shore height
6.1.2 Substrate properties
6.1.3 Shading
6.2 Individual spatial distribution
6.3 Ecological considerations
6.4 Understanding coexistence and future work

Reference List
Appendix
List of Tables

Chapter 2 - Morphological and Phylogenetic Analysis of Ten Species of Sympatric Fiddler Crab (Crustacea: Decapoda: Brachyura: Ocypodidae) in the Indo-West Pacific

2.1 BLAST analysis of the COI sequences compared with those of deposited fiddler crab sequences on GenBank 62

Chapter 3 - The Drivers of Coexistence of Ten Species of Sympatric Fiddler Crab in the Indo-West-Pacific

3.1 Sediment particle size scale from the GRADISTAT program 71
3.2 Analysis of variance (ANOVA) of sediment types across all transects 74
3.3 The sex ratios of the species recorded at Ambeua, all ratios are males to females 78
3.4 The species recorded at Ambeua, their habitat type and the percent of the total abundance of each 83

Chapter 4 - A Comparative Study of Maxilliped Adaptations of Fiddler Crab Species in Relation to Habitat

4.1 The total number of setae (Total S), the mean number of plumose (Mean P) setae and spoon-tipped (Mean ST) found on the inner face of the second maxillipeds of ten species of fiddler crab, with the mean spoon-tipped setae as a percent of total setae (Meant %ST) and mean carapace width (CW) of species 98

Chapter 5 - An Investigation into the Spatial Distribution and Home Ranges of Sympatric Fiddler Crabs

5.1 Mean home range size (m²) and mean carapace width (mm) of each species 121
5.2 Mean number of crabs living in the home range of each species 123
5.3 Mean distance of the nearest neighbour in the home range of each species 127
Chapter 6 - General Discussion

6.1 Size, habitat characteristics and the number of spoon-tipped setae of fiddler crabs the Ambeua shore

List of Figures
Chapter 1 - General Introduction

1.1 The Wakatobi Marine National Park, southeast Sulawesi, Indonesia 7
1.2 Kaledupa island 8

Chapter 2 - Morphological and Phylogenetic Analysis of Ten Species of Sympatric Fiddler Crab (Crustacea: Decapoda: Brachyura: Ocypodidae) in the Indo-West Pacific

2.1 Diagrammatic ventral view of a male fiddler crab 14
2.2 Diagrammatic lateral view of a fiddler crab 15
2.3 Scanning Electron Microscope image showing the in-situ orientation of the gastric mill in the stomach. 15
2.4 Schematic illustration of CLSM mounting method 19
2.5 Tubuca coarctata, Kaledupa, Sulawesi Tenggara, Indonesia 22
2.6 Tubuca coarctata, male, first pleopod; a, lateral surface; b, mesial surface 23
2.7 Tubuca coarctata, male, apical part of first pleopod; a, mesial surface; b, lateral surface 24
2.8 Tubuca coarctata, male, gastric mill; a, median tooth plate, ventral surface; b, lateral tooth plate, mesial surface 24
2.9 Tubuca demani, Kaledupa, Sulawesi Tenggara, Indonesia 26
2.10 Tubuca demani, male, first pleopod; a, lateral surface; b, mesial surface 27
2.11 Tubuca demani, male, apical part of first pleopod; a, mesial surface; b, lateral surface 28
2.12 Tubuca demani, male, gastric mill; a, median tooth plate, ventral surface; b, lateral tooth plate, mesial surface 28
2.13 Tubuca dussumieri, Kaledupa, Sulawesi Tenggara, Indonesia 30
2.14 Tubuca dussumieri, male, first pleopod; a, lateral surface; b, mesial surface 31
2.15 Tubuca dussumieri, male, apical part of first pleopod; a, mesial surface; b, lateral surface
2.16 Tubuca dussumieri, male, gastric mill; a, median tooth plate, ventral surface; b, lateral tooth plate, mesial surface
2.17 Paraleptuca crassipes, Kaledupa, Sulawesi Tenggara, Indonesia
2.18 Paraleptuca crassipes, male, first pleopod; a, lateral surface; b, mesial surface
2.19 Paraleptuca crassipes, male, apical part of first pleopod; a, mesial surface; b, lateral surface
2.20 Paraleptuca crassipes, male, gastric mill; a, median tooth plate, ventral surface; b, lateral tooth plate, mesial surface
2.21 Gelasimus jocelynae, Kaledupa, Sulawesi Tenggara, Indonesia
2.22 Gelasimus jocelynae, male, first pleopod; a, lateral surface; b, mesial surface
2.23 Gelasimus jocelynae, male, apical part of first pleopod; a, mesial surface; b, lateral surface
2.24 Gelasimus jocelynae, male, gastric mill; a, median tooth plate, ventral surface; b, lateral tooth plate, mesial surface
2.25 Gelasimus tetragonon, Kaledupa, Sulawesi Tenggara, Indonesia
2.26 Gelasimus tetragonon, male first pleopod; a, lateral surface; b, mesial surface
2.27 Gelasimus tetragonon, male, apical part of first pleopod; a, mesial surface; b, lateral surface
2.28 Gelasimus tetragonon, male, gastric mill; a, median tooth plate, ventral surface; b, lateral tooth plate, mesial surface
2.29 Austruca cryptica, Kaledupa, Sulawesi Tenggara, Indonesia
2.30 Austruca cryptica, male, first pleopod; a, lateral surface; b, mesial surface
2.31 Austruca cryptica, male, apical part of first pleopod; a, mesial surface; b, lateral surface
2.32 Austruca cryptica, male, gastric mill; a, median tooth plate, ventral surface; b, lateral tooth plate, mesial surface
2.33 Austruca mjoebergi, Kaledupa, Sulawesi Tenggara, Indonesia
2.34 Austruca mjoebergi, male first pleopod; a, lateral surface; b, mesial surface
2.35 Austruca mjoebergi, male, apical part of first pleopod; a, mesial surface; b,
lateral surface

2.36 *Austruca mjoeberti*, male, gastric mill; a, median tooth plate, ventral surface; b, lateral tooth plate, mesial surface

2.37 *Austruca perplexa*, Kaledupa, Sulawesi Tenggara, Indonesia

2.38 *Austruca perplexa*, male, first pleopod; a, lateral surface; b, mesial surface

2.39 *Austruca perplexa*, male, apical part of first pleopod; a, mesial surface; b, lateral surface

2.40 *Austruca perplexa*, male, gastric mill; a, median tooth plate, ventral surface; b, lateral tooth plate, mesial surface

2.41 *Austruca triangularis*, Kaledupa, Sulawesi Tenggara, Indonesia

2.42 *Austruca triangularis*, male, first pleopod; a, lateral surface; b, mesial surface

2.43 *Austruca triangularis*, male, apical part of first pleopod; a, mesial surface; b, lateral surface

2.44 *Austruca triangularis*, male, gastric mill; a, median tooth plate, ventral surface; b, lateral tooth plate, mesial surface

**Chapter 3 - The Drivers of Coexistence of Ten Species of Sympatric Fiddler Crab in the Indo-West-Pacific**

3.1 Schematic of Ambeua mangrove and mudflat

3.2 Photograph of Ambeua, showing the position of T1 – T5.

3.3 Shore height profile Ambeua

3.4 Distribution of fiddler crab species recorded at Ambeua, within each quadrat of transects 1 – 5.

**Chapter 4 - A Comparative Study of Maxilliped Adaptations of Fiddler Crab Species in Relation to Habitat**

4.1 Mouthparts of *Uca maracoani* adult male, a; first maxilliped, b; second maxilliped, c; third maxilliped

4.2 Dorsal view of right second maxilliped; a, plumose setae of *P. crassipes*; b, spoon-tipped setae of *T. dussumieri*

4.3 Principle Component Analysis (PCO) plot of the distLM model, showing
results from the multivariate permutational analysis (PERMANOVA) of
differences in sediment particle size of transects 1 – 5.
4.4 Principle Component Analysis (PCO) plot of the distLM model, showing
results from the multivariate permutational analysis (PERMANOVA) of
differences in sediment particle size of transects 1 – 5, 6 & 7 and 8 & 9.
4.5 Spoon-tipped setae, plumose setae and carapace width (mm) for the ten
species of fiddler crab at Ambeua.
4.6 The average number of spoon-tipped setae standardised to crab size
(carapace width) per species.
4.7 Variation between species in size and number of spoon-tipped setae of the
second maxillipeds.
4.8 Variation between species in size and structure of spoon-tipped setae of the
second maxillipeds.
4.9 Variation between species in size and structure of spoon-tipped setae of the
second maxillipeds.

Chapter 5 - An Investigation into the Spatial Distribution and Home
Ranges of Sympatric Fiddler Crabs

5.1 Quadrat with counters next to each burrow containing a fiddler crab
5.2 Burrow of A. perplexa male adult, represented by green dot, with estimated
home range, represented by black circle
5.3 The mean carapace width (mm) for the female adults, male adults, female
5.4 The correlation between carapace width (mm) and home range area (m²),
with an r-squared value of 0.34
5.5 Graph showing the correlation between home range area and the number of
crabs within it, with an r-squared value of 0.55
5.6 Observed species of nearest neighbour for the ten species of fiddler crab at
Ambeua. The species of the nearest neighbour for each species of crab at
Ambeua, shown as a percent of the total for all individuals of each species
5.7 Percentages of nearest neighbours shown in terms of whether they were
male or female; a) females of each species, b) males of each species
Declaration

Whilst registered as a candidate for the above degree, I have not been registered for any other research award. The results and conclusions embodied in this thesis are the work of the named candidate and have not been submitted for any other academic award.

Laura Anne Michie
Chapter 1

General Introduction

1.1 Ecological Theory of Coexistence

All species, to some extent, interact with other species. Species coexistence occurs in most natural environments, it would be very difficult to find a community made up of a single species which has no interaction with members of another species. Communities can consist of hundreds of species which are all enmeshed in relationships with one another. Species are deemed sympatric when they exist in the same geographic area and thus regularly encounter one another. Explaining and understanding species coexistence is a key area of investigation in ecology. Coexistence theory requires the species present to have limited similarities (Levins, 1979) or for mortality (environmental or predation) to keep populations below carrying capacity to avoid competition (Huston, 1979). Species must use the environment in different ways in order to coexist so that one species does not out-compete another, as determined by the competitive exclusion principle (Hardin, 1960). If two species compete for the same resource and one species has an advantage over another, it is likely that the one with the advantage will dominate in the long term; on the basis that competition occurs in a stable environment for the same limiting resource at the same time. This will result in either the extinction of the less successful species or an evolutionary or behavioural shift toward a different ecological niche (Hardin, 1960). For an environment to sufficiently sustain a population the number of coexisting species cannot exceed the amount of resources present and species cannot be too similar in their resource utilization (MacArthur and Levins, 1967). Therefore, the total number of species in an ecosystem is estimated at being proportional to the total range of the environment divided by the niche size of each species (MacArthur and Levins, 1967).
It is very difficult to understand the precise factors that separate species and to attempt to theorise the outcome in different environments. All environments will have some or many differences and various discrepancies have been found between theoretical estimates from the classic niche theory (Nathan et al., 2013). One of the main theoretical niche concepts is the competitive exclusion principle, stating that two species competing for the same limiting resource cannot coexist if other ecological factors are constant (Hardin, 1960). This statement doesn’t always hold in reality, where ecosystems are often characterized by a wide abundance of different species, often exploiting the same limiting resource. Many mechanisms have been suggested to explain discrepancies between theory and reality, including niche differentiation due to heterogeneous space and time (Nathan et al., 2013). Competitive exclusion plays a crucial role in how communities are structured and therefore has been highly studied (Amarasekare, 2003; Levins and Culver, 1971; Tilman, 1994). Yet, competition in the structuring of ecological communities has proven hard to evaluate and remains a contentious subject. Niche differentiation has long been recognised as a mechanism of species coexistence. Coexisting species frequently differ in resource use in at least one niche dimension and therefore avoid competition (Chesson, 2000).

1.2 Fiddler Crabs

Fiddler crabs are small intertidal crustaceans associated with warm-temperate to tropical soft-sediment intertidal shores, particularly mudflats within and adjacent to mangrove forests or temperate salt marshes (Crane, 1975). Their latitudinal range extends from roughly 37°46′S in Mar Chiquita Lagoon, Argentina (Spivak, 1991) to 42°14′ N in Massachusetts, USA (Sanford et al., 2006), which is an exceptional latitudinal distance to inhabit.

Fiddler crabs are most well known for their sexual dimorphism, with the striking asymmetry in males being the distinguishing trait of the genus. Male fiddler crabs have a small minor chela and a major chela that is greatly enlarged, sometimes representing up to 50% of the overall body weight (Crane, 1975; Rosenberg, 2002). Females have two small chelae which resemble the male minor chela. The minor chela is used in feeding to scoop sediment into the mouthparts. The male major chela
is used in mating displays and in agonistic interactions; for disputes over territory or when courting (Crane, 1975; Rosenberg, 2002). It is thought to have evolved by means of sexual selection (Swanson et al., 2013), which can occur directly through competition and indirectly through mate choice. The major claw is usually brightly coloured and striking; often the rest of the crab is as well (Crane, 1975; Rosenberg, 2002). The colouration of a male, the size and shape of the major claw and the ability to display (visually and acoustically) are thought to be the main factors behind mate choice (Von Hagen, 1984). Male fiddler crabs show complex displays, with each species having unique display characteristics that serve in mate choice, species recognition and combat (Crane, 1975; Von Hagen, 1984; Pope, 1997). Males have been known to adjust courtship displays depending on the distance of signal receiving females (How et al., 2007), showing the ability to gauge spatial and temporal ranges. Males often produce background waving to attract females from a distance and more intricate courtship waving at advancing females (Murai and Backwell, 2006). Female fiddler crabs have been shown to approach males with large major claws more often than smaller ones, with male handedness having no effect on female responses (Oliveira & Custodio, 1998).

The intertidal environment experienced by fiddler crabs governs their life in many ways; they must be capable of survival in both the terrestrial and marine environments and the majority of their activities are confined to the low tide period. The life of a fiddler crab is concentrated around its individual burrow; which protects it from predation, desiccation and acts as a refuge from heat (Crane, 1975; Powers & Cole, 1976). Male burrows are a mating ground and often females will wander through male territories looking for a suitable partner, they will choose a mate which will often give up its burrow for the female to incubate their eggs in (Crane, 1975; Nakasone & Murai, 1998; Salmon, 1984). When this occurs, a male must either; dig a new burrow, find a vacant dwelling, or win over a suitable burrow from another crab, which is where fighting normally ensues. It is rarely favourable to dig a new burrow as this can take some time and with such brief periods of emersion, time is imperative.

The existence of fiddler crabs in such an extreme environment, along with their remarkable sexual dimorphism, makes them a highly interesting topic for
investigation. Fiddler crabs are among the best invertebrates for behavioural and comparative study as they are prevalent, accessible and largely active at low tide. Fiddler crabs can inhabit shores that are immersed for long periods of time so are often seen living above mid-tide levels, allowing for a greater amount of time out of water, on the surface of the substrate (Crane, 1975). Fiddler crabs rarely live in the middle of broad open mudflats of soft, deep mud, perhaps due to the deficiency of oxygen causing food shortages (Crane, 1975). Most fiddler crabs live on mudflats, on the fringe of a mangrove, at the mouth of a brackish river or in tidal lagoons. There are only a few exceptions, one being *Gelasimus tetragonon*, which in some areas is found on shores where a thin layer of sandy mud covers a firm substrate of coral or rock (Crane, 1975).

Fiddler crab species are frequently seen coexisting, with two or three species often living sympatrically (Booksmythe et al., 2010; Lim, 2004; Lim and Kalpana, 2011; Teal, 1958). Higher alpha (local) diversities have been recorded, with four (Bezerra et al., 2006; Icely and Jones, 1978; Koch, 2005) and five species (Frith and Brunenmeister, 1980) seen coexisting. Seven species of fiddler crab were recorded living sympatrically by Barnes (2010) which was then surpassed by ten species found coexisting in Brazil (Thurman et al., 2013). These ten species were recorded on a 2 m by 8 – 10 m transect on the coast of Rio de Janeiro (Thurman et al., 2013) and the same ten species were reported in mangroves a short distance away (Bedé et al., 2008), however the factors driving this coexistence were not studied. Sixteen species of fiddler crab were recorded coexisting at a site in Panama, on the edge of a mangrove forest, near to a sewer outlet (Crane, 1975). This is the highest number of fiddler crab species seen living in one place, but no record was made of which species were present or the size of the sympatric area and no investigation was undertaken. Although many accounts of sympatric behaviour have been recorded, the sympatry observed in this study is remarkable as the coexistence of this many species is rarely seen. The current study offers the opportunity to investigate this high biodiversity in detail and consider the factors that may be driving this coexistence. Sympatry is used here to describe the coexistence of species in a small area (on Ambeua mudflat), where they exist in the same geographic area and thus frequently encounter one another.
Fiddler crabs, like most organisms, are able to coexist when sufficient resources are available. Heck and Whetstone (1977) stated that habitat heterogeneity is thought to stabilise competitive interactions, enhancing the coexistence of apparent competitors. Therefore, they will not be forced into segregated niches due to the high availability and variability of resources provided. In marine phytal environments, competition is generally considered unlikely, due to the continuously available food supplies (Hicks, 1977) and the capability of animals to vary in their use of resources and feeding strategies; being able to shift from preferred to less preferred food in situations of shortage (Heck and Orth, 1980).

In this study, ten species of fiddler crab have been recorded living in sympatry on the interface between a mudflat and a mangrove at Ambeua on the Indonesian island of Kaledupa, Southeast Sulawesi: *Tubuca coarctata* (H. Milne Edwards, 1852), *Tubuca demani* (Ortmann, 1897), *Tubuca dussumieri* (H. Milne Edwards, 1852), *Paraleptuca crassipes* (White, 1847), *Gelasimus jocelynae* (previously *G. neocultrimana*) (Shih, Naruse and Ng, 2010), *G. tetragonon* (Herbst, 1790), *Austruca cryptica* (Naderloo, Türkay and Chen 2010), *Austruca mjoebergi* (Rathbun, 1924), *Austruca perplexa* (H. Milne Edwards, 1837), *Austruca triangularis* (A. Milne-Edwards, 1873). It is worth noting that, although not coexisting with the other species, *Tubuca paradussumieri* (Bott, 1973) is present on the nearby mudflats.

Four species of fiddler crab were recorded living sympatrically in Mexico (Bezerra *et al*., 2006), finding that *Leptuca thayeri* and *Uca maracoani* had similar requirements in relation to sediment particle size, organic matter and water content of sediment, but could coexist as *L. thayeri* prefers vegetated habitats, while *U. maracoani* prefers open areas. The study also revealed that *Leptuca leptodactyla* and *Minuca rapax* had similar requirements in relation to sediment particle size, but could coexist due to differences in organic content of the substrate; *M. rapax* had a positive correlation with organic content and therefore did not inhabit areas with low values, where *L. leptodactyla* was found.

A preliminary attempt was made to assess the scale and nature of the alpha diversity of fiddler crabs across the research site at Ambeua (Barnes, 2010) by means of visual counts within 2 m² quadrats. An astounding diversity of seven species within an area
of 4 m² was revealed. The quadrats (Barnes, 2010) were dominated by *G. jocelynae* (as was the mudflat as a whole) and *A. perplexa*, which together constituted 67% of the individuals observed. Five other species were also present: *P. crassipes*, *T. demani*, *T. dussumieri*, *A. mjoebergi* and *G. tetragonon*. Since the study carried out by Barnes, the three other species in this study have additionally been observed at the site – *T. coarctata*, *A. cryptica* and *A. triangularis*.

Anthropogenic changes to natural habitats have been negatively linked to many ecological issues including the reduction of ecosystem biodiversity (Duke *et al.*, 2007; Maiti & Chowdhury, 2013). It is evident that human activity can influence the distribution and survival of many organisms, including fiddler crabs but surprisingly, some of these effects appear to benefit fiddler crabs. At Ambeua, these anthropogenic alterations appear to be creating additional environments for fiddler crabs to inhabit and therefore increasing habitat heterogeneity. As Crane (1975) states, it seems some of the richest sites for fiddler crabs are not shores lacking in any human activity but rather where there is some sort of anthropogenic influence, be it at the edge of a village, a refuse site or the outlet of a sewer. Crane (1975) even states herself that the ‘richest site for a fiddler crab is likely to be close to a thriving village, preferably with stilt-legged huts and no plumbing’, which is exactly what is occurring at this site. However, The negative side of human activity is usually inevitable; in areas where human influence occurs, the sheer number of people, industrialisation, deforestation and pollution are just a few things that are drastically affecting many organisms, including fiddler crabs.

1.3 Study Site; the Wakatobi Marine National Park

This study took place within the Wakatobi Marine National Park (WMNP), located off the south-east coast of Sulawesi. The park is situated in the middle of the Wallacea region and the coral triangle which contains some of the world’s richest marine biodiversity and is a priority for marine conservation (Tomascik *et al.*, 1997). The WMNP was established in 1996, became a World Biosphere Reserve in 2012 and covers 1.39 million hectares (Tomascik *et al.*, 1997). The WMNP is comprised of four main islands: Wangi-Wangi, Kaledupa, Tomia and Binongko, and several smaller islands. The WMNP includes coral reef, mudflat, seagrass and mangrove
habitats, all with high conservation value which provide fundamental resources to local communities (Unsworth et al., 2007).

Figure 1.1 The Wakatobi Marine National Park, southeast Sulawesi, Indonesia; a, Indonesia, showing Sulawesi; b, the four main islands that make the Wakatobi; Wangi-Wangi, Kaledupa, Tomea and Binongko; c, Kaledupa island, indicating Ambeua.

The site of coexistence studied in this investigation was located within and adjacent to the village of Ambeua (Figure 1.1c, 1.2a, b). The mangroves of Ambeua are dominated by *Avicennia* with a fringe of *Rhizophora*. The tree canopy remains relatively low throughout the mangroves and there is a maximum tidal range of two metres. The relatively low turbidity and high salinity of the tidal waters permits reefs to grow in close proximity to the mangroves and sea grass beds to form between the reefs and mangroves (Tomascik et al., 1997).
This study is an investigation into the morphology, ecology and behaviour of fiddler crabs in the Wakatobi Marine National Park, Indonesia, where ten species have been found living sympatrically. The factors driving coexistence can be extensive and multi-layered and there is the accepted possibility that some factors cannot be comprehended. It should also be noted that, regardless of the data collected and the evidence given, there will always be a certain amount of assumption when trying to understand species interactions. There are numerous theories about coexistence and this project focuses on those acting at local scales, however, broader scales are also referred to since they are important in understanding regional and global species diversity. The focus of this research is the fiddler crab species present at Ambeua, yet the concepts of coexistence investigated can also be applied to other organisms. This thesis comprises a comprehensive look at the coexistence of species utilising similar resources, a multidisciplinary and multi-technique approach has been adopted due to the number of factors that could be driving and affecting the coexistence observed. The factors studied in this thesis are morphological and ecological differences among species, interspecific and intraspecific variability, biotic and abiotic parameters and environmental heterogeneity.

The research begins with detailed descriptions of the different species investigated throughout the study, then goes on to look at their distribution across the Ambeua shore. The factors affecting their distribution were studied in an endeavour to understand how this many species can live sympatrically. The distribution of each species and individual crabs was then further investigated to look closely at inter- and intraspecific interactions.

With the ever-increasing destruction of natural environments, the extinction rate of species is growing and assemblages of species are being threatened at an unprecedented level. It is essential to develop a better understanding of how species coexist and this knowledge can be used in management and conservation strategies. Under natural conditions, most organisms are involved in a complex web of
relationships with other organisms. Coexistence can occur at many taxonomic levels, with competition highest between closely related species (Tokeshi, 1999). Though there are other species, including other decapod crustaceans (*Macrophthalmus* sp.), living sympatrically with the fiddler crabs, this project will, in detail, only consider the means of coexistence for the fiddler crab species.

1.5 Research Aims and Objectives

This study aims to help further understand the distribution of sympatric fiddler crab species and the diversity of form and function between coexisting species. The main aims of this investigation were to identify, through anatomy, morphology and genetic barcoding, the ten species of fiddler crab living at Ambeua (Chapter 2). This study aims to establish the distribution of the crabs across the site and to determine the factors that affect this distribution (Chapter 3). The research will look at niche differentiation between species and the means by which they can partition resources to enable survival. When species differentiate their niches, they tend to compete less and are thus, more likely to coexist. There are many ways in which fiddler crab species could differentiate their niches, such as by inhabiting different substrates, shore heights or areas of shade. This study will compare the major differences in mouthpart morphology, specifically the second maxilliped, in the ten sympatric species, with a focus on setae shape and abundance (Chapter 4). This work also aims to determine whether differences in mouthpart morphology are linked to species habitats, with a particular focus on the substrate on which the crabs are feeding. The distribution of individual crabs was studied to determine whether crabs are more likely to live nearest to a conspecific or an allospecific, and whether this varies between males and females and adults or juveniles to determine levels of competition (Chapter 5).
Chapter 2

Morphological and genetic analysis of ten species of sympatric fiddler crab (Crustacea: Decapoda: Brachyura: Ocypodidae) in the Indo-West Pacific

2.1 Introduction

The taxonomic history of fiddler crabs is complicated and has long been a debated subject. Shih et al. (2016) recently reviewed the taxonomic status of the genus *Uca* (to which all fiddler crab species belonged) and split it into multiple genera. Several taxa formerly treated as subgenera are now considered genera in their own right. Eleven genera now belong to two subfamilies; Gelasiminae and Ocypodinae. Much of the confusion and disagreement regarding the genus *Uca* was due to historical complications. A short synopsis (from Rosenberg et al., 2001) is that the earliest description of *Uca* came from an illustration by Seba (1758); from this image the species was named *Cancer uka una*. A number of other authors used this figure to name the species and several names were suggested, including *Cancer vocans major* (Herbst, 1782) and *Uca una* (Leach, 1814). The type species of *Uca* is now known as *Cancer vocans major*. The earliest actual specimens of the species were *Gelasimus platydactylus* (H. Milne Edwards, 1837) so the genus became known as *Gelasimus* for 60 years. Rathbun (1837) noted that the rejection of the name *Uca* did not conform to zoological nomenclature, so the genus became *Uca* once again. *Uca heterochelos* (Rathbun, 1918) was suggested as the type species, but 50 years later it was pointed out that the name was synonymous with *Uca major*, so this was adopted and the type species has been *Uca major* ever since. Bott (1973) discovered that the species pictured by Seba (1758) was not actually the American species known as *U. major*, but the species known as *Uca tangeri* from Africa and Portugal. Changing the name again would have been difficult, so the type species is still *U. major* but it is noted that this actually refers to *U. tangeri*. 
Although there have been many studies on fiddler crabs, few have studied the genus as a whole. There were two major revisions in the 1970’s, one by Bott (1973) and the other by Crane (1975), neither included phylogenetics, but relied on morphology to review the genus. Bott (1973) used the male pleopod as the primary means of classification, also suggesting that the genus be divided into numerous genera. Crane (1975) did a much more thorough study, but had missed the previous classifications by Bott, which took priority over Cranes’ classifications. Crane mostly used the carapace and cheliped morphology and front width for classification (Beinlich and Von Hagen, 2006; Naderloo, 2010; Rosenberg, 2001). Crane (1975) tended to put similar taxa, especially those with different male pleopods, into subspecies rather than present them as separate species, subsequent studies have raised nearly all these subspecies to specific species status (Green, 1980; Thurman, 1979, von Hagen and Jones, 1989; Rosenberg, 2001; Shih et al., 2009).

More recent studies attempted to clear up the taxonomic confusion of *Uca* (Beinlich and Von Hagen, 2006; Shih et al., 2009; Naderloo et al., 2010; Shih 2015) and the most recent (Shih et al., 2016) reviewed the genus and split it into separate genera, most of which were raised from the original subgenera. The genera belong to the family Ocypodidae and two subfamilies; Gelasiminae and Ocypodinae, with the genera being; *Afruca* Crane, 1975; *Austruca* Bott, 1973; *Cranuca* Beinlich & von Hagen, 2006; *Gelasimus* Latreille, 1817; *Leptuca* Bott, 1973; *Minuca* Bott, 1954; *Paraleptuca* Bott, 1973; *Petrucu* Shih, Ng & Christy, 2015; *Tubuca* Bott, 1973; *Xeruca* Shih, 2015, and *Uca* Leach, 1814.

The present chapter aims to identify through descriptions and imaging, the species investigated in this thesis. Descriptions of the gonopod, gastric mill and colouration are given, with additional information on the taxonomy and distribution of each species.

Species of fiddler crab have been known to differ in colouration and patterning depending on geographical location and sympatric associates (Crane, 1975; Michie et al., 2015). Due to the colour potentially being specific to site, it should not be used as an initial identification practice. Differences in colouration and patterning at separate geographical locations could be due to environmental factors or potentially
sympatric associates; the important objective is that species can discern conspecifics to avoid confusion between species. The colour and pattern of a males’ major cheliped is used for species recognition (Detto et al., 2006). The colour and pattern of the posterior carapace is often used for recognizing neighbours but is also used by females when choosing a mate (Detto et al., 2006). Females have been shown to use colour vision when selecting a mate (Detto, 2007), so being discernible can be vital for successful reproduction. The colour of the ventral (external) surface of the third maxillipeds could also be important in mate choice; when male crabs perform courtship displays they often fully extend their legs and elevate their carapace, exposing the ventral surface of their carapace and third maxillipeds.

When collecting data on transects, it is important to identify crabs from a distance so as not to disturb them and to aid in efficiency of data gathering. Therefore, identification needs to be done via discernible external morphology and can be supported by colouration. At Ambeua it is possible to distinguish species by claw/carapace morphology, size and colour, especially in the case of adult males.

The most important morphological characters used in fiddler crab identification, due to interspecific differences, are; the male pleopod, carapace, major cheliped and gastric mill. All of these are either shown or described here. The male pleopods were initially drawn using a camera Lucida, confocal imaging then proved to be more effective in capturing specific detail and structure. Due to drawings being the conventional method of presenting morphological evidence and for the purpose of comparison, both drawings and microscope images are shown here. The gastric mill was suggested as a valuable character in the systematics of brachyuran crabs (Naderloo, 2010) due to its morphology not appearing to be affected by diet (Felgenhauer and Abele, 1990). The morphology of the median tooth plate of the gastric mill was found to be one of the most useful features for distinguishing species in the lactea-group by Naderloo et al., (2010). Due to this, descriptions and Scanning Electron Microscopy (SEM) images of the gastric mills are given in this study.
Figure 2.2. Diagrammatic ventral view of a male fiddler crab. Modified from Crane, 1975.
Figure 2.2. Diagrammatic lateral view of a fiddler crab. Modified from Crane, 1975.

Figure 2.3. Scanning Electron Microscope image showing the in-situ orientation of the gastric mill in the stomach.
**Glossary of identification features to be used in the chapter:**

Anterior and Posterior: a position: Anterior indicates that an appendage is located near the head end of the body as opposed to the rear end of the body (the posterior).

Antero-lateral angle: The shape of the front corners of the carapace, where the anterior edge of the carapace meets the lateral edge of the carapace (Fig. 2.2). The shape of this angle can range in different species from moderately square to quite an acute triangular point.

Antero-lateral margin: The edge of the carapace which runs from the tip of the antero-lateral angle to the dorsal-lateral margin (Fig. 2.2).

Dactyl (dactylus): Terminal or distal part of leg, modified into the movable finger of the cheliped (Fig. 2.1)

Distal: a position: (usually relates to the appendages and their position in regards to the body) the part of the article furthest from the body

Dorsal and Ventral: a position: (relates to the body) dorsal relates to the topside of the animal as opposed to the underside (ventral).

Dorso-lateral margin: margin on the carapace (Fig. 2.2), from the junction between the antero-lateral margin, across the dorsal surface of the carapace.

Flange: A protruding rim or edge, often appearing to be calcified, when present, occurs on both sides of the terminal part of the sperm channel.

G1: first pleopod (male gonopod).

Pollex: Terminal or distal article of leg, modified into the fixed finger of the cheliped (Fig. 2.1).

Pore: Terminal opening of the sperm channel at the tip of the gonopod.

Thumb: A thumb like protrusion towards the distal end of the gonopod. Often differing in size and sometimes not present.
The study of morphology is enhanced using microscopy; SEM is one such technique that has been used here to image the gastric mill. SEM offers the ability to image surface materials and three-dimensional structures.

Confocal Laser Scanning Microscopy (CLSM) is an optical imaging technique that increases resolution and contrast by means of adding a spatial pinhole located at the confocal plane. A confocal pinhole excludes out of focus light from above and below the focal plane which causes an increase in image resolution. By moving the focal plane of the instrument step by step through the z plane of the specimen, a series of optical sections can be recorded. The individual z-stack images can subsequently be reconstructed in three dimensions by means of a maximum image projection. This technique was used here to analyse the structure and shape of the first male pleopod to determine interspecies differences.

One great advantage of CLSM is that it offers considerably enhanced imaging of biological structures, mainly due to the ability to collect optical slices of the object for use in creating a three-dimensional representation of the sample. Enhancements are such that it is possible to visualise interior sections (Michels, 2007). This technique offers an accurate representation of the form of the first pleopod (G1) and offers greater detail than could be gained from using scientific drawing techniques; the detail picked up by the microscope could easily go unnoticed when drawing, or be miss-interpreted. Some structures can be distinguished from one another due to the dominance of different types of fluorescence, caused by the different lasers. These differences in fluorescence very likely reflect different material compositions (Michels, 2007). CLSM also offers benefits in terms of ease of sample preparation and the ability to reverse the prepared samples back to preservation in alcohol, whereas with SEM the sample is permanently set.
2.2 Materials and Methods

2.2.1 Measuring crabs
Carapace width (CW) and carapace length (CL) were measured, using a digital caliper and measured in mm. Twenty male and twenty female adults of each species were measured. Bias in picking specific crabs was avoided by numbering burrows of each species and using a random number generator to select crabs. Male adults were established as any crab that exhibited mating display behaviour and attempted to actively court females. Female adults were established as ‘any larger than or equal to the smallest found to be carrying eggs or any female actively interested in courting a male’.

2.2.2 Taxonomy
The taxonomy and systematics in this thesis follows Shih et al., (2016).

2.2.3 Drawing using camera lucida
Each male first pleopod was placed in a deep cavity slide, with the dorsal side facing upwards, and covered with a layer of polyvinyl lactophenol to the top of the cavity, then covered with a rectangular coverslip. This was left overnight, to allow the polyvinyl lactophenol and the sample to set. Bubbles often appeared overnight from the larger pleopods, due to excess air inside being displaced by the polyvinyl lactophenol, so the process was often repeated to remove the bubbles. Once the sample was set, it was drawn using a dissection microscope with a camera lucida attachment. This was then repeated for the ventral side of the pleopod. The left drawing of each pair of gonopods is roughly the lateral view, while the right is approximately the mesial view. Minor differences in orientation, not specially noted, were occasionally required because of torsion, in order to facilitate interspecific comparisons.

2.2.4 Confocal Laser Scanning Microscopy (CLSM)
Samples were cleaned using a sonic bath, for 30 – 60 seconds per sample and for more persistent soiling, 90 seconds. Samples were initially imaged using autofluorescence but were then stained using Congo Red, due to better imaging results. The specimens were thoroughly washed in distilled water, added to the Congo red solution and left at room temperature for 24 hours. Afterwards, they were transferred to distilled water, left for five minutes and then washed thoroughly with distilled water to remove any excess stain.
Figure 2.4. Schematic illustration of the mounting method, modified from Michels and Buntzow, 2010; a, Adhesive reinforcement rings are glued on the microscope slide; b, the specimen is placed inside the cavity; c, polyvinyl lactophenol is transferred into the cavity; d, a cover slip is placed over the cavity.

The method for setting samples was adapted from the methods by Kihara and Falavigna da Rocha (2009). Adhesive reinforcement rings (typically for stationery use) were fixed one at a time onto a rectangular cover slide (Fig. 2.4), for larger specimens they were first cut into quarters to make the resulting cavity larger. Depending on the size of the pleopod and therefore the required size of the resulting cavity, 5-12 adhesive rings were used. The pleopod was then placed in the cavity and covered with a layer of polyvinyl lactophenol. The cavity was carefully covered with a small square coverslip and left to dry. Each sample was left overnight, allowing the polyvinyl lactophenol to set. If bubbles appeared overnight, the coverslip was removed to release trapped air and a new coverslip was placed over the cavity, sometimes with more polyvinyl lactophenol being added.

All specimens were viewed on a Nikon Eclipse upright microscope with A1-Si confocal microscope and four lasers (405 nm, 488 nm, 561 nm and 640 nm). For imaging, all four lasers were tested individually to determine if each of them captured different information for the final image. Different nanometre scales for each laser were tested to find the optimum range. The objective lens was set to a magnification of 10×. Pixel dwell was set to 2.3µm and images were produced at a pixel size of 1024 ×1024 dpi. Specimens were scanned using the z-projections feature with adjustments made for laser saturation. Series of stacks were obtained, collecting overlapping optical sections throughout the specimen; the number of stacks depended on the size of the specimen. Final images were obtained by maximum intensity projection. Dorsal and ventral sides were scanned for each
specimen. The confocal images are depicted in this chapter alongside the camera Lucida drawings to show the effectiveness of the microscopy.

2.2.5 Scanning Electron Microscope methods
SEM was used to image the gastric mills of all species. Samples were sonicated in 70% ethanol for 20 seconds to remove any inorganic or organic matter. Dehydration was achieved through a graded ethanol series of 30%, 50%, 70%, 90% and absolute ethanol. Material was transferred to 50% and then 100% Acetone, both for half an hour. Material was then covered with 100% hexamethyldisiloxane (HMDS) and placed in a fume hood overnight to allow for evaporation of the HMDS and full drying of the material. Each gastric mill was then carefully mounted onto carbon conductive adhesive tabs, which had previously been placed onto aluminium stubs. Material was sputter coated for observation in a JEOL 6060LV Scanning electron microscope at voltages of 15 kV using secondary modes.

2.2.6 Phylogenetic methods
DNA was extracted from leg tissue using the ‘Genomic DNA from tissue’ kit from Macherey-Nagel, deviating from the manufacturer’s protocol as follows: tissue was lysed overnight at 56 °C and DNA was eluted from the spin column using molecular grade water (2 × 70 μL washes) as opposed to elution buffer. Concentration, yield and purity of DNA were determined by UV spectrophotometry.

A 658 basepair fragment from the 5’ end of the cytochrome oxidase subunit I (COI) was amplified using the primer pair LCO1490 (forward 5’ GGT CAA CAA ATA ATA AAG ATA TTG 3’) and HCO2198 (reverse 5’ TAA ACT TCA GGG TGA CCA AAA AAT CA 3’) (Folmer et al., 1994). A ~550 basepair fragment of the 16S gene was amplified using the primer pair 16Sar (forward 5’ CGCCTGTTTATCAAAAACAT - 3’) and 16Sbr (reverse 5’ - CCGGTCTGAACCTACGATCACG - 3’). Amplifications were performed in 50 μL reactions using the DreamTaq PCR Master Mix, with each reaction containing: DreamTaq DNA Polymerase, 2X DreamTaq buffer, dNTPs, and 4 mM MgCl₂, 1 μL of each primer (10 mM) and 1‒2 μL DNA template (10–20 ng/μL) then brought to volume with nuclease-free water.
The PCR conditions were as follows: denaturation for 60 s at 94°C, annealing for 60 s at 50°C, and extension for 60 s at 72°C (35 cycles), followed by extension for 10 min at 72°C. A 5 μL aliquot of PCR product was then electrophoresed in a 1.5 % agarose gel. To load, the PCR product was mixed with DNA gel loading dye (containing Bromophenol Blue, Xylene Cyanol FF) and molecular water. Amplified products were purified using a NucleoSpin Gel and PCR Clean-up kit (Macherey-Nagel, Duren, Germany) according to the manufacturer’s guidelines. All PCR products (COI and 16S) were sent for Sanger sequencing at GATC biotech.

COI and 16S chromatograms were then edited and formatted using Chromas DNA sequencing software. Sequences were uploaded onto GenBank and compared with those on the GenBank database to confirm species identity.
2.3 Systematic account

**Superfamily Ocypodoidea Rafinesque, 1815**

**Family Ocypodidae Rafinesque, 1815**

All descriptions, measurements and material examined in this study are from Kaledupa, Sulawesi Tenggara, Indonesia.

**2.3.1 Tubuca coarctata** (H. Milne Edwards, 1852)
(Figs. 2.5a–d, 2.6a–b, 2.7a–b, 2.8a–b)

Males: (19.08 × 11.24 mm), (20.04 ×12.55 mm), (21.43 ×13.02 mm), (17.38 × 10.31 mm), (19.14 × 11.56 mm)

Females: (16.81 × 10.23 mm) (12.99 × 8.34 mm), (14.17 × 8.92 mm) (14.36 × 10.15 mm) (14.12 × 9.87 mm)

![Figure 2.5. Tubuca coarctata; Kaledupa, Sulawesi Tenggara, Indonesia; a, male adult, anterior view; b, male adult, posterior view; c, female adult, anterior view; d, female adult, posterior view. Photos by author.](image)

**Identification characters:** Front narrow. Carapace smooth. Antero-lateral angles acute. Antero-lateral margins practically absent with the dorso-lateral margins starting almost immediately after antero-lateral angle. Major cheliped with hook like projection at distal end of dactylus.
Gonopod morphology: G1 with stem curved in dorso-lateral direction (Figure 2.6). Distal end (from base of thumb) relatively short, tapering towards end. Whole distal end curved in slightly lateral direction (Figure 2.7). Pore of sperm channel in midline.

Figure 2.6. *Tubuca coarctata*, male, first pleopod; a, lateral surface; b, mesial surface.
Figure 2.7. *Tubuca coarctata*, male, apical part of first pleopod; a, mesial surface; b, lateral surface.

**Gastric mill**: Median tooth plate of gastric mill with 6 teeth (Figure 2.8a); different in shape; first two arched; 1 – 5 decreasing in size distally. Last one broader and longer. Lateral tooth plate with 19 comb-shaped teeth (Figure 2.8b).

Figure 2.8. *Tubuca coarctata*, male, gastric mill; a, median tooth plate, ventral surface; b, lateral tooth plate, mesial surface.
**Colouration:** The adult male carapace varies greatly; a fully grown adult male is most often largely black, with either a small white/yellow square (~2 mm) or three blue markings across the central carapace (Figure 2.5b). There are some adult males (known due to observed mating) that have a largely blue carapace with black markings (often associated with juveniles) and some that have a half black and half white carapace. The dactyl and pollex are white at the distal end and orange at the proximal end (Figure 2.5a). The orange extends across the manus with the lower half darker, sometimes being red/maroon. The top of the manus can be white. The carpus is usually the same colour as the adjacent manus (Figure 2.5a). The merus, ischium, basis and coxa are generally orange or light brown. The anterior ventral surface of the carapace and the third maxilliped are generally black. The walking legs and minor chelae tend to be black. The colouration of adult females also varies greatly. The fully grown adult carapace is similar to males; being largely black, with either a small white/yellow square (~2 mm) or three blue markings across the central carapace (Figure 2.5c). Sometimes the carapace is blue with black markings or with the lower carapace dark orange and the upper carapace light orange. The walking legs in females are like those in males (Figure 2.5c, d). In juvenile males, the carapace tends to be blue, with black markings across the top and centre. The dactyl and pollex of the major chela tend to be white, with the rest being orange. The walking legs and the minor chela are black, occasionally the whole crab can be blue. Juvenile females tend to have the same colour pattern as juvenile males, but with some having an orange carapace instead of blue.

**Distribution:** Australia, Indonesia, Philippines, New Guinea, Taiwan
2.3.2 Tubuca demani (Ortmann, 1897)
(Figs. 2.9a–d, 2.10a–b, 2.11a–b, 2.12a–b)

Males: (21.87 × 13.94 mm), (28.96 × 17.21 mm), (26.72 × 16.39 mm), (23.41 × 14.87 mm), (24.58 × 15.58 mm)

Females: (13.10 × 8.37 mm), (20.89 × 13.16 mm), (17.53 × 8.65 mm), (16.72 × 12.88 mm), (15.83 × 10.19 mm)

Figure 2.9. Tubuca demani, Kaledupa, Sulawesi Tenggara, Indonesia; a, male adult, anterior view; b, male adult, posterior view; c, female adult, anterior view; d, female adult, posterior view. Photos by author.

Identification features: Front narrow. Antero-lateral angles strongly acute, produced. Antero-lateral margins short to absent, developing gradually into dorso-lateral margins which are distinct and converge strongly. Carapace broader than long. Major cheliped with manus an equal length to dactyl and pollex. The dactyl is often slightly longer than the pollex. Pollex almost straight, dactyl slightly wider than fixed finger at proximal end. Both dactyl and pollex have a tooth midway between the proximal and distal ends.
Figure 2.10. *Tubuca demani*, male, first pleopod; a, lateral surface; b, mesial surface.

**Gonopod morphology:** G1 with stem curved in dorso-lateral direction (Figure 2.10). Distal end short with rounded tip. Thumb short, barely reaching base of flanges (Figure 2.11). Flanges moderately short. Pore of sperm channel large.
Figure 2.11. *Tubuca demani*, male, apical part of first pleopod; a, mesial surface; b, lateral surface.

**Gastric mill:** Median tooth plate of the gastric mill with 4 teeth (Figure 2.12a); all of similar shape and size; first one slightly larger, all marginally arched. Lateral tooth plate with 18 comb-shaped teeth (Figure 2.12b).

Figure 2.12. *Tubuca demani*, male, gastric mill; a, median tooth plate, ventral surface; b, lateral tooth plate, mesial surface.
**Colouration:** The adult male carapace is variable, it can be entirely grey, sometimes grey with a black transverse band across the bottom and occasionally the top half is grey and the bottom is black with the black creating a ‘W’ shape in the grey (Figure 2.9b). Eyestalks red to dark brown. The dactyl and pollex of the major chela generally have white tips, with the base having orange colouration (Figure 2.9a) which extends to the manus, the proximal end of the manus is a red/brown. The carpus is usually the same colour as the adjacent manus and the rest of the cheliped is more russet. The walking legs and the minor chela are dark brown, grey or black (Figure 2.9a, b). The anterior ventral surface of the carapace and the third maxillipeds are generally grey or dark brown. The colouration of adult females also varies; the carapace is most often grey with a black transverse band across the bottom, sometimes being orange and occasionally dark brown (Figure 2.9d). The walking legs in females are like those in males (Figure 2.9c, d). In juvenile males, the carapace is usually grey, and the bottom is black with the black creating a ‘W’ shape in the grey, sometimes it is grey with a black transverse band across the bottom. The dactyl and pollex of the major cheliped tend to be white/ light brown at the juvenile stage and the rest of the cheliped is orange, becoming darker with maturation. Juvenile females tend to have a grey or dark orange carapace and orange, brown or grey walking legs, with dark brown chelipeds.

**Distribution:** Indonesia, Southern Philippines
2.3.3 *Tubuca dussumieri* (H. Milne Edwards, 1852)
(Figs. 2.13a–d, 2.14a–b, 2.15a–b, 2.16a–b)

Males: (24.32 × 16.85 mm), (23.19 × 14.34 mm), (28.18 × 17.10 mm), (23.91 × 14.67 mm), (25.60 × 16.03 mm)

Females: (20.37 × 14.23 mm), (17.15 × 9.98 mm), (24.83 × 15.84 mm), (24.82 × 15.85 mm), (21.78 × 13.96 mm)

![Figure 2.13. *Tubuca dussumieri*, Kaledupa, Sulawesi Tenggara, Indonesia; a, male adult, anterior view; b, male adult, posterior view; c, female adult, anterior view; d, female adult, posterior view. Photos by author.](image)

**Identification features:** Front narrow. Fronto-orbital margins practically straight. Antero-lateral angles acute but not produced, antero-lateral margins short. Dorso-lateral margins distinct and converging. Carapace broader than long. Carapace smooth. Major cheliped with elongated dactyl and pollex. Pollex with tooth midway between proximal and distal ends. Gape between dactyl and pollex is triangular in shape, due to the distinct angle of the dactyl. Breadth of the dactyl consistent along its entire length and only tapers at the very tip.
Figure 2.14. *Tubuca dussumieri*, male, first pleopod; a, lateral surface; b, mesial surface.

**Gonopod morphology:** G1 with stem curved in dorso-lateral direction (Figure 2.14). Large anterior flange, with dark spine at tip of anterior edge. Thumb short (Figure 2.15). Pore of sperm channel in midline.
Gastric mill: Median tooth plate of the gastric mill with 5 teeth (Figure 2.16a); 1 – 4 decreasing in size distally, fifth one larger and attached to the plate dorsally. Lateral tooth plate with 18 comb-shaped teeth (Figure 2.16b).
**Colouration:** The adult male carapace is black (Figure 2.13b), sometimes with two or three central white markings. The dactyl of the major chela is generally white extending to the top of the manus, the pollex has a white tip and the rest is orange which extends to the bottom of the manus, usually becoming a darker (Figure 2.13b a). The carpus is a mixture of white, orange and brown and the rest of the cheliped is usually orange. The walking legs and the minor chela are generally black (Figure 2.13b a, b), sometimes with the back pair being blue/white. The anterior ventral surface of the carapace and the third maxillipeds are normally black. The colouration of the adult female carapace is similar to male (Figure 2.13b c, d). The walking legs in females are similar to those in males, except sometimes all of the legs are blue/white, not just the back pair (Figure 2.13b c, d). In juvenile males, the carapace is most often black with two or three central white markings. The dactyl and pollex of the major cheliped tend to be white at the juvenile stage and the rest of the cheliped is orange. Juvenile females tend to be similar to adult females, but nearly always with all the walking legs being blue/white.

**Distribution:** China, Taiwan, Thailand, Indonesia, Northwestern Australia, Papa New Guinea
2.3.4 *Paraleptuca crassipes* (White, 1847)  
(Figs. 2.17a–d, 2.18a–b, 2.19a–b, 2.20a–b)

Males: (10.08 × 14.67 mm), (9.34 × 15.13 mm), (10.47 × 16.22 mm), (10.36 × 16.19 mm), (9.93 × 15.26 mm)  
Females: (10.22 × 16.17 mm), (9.20 × 13.84 mm), (9.15 × 12.17 mm), (9.56 × 14.09 mm), (10.44 × 11.29 mm)


*Figure 2.17. Paraleptuca crassipes*, Kaledupa, Sulawesi Tenggara, Indonesia; a, male adult, anterior view; b, male adult, posterior view; c, female adult, anterior view; d, female adult, posterior view. Photos by author.
**Figure 2.18.** *Paraleptuca crassipes*, male, first pleopod; a, lateral surface; b, mesial surface.

**Gonopod morphology:** G1 with stem curved strongly in dorso-lateral direction (Figure 2.18); distal end long and strongly curved in lateral direction. Distal end tapers before bulging slightly at tip, causing a rounded end. Sides folded causing a twisting longitudinal groove visible on posterior surface. Pore of sperm channel in midline. Thumb short (Figure 2.19).
Figure 2.1. *Paraleptuca crassipes*, male, apical part of first pleopod; a, mesial surface; b, lateral surface.

**Gastric mill**: Median tooth plate of the gastric mill with 6 teeth; different in shape; 1 and 2 strongly arched. 1 – 4 decreasing in size distally. Larger gap between fourth and fifth (Figure 2.20a). 5 and 6 are fused and appear almost as one. Lateral tooth plate with 22 comb-shaped teeth (Figure 2.20b).

Figure 2.20. *Paraleptuca crassipes*, male, gastric mill; a, median tooth plate, ventral surface; b, lateral tooth plate, mesial surface.
**Colouration:** The adult male carapace is generally red (Figure 2.17b), sometimes with black or blue markings and occasionally all black with blue and red markings. The dactyl and pollex of the major chela are pale pink/red, the manus is pink/red and the rest of the chela is red (Figure 2.17a). The walking legs and the minor chela are generally black or red (Figure 2.17a, b). The anterior ventral surface of the carapace is often black but sometimes red. The ventral surface of the third maxilliped is almost always blue. The colouration of adult females varies greatly; sometimes the entire crab is red, often the carapace is red with blue and white markings, the carapace can be black with white or blue transverse markings or the carapace can be black with a few white or blue markings. The walking legs in females are similar to those in males (Figure 2.17c, d). In juvenile males, the carapace is similar to adults; usually red or black and often has white and blue markings. The major chela tends to be light brown or pink at the juvenile stage and becomes brighter with maturation. Juvenile females have the similar colour patterns to juvenile males. The colour variation in *P. crassipes* is one of the highest seen at this sight, but generally the colour of the male major chela is the same.

**Distribution:** China, Japan, Philippines, Thailand, Indonesia, Papa New Guinea, Melanesia, Micronesia
2.3.5 *Gelasimus jocelynae* (Shih, Naruse and Ng, 2010)

(Figs. 2.21a–d, 2.22a–b, 2.23a–b, 2.24a–b)

Males: (16.41 × 10.60 mm), (15.13 × 10.41 mm), (14.94 × 10.56 mm), (14.74 × 10.67 mm), (15.33 × 10.50 mm)

Females: (13.33 × 9.55 mm), (14.28 × 9.44 mm), (11.97 × 7.86 mm), (12.03 × 8.37 mm), (13.07 × 8.87 mm)

![Gelasimus jocelynae](image)

**Figure 2.21.** *Gelasimus jocelynae*, Kaledupa, Sulawesi Tenggara, Indonesia; a, male adult, anterior view; b, male adult, posterior view; c, female adult, anterior view; d, female adult, posterior view. Photos by author.

**Identification features:** Front narrow. Fronto-orbital margins practically straight. Antero-lateral angles acute, slightly produced. Antero-lateral margins short, dorso-lateral margins short or nearly absent. Carapace slightly broader than long. Carapace smooth. Major cheliped with elongated dactyl and pollex. Pollex has a distinct wave pattern occurring near the middle of the gape and a hook like tooth close to the distal end.
**Gonopod morphology**: G1 with stem curved in dorso-lateral direction (Figure 2.22). Simple flanges and a small pore. Thumb relatively short, but wide, can vary, often reaching beyond base of flange (Figure 2.23).
Figure 2.23. *Gelasimus jocelynae*, male, apical part of first pleopod; a, mesial surface; b, lateral surface.

**Gastric mill**: Median tooth plate of the gastric mill with 4 teeth; 1 – 3 decreasing in size distally, larger gap between third and fourth (Figure 2.24a). Fourth large and attached to the plate dorsally. Lateral tooth plate with 18 comb-shaped (Figure 2.24b).

Figure 2.24. *Gelasimus jocelynae*, male, gastric mill; a, median tooth plate, ventral surface; b, lateral tooth plate, mesial surface.
**Colouration:** The adult male carapace can vary in colouration, nearly always being a light/dark brown or grey colour but with varying degrees of markings in a range of colours: blue, aqua, orange and white, sometimes with a pearly effect (Figure 2.21b). The dactyl of the major chela is generally white extending to the top of the manus, the pollex sometimes has a white tip, the rest is orange which extends to the bottom of the manus, usually becoming a darker hue (Figure 2.21a). The lower manus or pollex of young adult males often has a brown mark, which can look like a spot of mud. The upper carpus is grey or white and the lower orange. The rest of the cheliped is either white or orange. The walking legs and the minor chela are generally brown or grey (Figure 2.21a, b). The anterior ventral surface of the carapace and the third maxillipeds are normally light brown or grey. The colouration of the adult female is similar to males (Figure 2.21c, d). The chelipeds in adult females are often orange (Figure 2.21c). In juvenile males, the carapace is most often light brown. The major cheliped tends to be white at the juvenile stage, often with a brown mark that looks like a smudge of mud, similar to the adults. Juvenile females tend to be like juvenile males.

**Distribution:** Pacific islands west of Fiji, including Japan, Taiwan, the Philippines, Indonesia, Papua New Guinea, and Vanuatu.
2.3.6 *Gelasimus tetragonon* (Herbst, 1790)

(Figs. 2.25a–d, 2.26a–b, 2.27a–b, 2.28a–b)

Males: (21.18 × 15.72 mm), (22.37 × 14.30 mm), (23.61 × 17.11 mm), (21.97 × 16.43 mm), (22.94 × 16.79 mm)

Females: (20.14 × 15.82 mm), (20.03 × 13.56 mm), (23.16 × 16.70 mm), (21.75 × 14.89 mm), (22.23 × 15.68 mm)

![Images of Gelasimus tetragonon](image_url)

Figure 2.25. *Gelasimus tetragonon*, Kaledupa, Sulawesi Tenggara, Indonesia; a, male adult, anterior view; b, male adult, posterior view; c, female adult, anterior view; d, female adult, posterior view. Photos by author.

**Identification features:** Front moderately broad. Antero-lateral angles acute, slightly produced. Antero-lateral margins short, converging from the angles. Dorsolateral margins short, sometimes absent. Sides of carapace slightly converging, but practically straight in posterior half. Carapace broader than long. Carapace smooth. Major cheliped with long, slender dactyl and pollex. Pollex almost straight, dactyl moderately arched. Both dactyl and pollex with one or more sub-distal teeth.
Gonopod morphology: G1 with stem strongly curved in dorso-lateral direction (Figure 2.26). Distal end slightly curved in lateral direction. Anterior flange slightly narrower than posterior, with a large pore. Thumb moderately large, not reaching base of flanges (Figure 2.27).

Figure 2.26. Gelasimus tetragonon, male first pleopod; a, lateral surface; b, mesial surface.
**Gastric mill**: Median tooth plate of the gastric mill with 4 teeth (Figure 2.28a); decreasing in size distally. Different in shape, first two arched, 3 and 4 straighter. Fourth large and attached to the plate dorsally. Lateral tooth plate with 19 comb-shaped teeth (Figure 2.28b).
**Colouration:** The adult male carapace is blue with a black mottled pattern (Figure 2.25b). The black markings vary in size and shape. The whole of the major chela is orange, with the dactyl often having a lighter tip and the proximal end of the pollex having a darker hue of orange which extends to part of the manus. The manus has a dark brown mottled pattern at the proximal end which extends to the carpus and merus (Figure 2.25a). The walking legs and the minor chela are black or dark brown and the back pair of walking legs often has a black and blue mottled pattern (Figure 2.25b). The anterior ventral surface of the carapace is generally black, dark brown or navy blue and the ventral surface of the third maxillipeds is nearly always the same blue as the carapace. The adult female carapace is similar to the male but often with a darker hue of blue (Figure 2.25d). The walking legs are orange and the minor chela often have a blue dactyl and pollex (Figure 2.25a). In juvenile males, the carapace has a mottled black and white pattern with the colours developing with maturity. The major chela tends to have a similar coloration to the adults but with white distal end to the dactyl and pollex. The legs are black, and the minor chela has a blue dactyl, pollex and manus. Juvenile females have the same colour pattern as juvenile males.

**Distribution:** South Africa to Iran, Madagascar, Thailand, Malaysia, Australia, Indonesia, Philippines, Papa New Guinea, Taiwan, Micronesia, Melanesia
2.3.7 *Austruca cryptica* (Naderloo, Türkay and Chen 2010)

(Figs. 2.29a–d, 2.30a–b, 2.31a–b, 2.32a–b)

Males (15.13 × 9.09 mm), (15.07 × 8.94 mm), (14.58 × 7.52 mm), (14.88 × 7.97 mm), (15.60 × 8.23 mm)

Females (12.16 × 8.23 mm), (13.29 × 8.95 mm), (13.30 × 8.84 mm), (9.14 × 6.80 mm), (9.12 × 6.85 mm)

![Austruca cryptica images](image_url)

**Figure 2.29.** *Austruca cryptica*, Kaledupa, Sulawesi Tenggara, Indonesia; a, male adult, anterior view; b, male adult, posterior view; c, female adult, anterior view; d, female adult, posterior view. Photos by author.

**Identification features:** Front broad. Anterolateral margin moderately convergent. Ventero-lateral margin of carapace moderately convergent. Outer surface of carpus smooth, manus with outer surface slightly granular. Both fingers can have enlarged proximal tooth and sub-distal teeth, yet other individuals can lack teeth altogether.
**Gonopod morphology:** G1 with stem curved in dorso-lateral direction (Figure 2.30); distal end relatively short with pore in midline. Thumb moderately long, reaching base of flange. Distal end has largely protruding dorsal lobe and ventral lobes with the dorsal lobe extending slightly beyond ventral one; whole distal end curved slightly in lateral direction (Figure 2.31).
Figure 2.31. *Austruca cryptica*, male, apical part of first pleopod; a, mesial surface; b, lateral surface.

**Gastric mill**: Median tooth plate of the gastric mill with 6 teeth (Figure 2.32a); first two massive, different in shape with first being strongly arched and the second much broader than the other teeth. 3 – 5 decreasing in size distally; large gap between second and third teeth; 4 and 5 nearly the same size, small gap between them; last one shorter, attached to plate dorsally. Lateral tooth plate with 17 comb-shaped teeth (Figure 2.32b).

Figure 2.32. *Austruca cryptica*, male, gastric mill; a, median tooth plate, ventral surface; b, lateral tooth plate, mesial surface.
**Colouration:** The adult male carapace is largely black, with white transverse markings (Figure 2.29b). Transverse bands vary in size and number. There are some individuals where there is as much white as black, and occasionally the pale region is turquoise rather than white. The dactyl and pollex of the major chela generally have white tips, with the base having orange or pink colouration (Figure 2.29a) which extends to the manus and carpus. The dactyl and pollex can nevertheless be all white or in equal parts white and orange or pink. The merus is usually the same colour as the adjacent manus and carpus, but a darker shade (Figure 2.29a). The walking legs and the minor chela are black, dark brown or grey (Figure 2.29a, b). The anterior ventral surface of the carapace and the merus of the third maxilliped are generally black. The ventral surface of the ischium of the third maxilliped is almost always white or pink. The colouration of adult females varies greatly. The carapace is most often a pale shade of pink or orange and can occasionally be grey (Figure 2.29c, d). There is frequently a white band across the lower posterior region. The walking legs in females are similar to those in males (Figure 2.29c, d). In juvenile males, the carapace is usually a mottled light brown/russet, with the patterns and darker colours developing with maturity. The major chela likewise tends to be light brown and pink at the juvenile stage but becomes darker with maturation. Juvenile females have the same colour pattern as juvenile males.

**Distribution:** Indonesia, Philippines
2.3.8 *Austruca mjoebergi* (Rathbun, 1924)

(Figs. 2.33a–d, 2.34a–b, 2.35a–b, 2.36a–b)

Males: (7.32 × 11.27 × mm), (7.16 × 10.89 × mm), (6.93 × 10.54 × mm), (6.14 × 9.80 × mm), (8.25 × 10.23 × mm)

Females: (10.89 × 6.87 mm), (10.80 × 6.85 mm), (10.16 × 6.64 mm), (10.38 × 6.71 mm), (8.42 × 5.89 mm)

![Figure 2.33. Austruca mjoebergi, Kaledupa, Sulawesi Tenggara, Indonesia; a, male adult, anterior view; b, male adult, posterior view; c, female adult, anterior view; d, female adult, posterior view. Photos by author.](image)

**Identification features:** Front broad. Antero-lateral angles moderately acute and slightly produced. Antero-lateral borders of carapace slightly convergent. Carapace slightly broader than long. Carapace smooth. Antero-lateral as well as dorso-lateral margins short or nearly absent. Major cheliped with dactyl and pollex twice as long as manus. Pollex almost straight, dactyl slightly wider than fixed finger, with sub-proximal tooth.
Figure 2.34. *Astruca mjoeborgi*, male first pleopod; a, lateral surface; b, mesial surface.

**Gonopod morphology**: G1 with stem curved in dorso-lateral direction (Figure 2.34). Border of stem between thumb and endpiece bulging outward. Thumb moderately short, sometimes reaching base of endpiece, longer than broad. Distal end has largely protruding dorsal lobe (Figure 2.35). Pore marked by a distinct indentation.
**Gastric mill**: Median tooth plate of the gastric mill with 7 teeth (Figure 2.36a); first two massive, different in shape with first being strongly arched and the second much broader than the other teeth. 3 – 5 of similar size and shape, 6 and 7 decrease in size. Lateral tooth plate with 17 comb-shaped teeth (figure 2.36b).
**Colouration:** The adult male carapace is light brown with a yellow/brown mottled pattern (Figure 2.33b). The dactyl and pollex of the major chela are yellow with white tips (Figure 2.33a). The rest of the major cheliped is yellow. The walking legs and the minor chela are black or grey with a mottled pattern, occasionally grey/white/yellow striped. The ventral surface of the carapace and the merus of the third maxilliped are generally similar to the carapace. The colouration of adult females is similar to the male (Figure 2.33d). The walking legs in females are like those in males. In juvenile males, the carapace is usually light brown, with the patterns and colours developing with maturity. The major chela tends to be light brown or yellow at the juvenile stage. Juvenile females have the same colour pattern as juvenile males.

**Distribution:** Australia (Northwest Territory to Western Australia), New Guinea and central Indonesia
2.3.9 *Austruca perplexa* (H. Milne Edwards, 1837)
(Figs. 2.37a–d, 2.38a–b, 2.39a–b, 2.40a–b)

Males: (14.80 × 9.00 mm), (14.64 × 10.18 mm), (14.40 × 9.80 mm), (16.20 × 10.30 mm), (14.80 × 8.70 mm)

Females: (11.30 × 7.30 mm), (12.00 × 7.20 mm), (11.20 × 8.10 mm), (10.30 × 6.80 mm), (12.80 × 7.60 mm)

![Austruca perplexa images](image)

**Figure 2.37.** *Austruca perplexa*. Kaledupa, Sulawesi Tenggara, Indonesia; a, male adult, anterior view; b, male adult, posterior view; c, female adult, anterior view; d, female adult, posterior view. Photos by author.

**Identification features**: Front broad. Antero-lateral angles moderately acute and slightly produced. Antero-lateral as well as dorso-lateral margins short or nearly absent. Antero-lateral borders of carapace slightly convergent. Carapace slightly broader than long. Carapace smooth. Major cheliped with long dactyl and pollex. Pollex has a tooth near the centre.
Gonopod morphology: G1 with broad endpiece, directed in dorsolateral direction (Figure 2.38), mesial border of stem between thumb and endpiece bulging outward. Thumb moderately long, often reaching beyond base of flanges. Distal end relatively long mainly due to the largely protruding dorsal lobe (Figure 2.39).
Figure 2.39. *Austruca perplexa*, male, apical part of first pleopod; a, mesial surface; b, lateral surface.

**Gastric mill:** Median tooth plate of the gastric mill with 5 teeth; decreasing in size distally (Figure 2.40a). First one long and strongly arched. Fourth appears to be split on some occasions. Fourth and fifth are separate from the rest, completely different shapes and attached to the plate dorsally. Lateral tooth plate with 17 comb-shaped teeth (Figure 2.40b).

Figure 2.40. *Austruca perplexa*, male, gastric mill; a, median tooth plate, ventral surface; b, lateral tooth plate, mesial surface.
**Coloursation:** Similar to *A. cryptica* the adult male carapace is largely black, with white transverse markings (Figure 2.37b). Transverse bands vary in size and number. The dactyl and pollex of the major chela are white and the manus and carpus tend to be yellow, yet can be white. The merus, ischium, basis and coxa are all yellow. The walking legs and the minor chela are yellow, white, grey and occasionally black (Figure 2.37a, b). The anterior ventral surface of the carapace and the third maxilliped are white. The colouration of adult females varies greatly. The carapace is most often a mottled light brown/russet, grey or white (Figure 2.37d), but on occasion is the same as the male carapace. The walking legs in females are like those in males. The anterior ventral surface of the carapace and the third maxilliped is most often white or grey, occasionally being black, with the ischium of the third maxilliped being white. In juvenile males, the carapace is usually a mottled light brown/russet, with the patterns and darker colours developing with maturity. The major cheliped tends to be entirely white at the juvenile stage and develops colour with maturation. Juvenile females have the same colour pattern as juvenile males; it can be hard to distinguish between juveniles of *G. jocelynae*, *A. cryptica* and *A. mjoebergi*.

**Distribution:** Eastern Indian Ocean (from the Nicobar Is. eastward), Thailand to China, Taiwan, Japan, Philippines, Indonesia, Australia (east coast), Pacific islands.
2.3.10 *Austruca triangularis* (A. Milne-Edwards, 1873)

(Figs. 2.41a–d, 2.42a–b, 2.43a–b, 2.44a–b)

Males: (11.85 × 8.37 mm), (13.60 × 8.11 mm), (12.26 × 7.19 mm), (10.38 × 6.84 mm), (12.14 × 7.54 mm)

Females: (10.87 × 5.82 mm), (12.80 × 8.17 mm), (11.16 × 8.19 mm), (12.26 × 8.23 mm), (11.82 × 7.44 mm)

**Figure 2.41.** *Austruca triangularis*, Kaledupa, Sulawesi Tenggara, Indonesia; a, male adult, anterior view; b, male adult, posterior view; c, female adult, anterior view; d, female adult, posterior view. Photos by author.

**Identification features:** Front broad. Antero-lateral angles strongly acute and produced. Antero-lateral margins absent. Dorso-lateral margins proceeding directly from the antero-lateral angle, strongly converging. Carapace broader than long. Carapace smooth. Major cheliped dactyl and pollex relatively wide. Pollex arched, dactyl also arched, but less so than pollex. Both pollex and manus have teeth in proximal and distal portion.
**Gonopod morphology:** G1 with stem curved in dorso-lateral direction (Figure 2.42). Distal end long and narrow with flanges extend throughout the length of the distal part of the shaft; the distal edge of flanges is rounded. Pore large, extending beyond the overlapping flanges. Thumb well developed, elongated, ending above the base of flanges. Whole distal end curved in lateral direction (Figure 2.43).
**Figure 2.43.** *Austruca triangularis*, male, apical part of first pleopod; a, mesial surface; b, lateral surface.

**Gastric mill:** Median tooth plate of the gastric mill with 10 teeth; different in shape. 1 – 4 decreasing in size distally. 5 much larger; twice as broad as third tooth. 6 – 10 decreasing in size distally. Tenth is much shorter and attached to the plate dorsally (Figure 2.44a). Lateral tooth plate with 20 comb-shaped teeth (Figure 2.44b).

**Figure 2.44.** *Austruca triangularis*, male, gastric mill; a, median tooth plate, ventral surface; b, lateral tooth plate, mesial surface.
**Colouration:** The adult male carapace is largely black, with white transverse markings (Figure 2.41a). Transverse bands vary in size and number. The dactyl and pollex of the major chela are white/off-white with a darker hue (Figure 2.41b) extending to the manus and carpus. The manus, carpus, merus and ischium have a dark brown speckled pattern (Figs. 2.41b). The walking legs and the minor chela are black or grey (Figure 2.41a, b). The ventral surface of the carapace and the merus of the third maxilliped are black. The colouration of adult females is similar to males; the carapace is black with white transverse markings, but with a larger degree of variability than in males; sometimes the whole carapace is white/off-white and occasionally the bottom is black and the top is white/off-white with black transverse markings (Figure 2.41c). There are frequently turquoise transverse markings across the lower posterior region (Figure 2.41c). The walking legs in females are black or grey. In juvenile males and females, the carapace is usually white and sometimes light brown/russet with the patterns and darker colours developing with maturity. The major chela likewise tends to be light brown at the juvenile stage but becomes darker and develops patterning with maturation. The walking legs and minor chela in juvenile males and females are black or grey.

**Distribution:** Australia, Indonesia, Malaysia, Philippines, Taiwan, China, Papa New Guinea
2.4 Genetic sequencing

All sequences were confirmed to a species level by comparing homologies using the Basic Local Alignment Search Tool (BLAST) from GenBank. Sequences of CO1 were confirmed to species level when compared with known fiddler crab sequences previously uploaded to GenBank via other authors. Only three samples of the ten species successfully amplified enough DNA for sequencing and due to lack of specimens, no more DNA could be extracted. Further work would have to be carried out to obtain sequencing data for the other species. The CO1 sequences for *T. coarctata*, *T. dussumieri* and *G. jocelynae* showed a 99%, 100% and 98% match respectively with sequences from the corresponding species previously deposited in GenBank.

Table 2.1 BLAST analysis of the COI sequences from this survey, compared with those of deposited fiddler crab sequences on GenBank, including the closest GenBank species match, the total alignment score (Total Score) the query coverage, the E-value, the highest percent identity to the subject sequence (Max ID) and the Accession code.

<table>
<thead>
<tr>
<th>Specimen</th>
<th>GenBank Match</th>
<th>Total Score</th>
<th>Query Coverage</th>
<th>E-value</th>
<th>Max ID</th>
<th>Accession</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Tubuca coarctata</em></td>
<td><em>Tubuca coarctata</em></td>
<td>1116</td>
<td>97%</td>
<td>0.0</td>
<td>99%</td>
<td>LO053371.1</td>
</tr>
<tr>
<td><em>Tubuca dussumieri</em></td>
<td><em>Tubuca dussumieri</em></td>
<td>1147</td>
<td>97%</td>
<td>0.0</td>
<td>100%</td>
<td>LC150436.1</td>
</tr>
<tr>
<td><em>Gelasimus jocelynae</em></td>
<td><em>Gelasimus jocelynae</em></td>
<td>1109</td>
<td>97%</td>
<td>0.0</td>
<td>98%</td>
<td>AB535415.1</td>
</tr>
</tbody>
</table>

2.5 Discussion

The species identification inferred by both the anatomical and genetic aspects of this study largely corroborate the finding of Shih *et al* (2016) and conforms to the decision to divide the genus *Uca* into multiple genera. This study has offered the possibility to compare morphological and genetic identification methods. Of the species that were successfully sequenced, the genetic data coincided with the species identification using gonopod characteristics. Since the form of the first gonopod is characteristic of each species, it is usually the primary identification feature. The specimens from this study had gonopod characteristics matching those of the same species in other studies (Bott, 1973; Crane, 1975; Naderloo *et al.*, 2010; Serene,
From the genera studied, the gonopods of the *Austruca* genus have thumbs that are moderately long to long, also with elongated flanges. Whilst the gonopods of individuals of the *Tubuca* and *Gelasimus* genera having short or almost entirely absent thumbs, along with short flanges. Other common species identification characteristics can vary within and between species, the male major claw is used in identification, but has been noted to differ between individuals of *A. cryptica* (Michie *et al*., 2015) and between individuals of both *A. perplexa* and *A. annulipes* (Crane, 1975).

Prior to this study, for most species investigated, the gastric mill morphology and crab carapace colouration have not been described in detail. Populations of the same species in different geographical areas often differ in their coloration (Crane, 1975; Detto *et al*., 2008). Therefore, colouration should not be used as a primary means of identification and should only be used once there is an understanding of the population under study and its sympatric associates.

Whilst gonopod morphology is the main feature used in distinguishing species (Crane, 1975; Naderloo, 2010; Türkay, 1975) the value of the gastric mill in identification is more surprising. It has proven a useful feature in generic classification (Sakai *et al*., 2006) and was established as the most useful feature in distinguishing species in the *Austruca* genus (Naderloo *et al*., 2010) and has also proved useful in the present investigation. Therefore, as agreed by Sakai *et al*. (2006) and Naderloo *et al*. (2010), we also propose that it should be used as a routine character in fiddler crab systematics.

This work highlights the need for an extensive and integrative study of fiddler crabs, including details of the gonopod, major claw and gastric mill morphology along with molecular sequences, in order to accurately identify species from, what can often be, a taxonomically challenging group.

The molecular sequences were successfully obtained for three species of fiddler crab. These were combined with the sequences for fiddler crabs available on GenBank to determine species identification. The sequences provided strong evidence to support classification with matches of 98% and above. Further work would need to be done
on additional samples to obtain successful sequences for all species in this investigation.

In terms of biogeographic distribution, all species have a relatively wide distribution across the Indo-West Pacific, except for *A. mjoebergi* and *A. cryptica* whose distributions are much smaller. *A. mjoebergi* is mostly confined to the North-West coast of Australia, with only a few recordings from Indonesia (Rosenberg, 2014). For *A. cryptica* there is very little information regarding its biogeographic range and thus far is only known from Indonesia (Naderloo *et al.*, 2010, Michel *et al.*, 2015). *G. tetragonon* has a distribution stretching as far west as Africa, ranging from South Africa up to Egypt (Rosenberg, 2014).
Chapter 3

The drivers of coexistence of ten species of sympatric fiddler crab in the Indo-West-Pacific

3.1 Introduction

There are currently 102 known, extant species of fiddler crab, of which 38 species occur in the Indo-West-Pacific, 36 in the Eastern Pacific and 22 in the Atlantic (Barnes, 2010; Ng et al., 2008; Rosenberg, 2001). Of the 38 species found in the Indo-West-Pacific, 19 are known from Indonesia (Barnes, 2010; Rosenberg, 2014). This gamma (regional) diversity may not seem that high considering the length of the Indonesian coastline, yet intertidal habitats appear to exhibit high alpha diversity and the present study describes one such occurrence. This project investigated the distribution of ten species of fiddler crab at a single site, which equates to more than half those known from Indonesia, and more than one quarter of all the known fiddler crab species of the Indo-West-Pacific, in an area of only c. 1,500 m².

This specific site of coexistence, where all ten species occur together, measures roughly 50 m x 30 m, on the edge of the village of Ambeua (Figure 1.2, page 8) and is under the influence of anthropogenic factors. The landward fringe of the mangrove has a single fresh-water inlet coming from the centre of the island. This water contains large amounts of anthropogenic waste (personal observation) which, as well as the waste deposited directly onto the mudflat from the nearby houses, is likely to generate a high level of organic matter content within the substrate. The seaward mangrove, the forest fringe and the open mudflat along much of the Kaledupan coastline is inhabited by large populations of fiddler crabs, yet preliminary observations show that this is the only area that contains such a high species abundance.

Fiddler crabs, being deposit feeders, obtain their food by sifting through sediments and extracting organic matter (Crane, 1975). Like any mudflat adjacent to a mangrove forest, the organic matter in the substrate at Ambeua comes from multiple sources; the forest provides many nutrients through the breakdown of woody debris and leaf litter (Clifton, Unsworth & Smith, 2010), the adjacent seagrass bed and coral reef will also contribute through the breakdown of plant matter, phytoplankton
and microbes and excretion by animals (Moberg and Folke, 1999; Sorokin, 1990). At this Ambeua, organic matter is also deposited onto the mudflat from the fresh-water inlet at the landward edge of the mangrove and from the houses, located on stilts, directly above the mudflat. These houses also impose shade on the mudflat, which is in addition to the shade provided by vegetation. These anthropogenic factors are likely to be a factor influencing, at least partially, the high species abundance at Ambeua.

Along with the anthropogenic aspect, there are many elements that are likely to drive the distribution of the fiddler crabs and several will be investigated in this study. Environment components believed to play a major role in the distribution of fiddler crabs are shore height, vegetation, substratum, food, salinity, shading and presence of predators or other organisms, especially potential competitors (Bezerra et al., 2006; Icely and Jones, 1978; Nobbs, 2003; Rabalais and Cameron, 1985; Thurman, 1998). Substratum properties such as particle size, organic matter content and water content play an important role in fiddler crab distribution in mangrove forests (Frith and Brunnenmeister, 1980; Icely and Jones, 1978; Reinsel and Rittschof, 1995; Mouton and Felder, 1996).

The behaviour and ecology of four species of fiddler crabs at Ambeua has previously been described and studied by Weis & Weis (2004); who recorded *Paraleptuca chlorophthalmus*, *Tubuca dussumieri*, *Gelasimus tetragonon* and *Gelasimus vocans* at Ambeua. Of which, *T. dussumieri* and *G. tetragonon* were also recorded in the current study. *G. vocans* would have been the correct identification at the time for what is now known as *G. jocelynae* (Shih et al., 2010). *P. chlorophthalmus* is similar in morphology to *Paraleptuca crassipes* and could have been misidentified, or was present in 2004 but no longer so in 2009 when a study was undertaken by Barnes (Barnes, 2010).

Many studies have investigated the factors affecting the distribution of fiddler crabs (Bezerra et al., 2006; Frith & Brunnenmeister, 1980; Icely and Jones, 1978; Teal, 1958) with a variety of methods used. Excavation is considered the most precise method for determining the density and diversity of fiddler crab populations, but it is highly invasive and labour intensive. There is the potential for it to compromise the stability of the mudflat environment which could be most unfavourable in an area where there is not only high diversity but also the only known, living population of
Austruca cryptica. Burrow counting is another valid method of estimating fiddler crab density (Ens et al., 1993; Mouton & Felder, 1996), but can only be truly accurate when working with single species populations. When multiple species are present it can be virtually impossible to determine to which species a burrow belongs. Furthermore, burrow counting can cause an over estimation of crab densities as not all burrows may necessarily contain a fiddler crab. Therefore, the preferred method for this investigation was visual counts of surface-active individuals. This method does come with its limitations; determining accurate population numbers is difficult as not all crabs may be on the surface at the same time, which is why this method should be repeated at different times of day and at various tidal exposures. This method was certainly considered to be the best for non-invasive, large-scale work with multiple species.

**Aims and Objectives**

This chapter first aims to establish the distribution of the crabs across the Ambeua site via the use of transects. Secondly, to test whether this distribution was related to sediment grain size, shading or vegetation.

**3.2 Materials and Methods**

**3.2.1 Crab distribution**

The study was conducted on the mudflat of Ambeua, Wakatobi National Park, Indonesia (Figures 3.1, 3.2). Field-work was carried out in the months of July and August from 2011 to 2014. Ten species of fiddler crab were found in differing abundances and distributions across the shore. Preliminary observations revealed that above a shore height (vertical distance above sea level) of 1.9 m the distribution of crabs becomes sporadic and below 1.2 m only one species occurs. Due to the main focus of this study being the sympatric species, transects were conducted on the mudflat where all ten species occurred. The site of sympatry is roughly 50 m across the shore and 30 m down the shore. Five 30 m long belt transects (Figure 3.1; 1 – 5; Figure 3.2), each set 10 m apart, were established down the shore, from the mangrove fringe (or equivalent height on the mudflat) down towards the low tide line. These belt transects consisted of quadrats measuring 1.42 m x 1.42 m (2 m²).
Continuous recording was deemed necessary because of the differing assemblages of the ten species across the shore. Of the five transects, two started under the shade of houses and went through the shade of houses again towards the lower shore; the other three started in the mangroves and continued onto the open and unshaded shore.

**Figure 3.1.** Schematic of Ambeua mangrove and mudflat, showing houses (White rectangles) & Transects (1-9)
Figure 3.2. Photograph of Ambeua, showing the position of T1 – T5. T1 began underneath the two houses on the left and ended on the open mudflat, T2, 3 & 4 were on the open mudflat and T5 began underneath two houses on the right, ending on open mudflat, with the final quadrat in the shade of a tree. Photo by author.

The quadrats themselves were marked into the sand rather than using a conventional quadrat as the presence of this seemed to ‘unsettle’ the crabs and caused them to remain in their burrows. Individuals were counted ten minutes after marking the quadrats, giving the crabs time to re-emerge from their burrows. The quadrats were assessed using binoculars and, in each case, visual counts of individuals were made over a period of approximately ten minutes. Each quadrat was assessed whilst emersed at both ebb and flood tides; a third recording was conducted half way between the two and an average number was taken from the three recordings. The ebb tide observations were carried out within two hours of the receding tide and flood tide observations were carried out in the two-hour period before high tide. In order to obtain maximum information about the populations of crabs in each quadrat, the crabs on the surface were identified to species, gender was determined, and adult/juvenile distinctions were made.

An initial temporal study had been carried out to verify that assemblages within each quadrat remained constant throughout the day. Crabs were identified in five quadrats, each hour of the low tide period, for three days. This was to assess whether all species appeared on the surface simultaneously or if there was some degree of
temporal separation. The only differences were in the hour after the tide receded, due to crabs emerging at different times. Subsequently, observations were not made during this time.

Preliminary observations indicated that there were fewer species present in the mangrove and on the lower mud-flat at Ambeua. In order to clarify and quantify this, transects were established in the mangroves and on the lower mudflat. Two 60 m transects in the mangrove (Figure 3.1; 8 & 9) and two 30 m transects on the lower shore (Figure 3.1; 6 & 7) were established and crabs were recorded in 2 m² areas every 5 m along each transect.

3.2.2 Biotic and abiotic factors

Within all quadrats, some of the abiotic and biotic factors which had the potential to influence the distribution of the ten species of crab were measured; the degree of shading (average percent throughout the day), presence of vegetation and substrate type.

3.2.3 Shore height profile

A shore height profile was measured at Ambeua, with the use of an optical leveller. Shore height was measured from the low tide point to the landward fringe of the mangrove. Local tide tables were used to determine the level of the low tide and height above chart datum was used. The optical leveller was set on a tripod and used to establish the point in the same horizontal plane on a two-metre rule that was stationed five metres up the shore. The point on the rule which is intersected by the line of sight is a measure of the difference in elevation of the beach at the two points that are five metres apart.

3.2.4 Sediment grain size analysis

Within three quadrats, five sediment samples were initially taken (one from each corner and one from the centre) to determine whether there was any significant difference in grain size within the given area. No significant difference (p > 0.05) was found, therefore one 10 g sediment sample was collected from the centre of every quadrat on all transects at Ambeua. Each sample was taken from the surface
(top 1 cm) as this is where the crabs feed from. The sieve mesh sizes used for this study were 1000 µm (1 mm), 500 µm, 250 µm, 125 µm and 63 µm. The sieves were stacked in descending order and stood in a plastic tray to catch residual particles smaller than 63 µm. Each sample (10 g) was placed into the top sieve (1000 µm) and using a wash-bottle with a narrow dispensing nozzle the sample was washed with water to separate particles and allow them to pass through the sieve. The sieve was washed with 100 ml of water for roughly three minutes, or when all visible particles less than 1 mm had passed through. The sieve was then removed from the stack and the contents emptied into a folded filter paper with an aperture of <10 µm.

The process was then repeated for the rest of the sieves. When the final particles had passed through the 63 µm sieve and into the tray, the contents of the tray were emptied into a large (27 cm diameter) 5 µm aperture filter paper.

When the water had drained from the filter papers they were placed in an oven at 60°C for 48 hours to dry. Once dry, the fractions from each filter paper (the contents of each sieve) were emptied into a pre-weighed tray and the weight obtained to an accuracy of 0.001 g. The dry weights from each sieve were added to determine the dry weight of the whole sample and the water content was calculated from the initial wet weight.

Sediment size categories were adopted from the GRADISTAT program (Table 1), originally modified from Udden (1914) and Wentworth (1922).

**Table 3.1.** Sediment particle size scale from the GRADISTAT program

<table>
<thead>
<tr>
<th>phi</th>
<th>Grain size</th>
<th>Descriptive term</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1000</td>
<td>Very coarse</td>
</tr>
<tr>
<td>1</td>
<td>500</td>
<td>Coarse</td>
</tr>
<tr>
<td>2</td>
<td>250</td>
<td>Medium</td>
</tr>
<tr>
<td>3</td>
<td>125</td>
<td>Fine</td>
</tr>
<tr>
<td>4</td>
<td>63</td>
<td>Very fine</td>
</tr>
<tr>
<td>5</td>
<td>31</td>
<td>Very coarse</td>
</tr>
<tr>
<td>6</td>
<td>16</td>
<td>Coarse</td>
</tr>
<tr>
<td>7</td>
<td>8</td>
<td>Medium</td>
</tr>
<tr>
<td>8</td>
<td>4</td>
<td>Fine</td>
</tr>
<tr>
<td>9</td>
<td>2</td>
<td>Very fine</td>
</tr>
</tbody>
</table>

Sediment size categories were adopted from the GRADISTAT program (Table 1), originally modified from Udden (1914) and Wentworth (1922).
3.3 Data analysis

3.3.1 Sediment grain size analysis

The sediment grain size data were processed using the GRADISTAT software for sediment parameters (Blott and Pye, 2001). This program was chosen because of its suitability for analysing data obtained from sieving techniques and its capacity to analyse percentage data rapidly. The geometric Folk and Ward graphical (1957) method was chosen as the most appropriate statistical output for this study due to it being more likely to describe the general characteristics of the whole sample more accurately (Blott and Pye, 2001). The mean classification given by GRADISTAT was calculated considering all particle sizes of the sediment, not just the most abundant particle size in the sample. This mean was used in statistical analysis when determining the significance of size of sediment particles in species’ distribution.

Analysis of Variance (ANOVA) was used to determine differences in sediment type across transects. A Tukey’s post hoc comparison test was carried out when significant differences occurred. ANOVA was also used to test for differences in species’ abundances across the transects with a Tukey's comparison test used to highlight differences. Further multivariate analysis of the sediment data can be seen in Chapter 4.

3.3.2 Crab distribution

Crab distribution was analysed using generalised linear mixed models (GLMMs) in R. The fixed effects of ‘shore height’, ‘sediment type’, ‘shading’ and ‘presence of vegetation’ were tested against the dependent variable ‘crab species’, with random factors of ‘transect’ and ‘quadrat’. A Poisson distribution was used in the event of overdispersion. Alternative models were compared using the Akaike’s Information Criteria (AIC) which calculates the maximum log-likelihood of each model (Akaike, 1973). The ‘lmer’ function in the package ‘lme4’ was used to calculate the AIC’s. Crab sex ratios were tested for deviation from a 1:1 ratio with a Pearson Chi – Square test.
3.4 Results

3.4.1 Shore height

The shore height profile is presented in Figure 3.3, with transects indicated. Ambeua has a long, gradual slope up from the low tide line to the landward mangrove edge.

![Shore height profile Ambeua with green points (●) highlighting transects 6 and 7, red points (●) highlighting transects 1-5 and purple points (●) highlighting transects 8 and 9.](image)

**Figure 3.3.** Shore height profile Ambeua with green points [●] highlighting transects 6 and 7, red points [●] highlighting transects 1-5 and purple points [●] highlighting transects 8 and 9.

3.4.2 Sediment grain size analysis

The substrate in all quadrats from Transect 1 – 5 (T1 – T5) was predominantly very fine sand with varying degrees of sorting. Sediment properties varied significantly between some areas (ANOVA, p < 0.05, z = 3.01). Table 3.2 highlights these differences through a Tukey post hoc comparison test. The means represented in the table were calculated via the Folk and Ward method in the GRADISTAT programme – the larger the number the coarser the sediment. Of T1 – T5, only T5 had sediment particle sizes that were significantly different from the rest (with slightly finer sediment). The only transects where the sediment particle size was significantly different from all others were Transects 6 and 7 (T6 & T7) on the lower shore; both transects had, on average, coarser sediment.
Table 3.2. Analysis of variance (ANOVA) of sediment types across all transects; Means were calculated via the Folk and Ward method in the GRADISTAT programme. Grouping information using the Tukey method and 95% confidence, means that do not share a letter are significantly different (p ≤ 0.05).

<table>
<thead>
<tr>
<th>Site</th>
<th>F &amp; W Mean</th>
<th>Grouping</th>
</tr>
</thead>
<tbody>
<tr>
<td>T6</td>
<td>108.96</td>
<td>B</td>
</tr>
<tr>
<td>T7</td>
<td>101.17</td>
<td>B</td>
</tr>
<tr>
<td>T1</td>
<td>55.52</td>
<td>C</td>
</tr>
<tr>
<td>T4</td>
<td>54.45</td>
<td>C</td>
</tr>
<tr>
<td>T3</td>
<td>46.08</td>
<td>C D</td>
</tr>
<tr>
<td>T2</td>
<td>43.41</td>
<td>C D</td>
</tr>
<tr>
<td>T5</td>
<td>37.67</td>
<td>D</td>
</tr>
<tr>
<td>T8</td>
<td>36.46</td>
<td>D</td>
</tr>
<tr>
<td>T9</td>
<td>34.98</td>
<td>D</td>
</tr>
</tbody>
</table>

T1 – T5 showed no significant difference in the sediment particle size between the low (Q15 – 21), mid (Q8 – 14) and upper (Q 1 – 7) transect zones (p = 0.244). T1 – T5 differed significantly from that of the T6 – T9. Silt was the dominant sediment substrate on T8 & T9, with an average abundance greater than 52% in all samples. The average abundance of sand on Transects 8 and 9 (T8 & T9) was less than 40%. The silt fraction was lower across T1 – T5; its average abundance did not exceed 40%. The average abundance of sand was greater than 50% on T1 – T7. Further analysis of sediment data can be seen in the following chapter.

With respect to the water content in the sediment; samples from Ambeua were between 21.8% and 45.8% water, higher values were found in vegetated (shaded) areas on the upper zones of T1 – T5 (41.8 ± 3.73%) on the whole of transects T8 & T9 and in the areas under houses (38.5 ± 4.38%). Lower values were found on the open mudflat on the mid (31.6 ± 2.21%) and lower (29.5 ± 1.43%) zones of T1 – T5 and on the whole of transects T6 & T7. Higher water content values were associated with finer sediments and lower values were associated with coarser sediments.
3.4.3 Crab distribution

A total of 2270 fiddler crabs were identified across transects T1 – T5, with 53 T. coarctata (2.7%), 51 T. demani (2.9%), 79 T. dussumieri (3.5%), 271 P. crassipes (12.2%), 1254 G. jocelynæ (52.2%), 40 G. tetragonon (4.4%), 12 A. cryptica (0.5%), 10 A. mjoebergi (0.4%), 438 A. perplexa (18.1%) and 62 A. triangularis (3.2%), shown in Figure 3.4. There was a total of eight species observed on T1, eight species on T2, six species on T3, seven species on T4 and seven species on T5. There were seven and six species observed on the two Ambeua mangrove transects (T8 & T9), one species on the Ambeua lower shore transects (T6 & T7). The total abundance of crabs observed differed between transects, with a total of 350 crabs on T1, 535 on T2, 535 on T3, 296 on T4 and 554 on T5.

Distribution analysis by GLMM revealed that the mean abundance per quadrat of T. coarctata increased significantly with shore height (p < 0.05, z = 2.23), individuals were usually seen in the shade of the mangrove trees, however were also found lower on the transects on T5, where the shade is extended by the stilted houses of the village (Figure 3.4).

The mean abundance per quadrat of P. crassipes increased significantly with shore height (p < 0.05, z = 3.16) and with mangrove cover (p < 0.05, z = 5.67). Individuals were observed living in the shade of the mangrove trees, except on T5, where they were also recorded under the shade of the houses (Figure 3.4).

A. cryptica was only recorded on the upper shore of T2 and 3. The mean abundance per quadrat of A. cryptica increased with shore height (p < 0.05, z = 3.49). Individuals could often be seen living in the partial shade of the mangrove trees (Figure 3.4).

The mean abundance per quadrat of T. demani increased with shore height (p < 0.05, z = 3.03), with shade (p < 0.05, z = 3.21), with finer sediments (p < 0.05, z = 2.16). T. demani was found is the lowest quadrat (Q21) of T5, under the shade of a tree (Figure 3.4).

The mean abundance per quadrat of T. dussumierii increased with shore height (p < 0.05, z = 2.49) and with finer sediments (p < 0.05, z = 4.00) yet decreased with the
presence of mangrove cover (p < 0.05, z = -2.61). *T. dussumieri* was found is the lowest quadrat of T5 (as with *T. demani*, Figure 3.4).

*G. jocelynae* was recorded in all three shore zones of all five transects (Figure 3.4). The mean abundance per quadrat of *G. jocelynae* decreases significantly with shore height (p < 0.05, z = -2.35) and mangrove cover (p < 0.05, z = -4.31).

*A. mjoebergi* was only recorded on the upper shore zones of T2 – T4. The mean abundance per quadrat of *A. mjoebergi* increased with shore height (p < 0.05, z = 2.62) (Figure 3.4). The mean abundance per quadrat of *A. perplexa* increased with shore height (p < 0.05, z = 3.44) and mangrove cover (p < 0.05, z = 2.39). The mean abundance per quadrat of *G. tetracionon* also increased with shore height (p < 0.05, z = 2.70). The mean abundance per quadrat of *A. triangularis* increased significantly with mangrove cover (p < 0.05, z = 3.61). *A. triangularis* was only found in shaded areas, many of which were underneath the stilted houses (personal observation).

On T1 – T5, between 0 and 54 individual crabs (mean of 21.62 ± 1.21) were observed within each quadrat (at the time of sampling, either with a burrow or walking through), with a mean of 2.49 ± 0.16 species present per quadrat. 42% of quadrats contained three or more species, 27% of quadrats contained four or more species and 13% contained five or more species (Figure 3.4).

Between two and twelve individual crabs were observed within each quadrat of T6 & T7 (mean of 7.4 ± 1.07), with just one species present in each quadrat. Between zero and 38 individual crabs were observed within each quadrat of T8 & T9 (mean 11.5 ± 2.29), with a mean of 3.05 ± 0.27 species present per quadrat. Analysis of variance (ANOVA) showed that the total abundance of crabs (of all species) did not differ significantly on T1 – T5 (p = 0.534). However, the abundance of individual species across the transects did vary (p < 0.001). Tukey’s post-hoc test revealed three groups that were significantly different, with *G. jocelynae* in a distinct group with a significantly higher abundance than the other species.

All seven species that were present under the houses were also present in the mangrove at Ambeua, whilst the three species that were absent from the mangrove transects (*G. tetracionon*, *A. cryptica* and *A. mjoebergi*) were also absent from under the houses. Figure 3.4 shows the distribution of species along T1 – T5.
**Figure 3.4.** Distribution of fiddler crab species recorded at Ambeua, within each quadrat of the five Ambeua transects. Each square represents a quadrat with crab abundance represented by colour, the key showing that white squares = 0 crabs and red squares = 54 crabs (max. no found in a quadrat) and colours in between representing respective abundances.
The sex ratio of crabs varied between species at Ambeua (see Table 3.3). Most species had a sex ratio of 1:1, apart from *P. crassipes*, *A. cryptica* and *G. tetragonon* which had a higher ratio of males and only *A. triangularis* had a higher ratio of females to males.

**Table 3.3.** The sex ratios of the species recorded at Ambeua (T1 – T5), all ratios are males to females

<table>
<thead>
<tr>
<th>Species</th>
<th>Ambeua Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Tubuca coarctata</em> (n = 53)</td>
<td>1:1</td>
</tr>
<tr>
<td><em>Tubuca demani</em> (n = 51)</td>
<td>1:1</td>
</tr>
<tr>
<td><em>Tubuca dussumieri</em> (n = 79)</td>
<td>1:1</td>
</tr>
<tr>
<td><em>Paraleptuca crassipes</em> (n = 271)</td>
<td>1.5:1</td>
</tr>
<tr>
<td><em>Gelasimus jocelynae</em> (n = 1254)</td>
<td>1:1</td>
</tr>
<tr>
<td><em>Gelasimus tetragonon</em> (n = 40)</td>
<td>1.5:1</td>
</tr>
<tr>
<td><em>Austruca cryptica</em> (n = 12)</td>
<td>2:1</td>
</tr>
<tr>
<td><em>Austruca mjoebergi</em> (n = 10)</td>
<td>1:1</td>
</tr>
<tr>
<td><em>Austruca perplexa</em> (n = 438)</td>
<td>1:1</td>
</tr>
<tr>
<td><em>Austruca triangularis</em> (n = 62)</td>
<td>1:2.5</td>
</tr>
</tbody>
</table>

The Pearson Chi-Square test shows significant deviation from a 1:1 ratio for the whole data set (Chi-square = 19.507, DF = 7, p = 0.007), with *A. triangularis* (Chi-square = 10.0775) and *P. crassipes* (Chi-square = 6.1021) contributing to the Chi-Square value more than any of the other species. *A. cryptica* and *A. mjoebergi* were removed from the chi-squared test due to low sample sizes.

Equal samples of each species were not recorded as calculations were done using data from the transects at Ambeua. Sex ratios were explored, but the data was not collected for this purpose, however it pinpoints interesting further work to be done on fiddler crab sex ratios at this site.
3.5 Discussion
3.5.1 Crab Distribution

Ten species of fiddler crab were found on transects one to five, this is an increase from the four species found by Weis and Weis (2004) at Ambeua and the seven found by Barnes (2010). This could be due to further recruitment to the site or it could be a result of the greater area covered in this current study. *G. jocelynai* was by far the most abundant species and was found on all transects in all areas, showing that this species can live in a variety of habitats (a range of shore heights, sediment types and shaded/unshaded areas) and must therefore, be highly versatile/adaptable. On the other hand, *A. mjoeborgi* and *A. cryptica* occupy a small range on the shore and are found in low abundances suggesting they occupy more specific habitats, have recently recruited to the site, are susceptible to competition from other species or are (more) ‘sensitive’ to environmental variables. Few crabs are seen living below the mid-water line due to the reduced feeding time on the shore; *G. jocelynai* is the only species found here, which could be due to its apparent ability to feed rapidly (see *G. jocelynai* section below).

The distribution of each species of fiddler crab appears to be influenced by a variety of factors, some of which have likely not been covered in this study (see General Discussion, section 6.3). The vertical distribution via shore height analysis was investigated for each species, alongside substrate and shading preferences and each species does appear to inhabit a certain range for each of these factors (Table 3.4).

*G. jocelynai*

The only species present on all transects was *G. jocelynai*, which was most abundant at lower shore levels and in areas with least mangrove cover. They could often be seen feeding in groups by the low-tide line, this process is known as droving, a schooling behaviour observed when fiddler crabs are away from their burrows. This process was also seen in *G. vocans* (*G. jocelynai* was separated from *G. vocans* in 2010 (Shih *et al.*, 2010), where it was observed to be advantageous to move away from the burrow and forage in areas of higher organic content near the low tide level, where the number of feeding motions was seen to increase (Murai *et al.*, 1983). *G. jocelynai* was the only species observed on the lower shore, the
quadrats of which, on average, contained the lowest number of individual crabs. This suggests that when *G. jocelynae* lives monospecifically, each crab has a larger range, which could be due to reduced competition. *G. jocelynae* can also be found in high numbers on the mid and upper shore to around five metres into the mangrove. This result is consistent with those found by Weis and Weis (2004) at Ambeua, Lim *et al.* (2005) and Icely and Jones (1978).

**T. coarctata**

The distribution of *T. coarctata* was influenced by shore height, with none found on the mid and lower shore, except on Transect five under the houses. The abundance increased on the upper shore and in the mangrove and the species was often associated with shade, either in the form of buildings or mangrove trees. These findings were consistent with that of Shih *et al.* (2010) who found *T. coarctata* on the mid and upper shore. Shih *et al.* (2010) also found that *T. coarctata* was sympatric with *T. dussumieri* and *P. crassipes* which is consistent with the results of this study. *T. coarctata* has been observed living on the upper-shore and towards the landward zone of mangroves (Crane, 1975) close to *T. dussumieri*.

**T. demani**

The abundance of *T. demani* increased in shaded areas, both in the mangrove and under the houses. It also increased with shore height, which is associated with the presence of mangroves, except on Transects one and five, where it was found under the houses lower down the shore, suggesting that this species has a preference for shaded areas and may only appear at lower shore heights when shade is available. Previous studies showed similar distribution patterns for *T. demani* (Crane, 1975) which was observed living high up on the shore in the shade of mangrove trees, drainage ditches and even in the shade under stilted houses.

**T. dussumieri**

Associated with the mid-upper shore zones and can be found in the lower regions of the mangrove. Although statistically it decreases with the presence of mangrove trees it is found in the shade of the houses on Transects one and five. *T. dussumieri* has previously been observed living in mangrove areas (Frith & Brunenmeister, 1980).
and upper-shore mudflats on the edge of mangroves (Crane, 1975) often with *T. coarctata* and *T. demani* as sympatric associates.

**P. crassipes**
More abundant in the upper and mid shore and the mangrove, i.e. it was found more frequently on the finer substrate. It was often associated with shade, either in the form of buildings or mangrove trees which corresponds with observations made by Crane (1975) and by George and Jones (1982) who characteristically found *P. crassipes* living near to high tide levels on the fringe of mangrove estuaries.

**A. cryptica**
The abundance of *A. cryptica* increases with shore height; it was observed mostly in unshaded areas but was also seen under *Rhizophora* trees. *A. cryptica* is one of the species with the lowest abundance, with twelve individuals on the transects and only around thirty individuals across the whole of the Ambeua site. This population of *A. cryptica* is the first and currently the only known living population of this species (Michie et al 2015).

**A. mjoeberti**
Associated with the mid-upper shore and found in unshaded areas; this is similar to Booksmythe et al (2008) who found *A. mjoeberti* on the upper shore in Darwin, Australia, yet differs to findings by George and Jones (1982) who found *A. mjoeberti* on sandy mud on the landward edge of mangroves in Western Australia.

**A. perplexa**
The distribution of *A. perplexa* was also influenced by shore height, with more individuals found on the upper and mid shore than the lower shore. This result was consistent with observations reported by Crane (1975) who observed *A. perplexa* dominating the mid shore yet differed from that of George and Jones (1982) who found *A. perplexa* living on throughout the mangroves of Western Australia.

**A. triangularis**
*A. triangularis* is associated with shaded areas, living exclusively under the cover of either mangroves or houses. Personal observations showed that under one of the
houses on the lower shore of Ambeua (outside of the transect area), *A. triangularis* was the only species present. This could be due to the sediment under the house being much finer than that of the adjacent shore (at the same height above sea level). This data is consistent with that of Frith (1977), who found *A. triangularis* living exclusively on fine sediments in mangroves.

**G. tetragonon**

*G. tetragonon* is associated with both shaded and unshaded areas and was found at all shore heights, however its abundance does increase with shore height. Personal observations showed that *G. tetragonon* was associated with the presence of coral rock at Ambeua – crabs could often be seen feeding on small patches of algae on the surface of rocks and rubble. These findings correspond with the findings of Weis and Weis (2004) at Ambeua and Takeda *et al.*, (2004) on Phuket Island, Thailand, who found that *G. tetragonon* fed primarily on rocks covered by filamentous green algae. *G. tetragonon* was also found by Crane (1975) on open tidal flats, often on substrates with coral and shell, often covered in filamentous algae, mixed with muddy sand.
Table 3.4 The species recorded at Ambeua, their habitat type and the percent of the total abundance of each.

<table>
<thead>
<tr>
<th>Species</th>
<th>Shore Height Range</th>
<th>Mean Substrate Type</th>
<th>Shade</th>
<th>% of total crabs at Ambeua</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Tubuca coarctata</em></td>
<td>Mid – High</td>
<td>Silt</td>
<td>No Shade *</td>
<td>2.7</td>
</tr>
<tr>
<td><em>Tubuca demani</em></td>
<td>Mid – High</td>
<td>Sandy Silt – Silt</td>
<td>No Shade *</td>
<td>2.9</td>
</tr>
<tr>
<td><em>Tubuca dussumieri</em></td>
<td>Mid – High</td>
<td>Sandy Silt – Silt</td>
<td>No Shade *</td>
<td>3.5</td>
</tr>
<tr>
<td><em>Paraleptuca crassipes</em></td>
<td>High</td>
<td>Silt</td>
<td>No Shade *</td>
<td>12.2</td>
</tr>
<tr>
<td><em>Gelasimus jocelynae</em></td>
<td>Low – Mid</td>
<td>Sand – Silty Sand</td>
<td>No Shade *</td>
<td>52.2</td>
</tr>
<tr>
<td><em>Gelasimus tetragonon</em></td>
<td>Mid</td>
<td>Sand – Silty Sand</td>
<td>No Shade</td>
<td>4.4</td>
</tr>
<tr>
<td><em>Austruca cryptica</em></td>
<td>Mid</td>
<td>Silty Sand</td>
<td>No Shade *</td>
<td>0.5</td>
</tr>
<tr>
<td><em>Austruca mjoebergi</em></td>
<td>Mid</td>
<td>Silty Sand</td>
<td>No Shade *</td>
<td>0.4</td>
</tr>
<tr>
<td><em>Austruca perplexa</em></td>
<td>Mid</td>
<td>Silty Sand</td>
<td>No Shade *</td>
<td>18.1</td>
</tr>
<tr>
<td><em>Austruca triangularis</em></td>
<td>Mid - High</td>
<td>Silt</td>
<td>No Shade *</td>
<td>3.2</td>
</tr>
</tbody>
</table>
3.5.2 Sediment grain size analysis

When considering transects one to five, sediment particle size did not appear to affect the distribution of crabs, as there are very few differences between transects. When looking at the further regions of Ambeua (T6 – T9) the sediment type differed from the area of coexistence and fewer species were observed in each area. This suggests that, although these crabs can all exist in sympatry on a small patch of shore, there are potential limits to where they can live (further sediment analysis can be seen in Chapter 4).

Analysis of the sediment from transects one to five did not show any differences between shore heights and there were only minor differences between transects, meaning all species are coexisting on a similar sediment type. The distribution of crabs across the transects shows that although all species can occur in the same general area, there are, or may be, specific characteristics, of certain, (sub-) areas, which preclude some species. When considering other studies into the distribution of fiddler crabs (Crane 1975; Icely and Jones 1978; Lim 2005; Weis and Weis 2004), sediment particle size appears to be an important factor. The distribution in this investigation suggests sediment size could be having an effect; grain size is likely to affect distribution due to the morphology of the crabs’ mouthparts; this will, therefore, be investigated and considered in the following chapter. Sediment particle size generally changes with shore height (Bezerra, 2006; Lim & Kalpana, 2011) yet at Ambeua the houses alter the topography of the area which subsequently influences environmental factors such as the settlement of sediments, which is completely different under the houses than on the open mudflat, and shading.

The seven species that are found under the houses are also found in the mangrove (see section 3.4.3), meaning the presence of the houses may not be directly leading to an increase in the number of species. However, the presence of the houses increases the site heterogeneity which could therefore be reducing the competition for space in the mangrove. The houses are, in terms of height above sea level, lower on the shore than the mangroves, meaning the species living underneath them are occurring at lower shore heights than they might ‘normally’ occur. These anthropogenic factors are directly altering the ecosystem and are subsequently allowing crabs to dwell in places otherwise unoccupied by the species.
The water content of sediment samples differed: the upper shore and mangrove samples contained more water than the mid shore and lower shore which is likely due to the upper shore and mangrove being vegetated areas, these results are consistent with the findings of Bezerra et al (2006). To determine whether water content plays a role in fiddler crab distribution at Ambeua, further samples would need to be taken throughout the day to determine temporal fluctuations.

The majority of the species at Ambeua were found with 1:1 male to female ratios (T. coarctata, T. dussumieri, T. demani, G. jocelynae, and A. mjoebergi, A. perplexa), with P. crassipes and G. tetragonon and A. cryptica having ratios of more males to females. A. triangularis is the only species with a ratio of more females. Male-biased sex ratios are often common in fiddler crab populations (Crane 1975; Emmerson 1994; Frith & Brunenmeister 1980), but whether this is genetic, occurs during embryonic development, occurs subsequently or is site specific remains debated. Johnson (2003) found that male-biased sex ratios are likely a result of increased mortality rates in females, potentially due to the increased energy costs related to reproduction. The female-biased sex ratio of A. triangularis at Ambeua would be hard to understand without further investigation. It could be due to a very small sample size, or simply be due to the sampling area being where more females were living, or that more females have successfully recruited to Ambeua.

Although robust data could not be obtained, temperatures on the open mudflat often surpassed 37°C while they were almost 5°C lower in other areas where mangrove cover and housing provided shade (Michie, unpublished). Oxygen consumption measured at different water temperatures revealed that oxygen consumption of Uca maracoani increased up to a temperature of 35°C (Koch, 1999), while other species showed reduced oxygen consumption at temperatures above 32°C, suggesting physiological stress due to overheating. If crabs must retreat to their burrows to avoid heat-stress, feeding time is reduced and consequently these areas would not be a preferred habitat for these species. At the research site in this study, G. jocelynae, G. tetragonon, A. mjoebergi and A. perplexa are capable of coping with these conditions since they were observed inhabiting the open mudflat.

Crane (1975) observed that G. tetragonon proved is highly resistant to heat. Macnae (1967) recorded G. vocans (closely related to G. jocelynae) as tolerant of high
temperature. Species such as *A. triangularis*, *P. crassipes* and *T. coarctata* are usually found in shaded areas and an association with mangroves has been reported (Crane 1975; Macnae 1963, Weis and Weis 2004), although Crane (1975) indicates that, in the absence of mangrove cover, some species may live on the open shore. *G. jocelynae* and *G. tetragonon* are relatively similar in their habitat choice. The main factor separating these species is vertical zonation; *G. tetragonon* often had its distribution higher on the shore than *G. jocelynae*. *G. tetragonon* has been found associated with rocky areas and silt or sand overlying dead coral (Crane, 1975), whereas *G. jocelynae* has not been recorded in these areas.

Through studying the distribution of the fiddler crabs at Ambeua, it appears that each species can occupy a distinct niche, yet their habitats appear to overlap considerably. The overlap of their habitats has occurred due to the capability of all species to survive and persist in this specific area. For all species to succeed in this area, it requires a lack of interspecific competition and/or single-species dominance. Further work would be required to determine whether there is a lack of competition i.e. minimal negative / aggressive interactions between individuals of different species, despite their habitat overlaps and their spatial proximity to each other. To understand the relationships between environmental factors further, multivariate analysis needed to be done, this is reported in the following chapter. Thurman *et al* (2013) observed ten species of fiddler crab along an 8 to 10 m transect, which exceeded the biodiversity reported by Barnes (2010) at Ambeua. In this report, ten species were found at Ambeua; unfortunately, Thurman *et al* (2013) did not present detailed data regarding the distribution and environmental factors at the site in Brazil. These studies, alongside that of Crane (1975) in Panama, highlight the capacity of intertidal areas to support/maintain relatively large numbers of (closely-related) species.
Chapter 4

A comparative study of maxilliped adaptations of fiddler crab species in relation to habitat

4.1 Introduction

Fiddler crabs, being deposit feeders, meet their nutritional requirements by digesting organic matter removed from sediments. Most sediments consist largely of inorganic matter; even organically-rich sediments can be 95% inorganic matter, meaning fiddler crabs rely on a nutritionally-poor food source (Lopez and Levinton, 1987). Nevertheless, they survive and, characteristically, dominate temperate and tropical mudflats, often adjacent to mangroves. Most deposit feeders, due to this low-energy food-source, spend most their time feeding (Lopez and Levinton, 1987).

The diet of a fiddler crab consists mainly of detritus, bacteria and microalgae that are removed from the particles of sediment (Genoni, 1985). During feeding, fiddler crabs use the minor cheliped to transfer portions of sediment from the upper layers of the substrate to the buccal cavity, where food is removed by the mouthparts for ingestion. The mouthparts, especially the maxillipeds, bear a large number of setae, which are used to separate food from sediment by a scrubbing mechanism.

The mouthparts consist of a pair of mandibles, two pairs of maxillae and three pairs of maxillipeds, all ‘layered’ to form the buccal region. The outer mouthpart ‘layer’ is the pair of third maxillipeds (Figure 4.1c) which have a hardened chitinous ventral surface to protect the buccal cavity. The next ‘layer’ is the pair of second maxillipeds (Figure 4.1b) and then the first maxillipeds (Figure 4.1a). The dorsal surface of the second maxillipeds and the ventral side of the first maxillipeds are covered in setae; these surfaces face each other which enables the scrubbing of sediment particles for food. Through vibrating lateral sweeps, the second maxillipeds free particles from the first maxilliped, catching them between specialized setae including those with spooned-tips. During the sweeping process, the setae scrub the particles free of food material, which is caught in the setae of the first maxillipeds. From there the setae of the maxillae pass the material backward and it is drawn between the mandibles. This technique is aided via a flotation process, where less dense organic matter is
Food is further processed in the foregut of the digestive tract. The stomach contains a gastric mill capable of grinding ingested food into smaller particles. The gastric mill lines the inside of the muscular stomach wall and consists of rows of teeth, stiff bristles and setae. In the case of fiddler crabs, the shape of this gastric mill varies interspecifically, offering, amongst other things, a means of species differentiation.
Mocquard (1883) examined and described the foreguts of over 100 species of decapod and demonstrated that the foregut is built on a uniform plan and that the differences in form between species are the result of disappearance or fusion of ossicles.

Different species of fiddler crab are known to be specialised, morphologically and behaviourally, for handling particles of sediment of a certain size. The setae of the second maxillipeds have been described in various ways. The forms most often referred to are those described by Crane (1975); the ‘spoon-tipped’ setae (Figure 4.2b) having broadened tips that are cupped and arched inwards and ‘plumose’ setae (Figure 4.2a) that are feather-like in appearance. Crane (1941) stated that fiddler crab species that dwell in sandy habitats generally have abundant ‘spoon-tipped’ setae on the second maxillipeds, while those occurring in muddy habitats have more ‘plumose’ setae and less ‘spoon-tipped’ setae. The density and shape of these setae can differ depending on the preferred substrate (Crane, 1975). The number of ‘spoon-tipped’ setae is considered to be the most important factor when determining the sediment type that a species feeds on (Costa & Negreiros-Fransozo, 2001; Crane, 1975; Icely & Jones, 1978; Miller, 1961; Teal, 1958; Takeda et al., 2004).

Figure 4.2 Dorsal view of right second maxilliped; a, plumose setae of *P. crassipes*; b, spoon-tipped setae of *T. dussumieri*.
The mouthparts of three fiddler crab species (*Leptuca pugilator* Bosc, 1802, *Minuca pugnax* Smith, 1870, and *Minuca minax* LeConte, 1855) were studied in detail by Miller (1961). *L. pugilator* lived on sandy sediments, *M. pugnax* occurred in muddy regions, while *M. minax* preferred sandy and muddy habitats. For all three species, Miller (1961) reported several degrees of adaptation of the second maxilliped. *L. pugilator* had the highest total number of spoon-tipped setae, followed by *M. pugnax*, and, finally, *M. minax*. Miller did not include quantitative analysis of the second maxilliped in his study, but, since then, there have been numerous accounts with quantitative evidence to support Crane’s work (Ono, 1965; Salmon and Atsaides, 1968; Weissburg, 1991; Lim, 2004).

The relationship between feeding habit and morphological divergence of mouthparts has been studied (Icely & Jones, 1978; Lim, 2004; Ono, 1965), finding that the mouthparts of many fiddler crabs are specialised for a specific size-range of sediment particles. These adaptations appear to be partially responsible for the different distributions of species across different habitats. Icely & Jones (1978) studied four fiddler crab species co-existing in the mangrove fringes of Mida Creek, Kenya. They found that the four species (*Austruca lactea* De Haan, 1835, *Gelasimus vocans* Linnaeus, 1758, *Gelasimus tetragonon* Herbst, 1790 and *Paraleptuca chlorophthalmus* H. Milne Edwards, 1837) had only slight morphological differences between their mouthparts with the exception of the second maxilliped, which differed greatly. The differences in mouthpart structures seen between these four species appear to represent adaptations to the type of sediment upon which they feed: *A. lactea* had high numbers of spoon tipped setae on the second maxillipeds, whereas *G. tetragonon*, *G. vocans* and *P. chlorophthalmus* all have fewer spoon tipped setae which are associated with finer sediments.

When comparing the maxillipeds of males and females, both Weissburg (1991) and Lim (2004) counted numbers of spoon-tipped setae. Lim found that there was no significant difference in the total number of spoon-tipped setae of males and females, indicating no sexual dimorphism. However, Weissburg stated a difference of 10-50% more spoon-tipped setae in females than in males. These differences may be due to the disparity in the rates of feeding between the sexes - females feed faster than males, and therefore need increased numbers of setae to separate food from sediment...
particles efficiently (Weissburg, 1991). Although the results of Weissburg (1991) revealed that females had more setae per unit area of carapace width than males, his assessments were based on counts within randomly placed grids (10 × 20 μm and 20 × 20 μm) on different areas of the first and second maxillipeds, rather than total number of spoon-tipped setae.

If resource partitioning does occur at this site of sympatry, comparing the structure of feeding apparatus is one way to deduce whether species can live on different substrates (MacArthur & Levins, 1967). Differences in types and number of setae seem to imply a preference for a substrate type within a specific size interval of substrate-particles. Thurman (1982) found that both *Minuca longisignalis* Salmon & Atsaides, 1968 and *Minuca mordax* Smith, 1870, which inhabit fine sediments, have similar numbers of setae, with relatively few spoon-tipped setae and numerous ‘woolly’ hairs. Thurman also found that *Minuca rapax* Smith, 1870 and *Minuca pugnax* Smith, 1870, which inhabit muddy sands, have a greater relative abundance of spoon-tipped setae.

The distribution of males and females must be considered, due to the differences in feeding capacity - males must compensate for feeding at half the rate of females (Valiela *et al*., 1974). Males seem to compensate by extending the duration of feeding; Valiela *et al*., (1974) observed that, as high tide approaches, only males are still feeding. They also noted that the distribution of females was occasionally biased towards open areas; the need to expose eggs to a sufficient flow of well-oxygenated water can force ovigerous females to remain in relatively unprotected, open areas at high tide.

As well as the number of spoon-tipped setae, the shape of these hairs has also been studied (Colpo and Negreiros-Fransozo, 2013; Crane, 1975; Icely and Jones, 1978). In Crane’s (1975) extensive study, an interspecific difference was noted regarding the shape of the apical end of the spoon-tipped setae and illustrative examples were given. Icely and Jones (1978) studied the spoon-tipped setae of four species of fiddler crab to show how these structures may be related to the habitat occupied by each species. Colpo and Negreiros-Fransozo (2013) described and classified the setae on the dorsal surface of the second maxillipeds of nine species of fiddler crab, to determine whether there is a relationship between mouthpart morphology and
ecological distribution. In all three studies, it was concluded that morphological differences occurred between species and that this may influence ecological distribution.

The morphological characteristics of fiddler crab mouthparts and their relationship with the sediment that the crabs occupy is the focal point of this study. The relationship of the morphology of the mouthparts and the sediment upon which the crabs feed would result from the capacity of the mouthparts to be used to manipulate and sort sediment-grains of given sizes to remove algae and detritus for food. The second maxillipeds of fiddler crabs have specialized setae which vary in both number and shape among species (Crane, 1975; Colpo and Negreiros-Fransozo, 2013; Icely and Jones, 1978; Lim, 2004; Miller, 1961). Having more spoon-tipped setae is believed to enable the crabs to handle coarser sediments, whereas crabs with fewer of these specialised setae would be constrained to muddy sediments (Costa & Negreiros-Fransozo, 2001; Crane, 1975; Icely & Jones, 1978; Miller, 1961; Lim, 2004).

Studies by Lim (2004), Lim and Kalpana (2011) and Yamaguchi and Ogata (2000) showed that generally there is no intraspecific sexual dimorphism in the number of plumose setae and spoon-tipped setae for several species of fiddler crab. Due to this evidence, personal observations, and the fact that this study is concentrated on interspecific differences, males of each species were chosen for comparison in this investigation.

This chapter investigates the sediment characteristics that could influence the spatial distribution of ten species of fiddler crab (A. cryptica, A. mjoebergi, A. perplexa, A. triangularis, G. jocelynae, G. tetragonon, P. crassipes, T. coarctata, T. demani, T. dussumieri) found on a mudflat, at the fringe of a mangrove, on the Indonesian Island of Kaledupa. The morphology of the second maxilliped was studied considering the number of spoon-tipped and plumose setae to help explain the ecological distribution of the species.
**Aims:** The research presented here aims to compare the major difference of the second maxillipeds in the ten sympatric species, with a focus on setae shape and abundance. This work also aims to determine whether differences in mouthpart morphology are linked to species habitats, with a specific focus on the substrate on which the crabs are feeding.

### 4.2 Materials and Methods

#### 4.2.1 Sediment grain size analysis

See Chapter 3 (page 70) for method and data collection details.

A synopsis for results purposes: in the area of high coexistence, five 30 m long belt transects (T1 – T5) were established down the shore, from the mangrove fringe towards the low tide line. Two 60 m transects were also established in the mangrove (T8 & T9) and two 30 m transects on the lower shore (T6 & T7), with a quadrat sampled every 5 metres along each. One sediment sample was collected from the centre of every quadrat on all transects.

#### 4.2.2 Mouthparts

In August 2012, crabs were randomly collected from Ambeua shore, on the island of Kaledupa within the Wakatobi National Park, Indonesia. Specimens were first put into a cold environment to sedate them and then fixed and preserved in 75% ethanol. The crabs were brought back to the laboratory at the University of Portsmouth for analysis.

The carapace-width of each crab was measured with a pair of digital Vernier callipers and then all mouthparts were removed using a pair of forceps. For one specimen of each species, enumeration of all setae on the dorsal surface of both the left and right second maxilliped (Appendix, Table 1) indicated no difference (p > 0.05) between the two. Subsequently, the right second maxilliped of each specimen was chosen for this investigation, with ten males of each species being analysed. Studies of the mouthparts were facilitated by cleaning; each maxilliped was sonicated for 10-40 seconds, depending on size of sample and degree and tenacity of soiling. Samples were initially left in ethanol during the sonication process, yet this did not afford adequate results, so to aid in the cleaning process samples were transferred to a 2% Decon90 solution for the sonication process.
Dehydration was achieved through a graded ethanol series of 30%, 50%, 70%, 90% and absolute ethanol. Material was then transferred to 50% and then 100% acetone, each for half an hour. Material was covered with a small layer of 100% hexamethyldisilazane (HMDS) and placed in a fume-hood overnight to allow for infiltration then evaporation of the HMDS, leading to drying of the material without the damage associated with higher surface tension solvents (Schatten, 2011). Each maxilliped was then mounted onto carbon based, electronically conductive, adhesive discs, which had previously been placed onto aluminium stubs. Material was then sputter coated for observation in a JEOL 6060LV scanning electron microscope (SEM) at voltages of 15 kV using the electron imaging mode.

4.3 Data Analysis

A two-way non-parametric multivariate analysis of variance (PERMANOVA) was used to test for differences in sediment type across T1 – T5. Another PERMANOVA was performed to include sediment samples taken from the Ambeua mangrove (T8 & T9) and samples from the lower shore (T6 & T7). General Linear Models (GLM) were used to analyse the effects of multiple variables on the fiddler crab mouthparts. Individual GLMs were conducted for the responses ‘percentage of spoon-tipped (ST) setae’, ‘number of plumose (P) setae’, ‘number of ST setae’; all were modelled against ‘species’, ‘Carapace Width (CW)’ and ‘Species*CW’ with ‘CW’ as a covariate. The response ‘Number of ST setae’ with just Austruca perplexa, Austruca mjoebergi and Austruca cryptica (the three species with the highest number of spoon-tipped setae) was modelled against ‘Species’, ‘CW’ and ‘Species*CW’ with ‘CW’ as a covariate. An ANOVA using ‘number of ST setae’ versus ‘species’ was performed with a Tukey’s pairwise comparison test, to separate significant values into distinct subsets.

4.4 Results

4.4.1 Sediment grain size analysis

Ambeua has a gradual, shallow incline to the shore which causes a gradient of fine to coarse sediment from the landward fringe of the mangrove to the lower shore. Analysis of sediment particle-sizes shows that the sediment type is not consistent across the shore. In terms of the sediment samples at Ambeua, the majority (91.6% average) of each sample falls into the > 125 μm, 125 < > 63 μm and < 63 μm size
ranges. Figure 4.3 highlights the differences found, with T5 having significantly \((p = 0.025)\) more sediment in the \(<63\mu m\) range than T1 – T4. Vectors indicate direction of the parameter effect in the ordination plot; the six vectors indicated in the dbRDA plot show the sediment types related to the habitat. The vector for \(<63 \mu m\) is directed towards T5 on the Principle component analysis (PCO) plot (Figure 4.3), showing finer sediments in this area. Examination of the sediment particle size data for the quadrats of T5 shows that the finer sediment samples came from the quadrats underneath the houses.

![Figure 4.3](image)

**Figure 4.3** (a) Principle Component Analysis (PCO) plot of the distLM model, showing results from the multivariate permutational analysis (PERMANOVA) of differences in sediment particle size of transects 1 – 5. (a) The data are displayed across the two main principal coordinates (PCO 1 and 2). Each point represents a sediment sample and each colour represents a transect. Closer clustering between points indicates higher relative commonality with respect to sediment particle sizes (more particle sizes in common). Larger distances between points indicate lower relative commonality in sediment particle sizes. Including a vector (b) indicating the parameter effect in the ordination plot i.e. the sediment particle sizes associated with each area.
The sediment types between the main area of coexistence (T1 – T5), the mangrove (T8 - T9) and the lower shore (T6 & T7) were shown to be significantly different (Figure 4.4, PERMANOVA main test: \(F = 16.1, p = < 0.001\)). The vectors of Figure 4.4 represent sediment particle size and fiddler crab species. The vector for particle size range <63 µm is directed towards T8 & T9 indicating finer silty sediment. The 63 µm vector is directed towards the middle, indicating very fine sandy sediment on transects 1 - 5. All samples from T6 & T7 appear on the left side of the PCO plot, furthest from the samples from T8 & T9. The 125, 250, 5000 and 1000 µm vectors are directed towards T6 & T7, indicating fine to coarse sand in this area.

**Figure 4.4** (a) Principle Component Analysis (PCO) plot of the distLM model, showing results from the multivariate permutational analysis (PERMANOVA) of differences in sediment particle size of transects 1 – 5, 6 & 7 and 8 & 9. (a) The data are displayed across the two main principal coordinates (PCO 1 and 2). Each point represents a sediment sample and each colour represents a transect. Closer clustering between points indicate higher relative commonality with respect to sediment particle sizes (more particle sizes in common). Larger distances between points indicate lower relative commonality in sediment particle sizes. Including vectors (b, c) indicating the parameter effect in the ordination plot (b) the sediment particle sizes associated with each area, (c) the crabs associated with each area.
The lower shore of Ambeua (T6 & T7), which *G. jocelynae* alone inhabits, has an average of 60% fine - very fine sand (<125 µm), whereas, on T1 – T5, where all ten species are found coexisting, there is an average of 89% fine - very fine sand. In the mangrove, where *T. coarctata, P. crassipes, T. demani, T. dussumieri, G. jocelynae* and *A. triangularis* are found, there is an average of 95% fine – very fine sand.

*G. jocelynae* appears to dwell in a wide range of substrate types, from sediment dominated by fine sand to that consisting of very coarse grains. *G. jocelynae* is the only species that lives on the lower shore of Ambeua (Figures 4.3 & 4.4). *G. tetragonon* is often found living on medium- to coarse-grained sediment and at Ambeua it is often found feeding on algae-covered rocks and persistently defends these feeding ranges.

### 4.4.2 Mouthparts

The mean number of spoon-tipped setae is significantly different between the species (GLM, \(F = 7.55, \ DF = 9, \ p < 0.001\)) and between crabs of different sizes (tested with carapace width). The mean number of spoon-tipped setae does not differ significantly (GLM, \(F = 5.04, \ DF = 2, \ p < 0.005\)) between *A. perplexa, A. mjoebergi* and *A. cryptica* (the three species with the highest number of spoon-tipped setae) except when tested alongside carapace width (GLM, \(F = 103.96, \ DF = 1, \ p < 0.005\)). The mean number of plumose setae does not differ between species (GLM, \(F = 7.25, \ DF = 9, \ p = 0.151\)) unless tested alongside carapace width (GLM, \(F = 51.58, \ DF = 1, \ p < 0.001\)). This is noticeable in Figure 4.5, where the size of the bars and therefore the ‘no. of plumose setae’ do not appear to differ between species. The mean number of spoon-tipped setae as a percent of total setae is significantly different between species (GLM, \(F = 14.13, \ DF = 9, \ p < 0.001\)) but not between crabs of different sizes (GLM, \(F = 0.26, \ DF = 1, \ p = 0.613\)).
Table 4.1 The total number of setae (Total S), the mean number of plumose (Mean P) setae and spoon-tipped (Mean ST) found on the inner face of the second maxillipeds of ten species of fiddler crab, with the mean spoon-tipped setae as a percent of total setae (Mean %ST) and mean carapace width (CW) of species. n = 10 males of each species.

<table>
<thead>
<tr>
<th>Species</th>
<th>Total S</th>
<th>Mean P</th>
<th>Mean ST</th>
<th>Mean %ST</th>
<th>CW (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Tubuca coarctata</em></td>
<td>472.2 ± 21.8</td>
<td>393.7 ± 15.0</td>
<td>78.5 ± 6.8</td>
<td>16.5 ± 0.6</td>
<td>18.2 ± 0.9</td>
</tr>
<tr>
<td><em>Tubuca demani</em></td>
<td>482.0 ± 12.8</td>
<td>371.0 ± 11.3</td>
<td>111.0 ± 5.4</td>
<td>23.0 ± 0.9</td>
<td>22.2 ± 1.5</td>
</tr>
<tr>
<td><em>Tubuca dussumieri</em></td>
<td>525.7 ± 19.4</td>
<td>438.0 ± 10.9</td>
<td>87.7 ± 9.2</td>
<td>16.5 ± 1.2</td>
<td>25.0 ± 1.1</td>
</tr>
<tr>
<td><em>Paraleptuca crassipes</em></td>
<td>472.7 ± 22.0</td>
<td>393.0 ± 19.3</td>
<td>79.7 ± 2.8</td>
<td>16.9 ± 0.2</td>
<td>16.1 ± 0.1</td>
</tr>
<tr>
<td><em>Gelasimus jocelynae</em></td>
<td>554.2 ± 47.0</td>
<td>381.7 ± 37.0</td>
<td>172.5 ± 10.1</td>
<td>31.3 ± 0.8</td>
<td>15.3 ± 0.5</td>
</tr>
<tr>
<td><em>Gelasimus tetragonon</em></td>
<td>545.2 ± 21.3</td>
<td>403.0 ± 15.7</td>
<td>142.2 ± 6.0</td>
<td>26.0 ± 0.3</td>
<td>22.6 ± 0.4</td>
</tr>
<tr>
<td><em>Austruca cryptica</em></td>
<td>1404.0 ± 38.0</td>
<td>437.7 ± 17.3</td>
<td>966.2 ± 32.5</td>
<td>68.8 ± 1.0</td>
<td>13.8 ± 0.4</td>
</tr>
<tr>
<td><em>Austruca mjoebergi</em></td>
<td>1149.2 ± 76.1</td>
<td>378.7 ± 32.2</td>
<td>770.5 ± 44.2</td>
<td>67.1 ± 0.7</td>
<td>10.6 ± 0.2</td>
</tr>
<tr>
<td><em>Austruca perplexa</em></td>
<td>1071.7 ± 60.3</td>
<td>428.0 ± 23.2</td>
<td>643.7 ± 37.1</td>
<td>60.0 ± 0.1</td>
<td>15.4 ± 0.4</td>
</tr>
<tr>
<td><em>Austruca triangularis</em></td>
<td>421.7 ± 20.7</td>
<td>362.0 ± 16.8</td>
<td>59.7 ± 4.8</td>
<td>14.1 ± 0.6</td>
<td>12.6 ± 0.2</td>
</tr>
</tbody>
</table>
Figure 4.5 illustrates the number of spoon-tipped setae, number of plumose setae and carapace width for each species, supporting the statistics that the number of spoon-tipped setae differs significantly but is not a result of carapace width. Figure 4.6 illustrates the number of spoon-tipped setae standardised for carapace width, also supporting the statistics that the larger crabs do not necessarily have more spoon-tipped setae. The second maxilliped analysis showed that *T. coarctata*, *P. crassipes*, *T. demani*, *T. dussumieri*, *G. jocelynae*, *G. tetragonon* and *A. triangularis* have low numbers of spoon-tipped setae, while in *A. cryptica*, *A. mjoebergi* and *A. perplexa* they occur in much higher numbers.

**Figure 4.5** Spoon-tipped setae, plumose setae and carapace width (mm) for the ten species of fiddler crab at Ambeua. Setae numbers are the amount found on the inner face of the second maxilliped. Showing the results of an ANOVA for ‘No. of ST setae’ versus ‘species’, with a Tukey’s pairwise comparison test: means that do not share a letter are significantly different (p = 0.002). n = 10 males of each species.
Figure 4.6. The average number of spoon-tipped setae standardised to crab size (carapace width) per species.

Figure 4.7 shows SEM photographs of the whole dorsal surface of the second maxilliped for all ten species. All species have layers of feather-like plumose setae which occur along the carpus and merus - the ten species all have similar numbers of these plumose setae. All species have spoon-tipped setae on the merus and it is evident from these figures that the number of spoon-tipped setae differ substantially between species, which conforms to the statistical evidence.

Figure 4.8 shows SEM photographs of the dorsal surface at a magnification of x 90 for all ten species. On the SEM photographs, the setae of *A. cryptica, A. mjoebergi* and *A. perplexa* are angled inwards, which obscures the shape of the spoon tips, but does not affect counting the number of setae. The setae are not angled like this *in situ*, just when dehydrated and prepared for SEM imaging. *A. cryptica, A. mjoebergi* and *A. perplexa* have spoon-tipped setae covering the merus of the second maxilliped, whereas the other species have fewer setae with lower densities and only small portions of the merus possessing spoon-tipped setae.

The ‘spoon-tipped’ setae can differ between species; the shape of the spoon tips is apparent in Figure 4.8 and at higher magnification in Figure 4.9. The shaft of these setae is roughly cylindrical, towards the tip it becomes flattened and slightly concave with curved edges and lateral denticles, giving them their ‘spoon-tipped’ name. The
apical end of these spoon-tipped setae appears to differ between species. Some setae have a much more ‘cupped’ tip with more obvious denticles, whereas some are flatter and have less pronounced denticles. Some have a greater length than width, whereas those of *A. cryptica*, *T. demani*, *T. dussumieri* and *A. perplexa* are equal in length and width.

Following the descriptions of shape by Colpo and Negreiros-Fransozo (2013); *T. coarctata* has setae with a slightly curved tip, *P. crassipes*, *G. jocelynae*, *A. mjoebergi*, *G. tetragonon* and *A. triangularis* all have setae with a moderately curved tip and *A. cryptica*, *T. demani*, *T. dussumieri* and *A. perplexa* have setae with a strongly curved tip.
Figure 4.7 Variation between species in size and number of spoon-tipped setae of the second maxillipeds. Dorsal (inner) face of whole right second maxilliped; a, *T. coarctata*; b, *P. crassipes*; c, *A. cryptica*; d, *T. demani*; e, *T. dussumieri*; f, *G. jocelynae*; g, *A. mjoeberti*; h, *A. perplexa*; i, *G. tetragonon*; j, *A. triangularis*. 
Figure 4.8 Variation between species in size and structure of spoon-tipped setae of the second maxillipeds. Dorsal (inner) face of right second maxilliped; a, *T. coarctata*; b, *P. crassipes*; c, *A. cryptica*; d, *T. demani*; e, *T. dussumieri*; f, *G. jocelynae*; g, *A. mjoeborgi*; h, *A. perplexa*; i, *G. tetragonon*; j, *A. triangularis*. 
Figure 4.9 Variation between species in size and structure of spoon-tipped setae of the second maxillipeds. Dorsal view of the apical end of the spoon-tipped setae on the second maxilliped; a, Tubuca coarctata; b, Paraleptuca crassipes; c, Austruca cryptica; d, Tubuca demani; e, Tubuca dussumieri; f, Gelasimus jocelynae; g, Austruca mjoebergi; h, Austruca perplexa; i, Gelasimus tetragonon; j, Austruca triangularis.
4.5 Discussion

The results show that on Transect 5, where there are houses, the substrate is finer. These houses have provided additional shade on the shore and have most likely caused the progressive replacement of different sediment types; the areas under the housing comprise fine sediment and outside these areas the substrate is coarser. The houses are built on stilts and concrete blocks and it is likely this anchors the soil; creating a similar effect (on sediment partitioning and accumulation) to that of the roots of mangrove trees.

Other than the areas under the houses, the sediment samples on transects 1 – 5 are relatively consistent in their particle size distribution. In comparison, the sediment from Ambeua mangrove (T8 & T9) is finer and the sediment on the lower shore (T6 & T7) is coarser. The number of species living in each area differed, with the lower shore of Ambeua being home to only \textit{G. jocelynae}, the mangrove being home to six species (\textit{T. coarctata}, \textit{T. demani}, \textit{T. dussumieri}, \textit{P. crassipes}, \textit{G. jocelynae} and \textit{A. triangularis}) and transects 1 – 5 being home to all ten species.

The distribution of fiddler crabs has been investigated in relation to many environmental factors. The features of the substratum on which they live and feed has previously been determined as one of the most important factors that can influence their spatial distribution (Bezerra, 2006; Crane, 1975; Frusher \textit{et al.}, 1994; Icely & Jones, 1978; Macintosh, 1988). Just as previous studies have shown, the results of this investigation found that the features of the substratum are a factor in understanding the distribution of species, however some species appear to have the ability to feed on a wider range of substrate types than previously thought; this is discussed further in Chapter 6. One limitation of my study is that if differential feeding is occurring below the < 63 µm size range, this would not have been detected due to the sieve sizes available.

The variation in the number of plumose setae between species was shown to be significant, but only when species and carapace width were tested together. There was no difference in the number of plumose setae between species when carapace width was not accounted for. This lack of variation between the number of plumose setae for species feeding on distinct particle sizes, suggests that these setae are not
modified for feeding on the different sediment types. This type of setation has also been studied by Colpo & Negreiros-Franzozo (2013) and appears to be less adaptive for feeding capabilities than the specialised spoon-tipped setae; their study showed that there are normally five different types of papposera setae on the second maxillipeds, four of which are plumose and the fifth being the spoon-tipped setae. The spoon-tipped setae were the only ones which differed in number, size and shape between species.

The general expectation is that crabs inhabiting coarser sediment possess more spoon-tipped setae than those occurring in areas with finer sediments (Bezerra, 2006; Crane, 1975; Icely & Jones, 1978; Lim, 2004; Lim & Kalpana, 2011). Altevogt (1957) observed that the spoon-tipped hairs aided in the removal of coarse sediment particles from the buccal cavity, suggesting the reason for the relationship observed in the studies above. The results for some species in this study conform to this general expectation, yet for others, the results indicate that the substrate the crabs are feeding on, and the number of spoon-tipped setae they possess do not always correlate. Although most species live in areas where their mouthparts fit the expected substrate type, they are also observed living and feeding in areas outside of this.

It is evident from the present results that, for any given carapace size, *A. cryptica* has more spoon-tipped setae than any other species, closely followed by *A. perplexa* and *A. mjoebergi*; these species tend to inhabit areas with intermediate/coarse sediment and are rarely found living in areas with a fine-grained substrate. *A. triangularis, T. coarctata, T. demani, T. dussumieri and P. crassipes* have mouthparts associated with finer sediments and were found in these areas. *G. tetragonon* and *G. jocelynae* also have mouthparts associated with finer sediments but at Ambeua inhabit coarser sediments.

Throughout this study it has been shown that *G. jocelynae* can inhabit a wide range of substrates and shore heights but is almost always found in lower tidal areas of open shore where the substrate is coarser. Icely and Jones (1978) also found *Gelasimus vocans* (previous name of *G. jocelynae*) was most abundant on shores with the least mangrove cover. They found that *G. vocans* had a preference for the
lower-shore region and extended only as far up as the mid-shore in areas of high organic content.

Throughout this study *G. tetragonon* and *G. jocelynae* were found on similar parts of the shore at Ambeua. Icely & Jones (1978) also found that *G. tetragonon* showed a similar preference to *G. vocans* for the lower tidal areas of open shore with high densities occurring below the mangrove zone. They found no difference in the particle size and organic content of the substrata occupied by *G. tetragonon* and *G. vocans* with the former showing a marginal selection for finer sediments, with similar results being found in this study at Ambeua. It should also be noted that *G. tetragonon* feeds on algae from the surface of stones and rocks at Ambeua and actively defends these feeding zones; this may compensate for the fact that it is not living in an area generally associated with the morphology of its mouthparts Hartnoll (1975) also found that *G. tetragonon* was associated with stony areas and Crane (1975) found *G. tetragonon* on silt and sand overlying dead coral.

In this study, *P. crassipes*, *T. coarctata* and *A. triangularis* have been found occupying fine sediments and have only been observed living in shaded areas. There are areas at Ambeua where the sediment is fine, yet there is no shading and these three species have not been found. This indicates that these species need shade, most likely to avoid desiccation and/or overheating.

According to previous studies, the relationship between substratum and the number of spoon-tipped setae on the second maxillipeds is one of the main factors affecting distribution (Icely & Jones, 1978; Lim, 2004; Lim & Kalpana, 2011). In this study, although these factors do appear to contribute to crab distribution, the results indicate that the substrate the crabs are feeding on and the number of spoon-tipped setae they possess does not always correlate with the substratum. Although most species live in areas where their mouthparts fit the expected substrate type, they are also found living in areas outside of this. There are areas where species are living that might not be considered ‘normal’ for those species to inhabit. Whatever the reason for this - may it be an exclusion from other, more preferred areas or a selection for this non-ideal substratum - they can live in these areas sufficiently well to survive and persist. These results suggest that at this research site, and potentially elsewhere, the relationship between the number of spoon-tipped setae on the second maxilliped and
the ability to manipulate specific sediment types is not the main factor affecting the ecological distribution of fiddler crabs. However, the nutritional richness of sediments is an important factor to be investigated. Colpo & Negreiros-Fransozo (2011) found that crabs with varying degrees of mouthpart adaptations (from high to low numbers of spoon-tipped setae) could all manipulate the same sediment types. The same is occurring at Ambéua; in some 2 m² quadrats, up to seven species are found on the same substrate type.

The mouthparts of *A. perplexa* and *G. vomeris* were studied (Lim and Kalpana, 2011), finding that *A. perplexa* had significantly more spoon-tipped setae; in similar quantities to this current study. The mouthparts of four species of fiddler crab were studied on the Kenyan coast (Icely and Jones, 1978), finding that *G. tetragonon* had very few spoon-tipped setae; similar to the results of this study. It appears, that wherever a species is found geographically, the number of spoon-tipped setae does not vary, suggesting that this degree of adaptation is a genotypic trait. However, this would need to be studied more critically, to determine whether crabs’ mouthparts are a genetic trait rather than phenotypic.

Colpo & Negreiros-Fransozo (2011) found that the relationship between number of spoon-tipped setae and substratum was not the only factor affecting the distribution of fiddler crabs, yet particle size and organic matter content could, together, be deciding factors. The spoon-tipped setae on both the first and second maxillipeds scrape sediment grains to release the organic matter, detritus and microorganisms adhered to them (Crane, 1975; Miller, 1961). The number of these setae could affect the efficiency of scraping the sediment grains and consequently the ability of the crabs to obtain food (Icely & Jones, 1978; Miller, 1961). Species with more spoon-tipped setae can clean sediment grains more efficiently than species with fewer as the spoon-tipped setae can scrape sediment particles more efficiently than plumose setae (Dye & Lasiak, 1987; Takeda *et al.*, 2004). Finer sediments have a greater surface area for the attachment of food which would, other things being equal; make such sediments more nutritious than coarser sediments (Gray, 1974; Raffaelli & Hawkins, 1996). Therefore, species with fewer spoon-tipped setae are limited to feeding from fine, organically rich sediments (Robertson & Newell, 1982), whereas species with more spoon-tipped setae can obtain food from coarse, nutritionally poor
sediments. The crabs with more spoon-tipped setae are adapted to feeding from coarser sediments as a high number of spoon-tipped setae can help avoid the ingestion of larger particles that might damage the gastric mill (Icely & Jones, 1978; Miller, 1961). Species with fewer spoon tipped setae do not have this advantage which may be why they are not associated with coarser sediments. However, ingesting smaller particles is less likely to harm the gastric mill and could even be advantageous; the ingestion of smaller inorganic particles is unlikely to cause significant damage and the organic content adhering to it could help crabs obtain more food. (Colpo & Negreiros-Fransozo, 2011)

Colpo & Negreiros-Fransozo (2011) found, for all four species they studied, that the particle size composition of feeding pellets was, on average, larger than in the substrate the crabs were feeding from. It could be that the crabs find it easier to pick up larger particles, so the feeding pellets have a higher proportion of these particles than the substratum generally, or that during the sifting process larger particles may end up on the surface more so than smaller ones and therefore get picked up by the crabs. It could also be that certain crabs are consuming some amount of the (smaller particles of) sediment whilst extracting organic matter for food.

The process by which fiddler crabs’ separate food substances from the substratum was studied by Altevogt (1957). It was found that they draw up water from the gill chamber and use it to separate the less dense food particles from the denser inorganic grains; when the mouthparts are filled with water the less dense particles will float upwards and the denser ones can be removed through the opening at the bottom of the maxillipeds. The crabs at Ambeua that have fewer spoon-tipped setae yet feed on fine sediment could consequently be using excess water to aid in the removal of these larger particles. The shape of the spoon-tipped setae could also contribute to manipulating different particles sizes.

The apical end of the spoon-tipped setae appears to differ between species with no apparent relation to the sediment type that is fed upon. Colpo & Negreiros-Fransozo (2013) studied the form of the spoon-tipped setae in nine species of fiddler crab, concluding that there are interspecific differences in the shape of these setae and noting that further work must be done to improve the understanding of the actual
function of each variant of spoon-tipped seta and their capacity to scrape sediment-
particles. This current study also revealed interspecific differences in the shape of
spoon-tipped setae that do not correlate with the substrate in the same way as the
abundance of spoon-tipped setae correlates with substrate. These shapes and their
potential significance cannot be fully understood without further investigation into
actual feeding mechanisms.

For any mangrove that has an adjoining mudflat, each time the tide recedes, new
organic matter is drawn down from the mangrove and replenishes the food supply
for any inhabiting deposit feeders. Due to the high levels of human activity at
Ambeua and the waste being deposited into the mangrove, it is probable that the
organic content of the sediment is high, which is likely to contribute to the capacity
of the site to sustain this high number of crabs. Further work would need to be done
to establish the organic content of the substrate at Ambeua.

One of the basic requirements for survival is the ability of an organism to obtain
food from its environment, which is thus a factor governing the distribution of any
animal (Miller, 1961). This study, and many that have come before, have shown that
the ability to feed and the morphological adaptations to an environment are factors in
the distribution of fiddler crabs. The ten species present at Ambeua have been there
for at least four years (Michie, personal observations) and considering that the life
span of these crabs is estimated at two years, more than one generation of each
species must have recruited and survived here. Crane (1975) noted seeing sixteen
species of fiddler crab in a cove at the entrance to the Panama Canal, on the edge of
a sewer outlet which would likely contribute to a high organic content; a prime
example of how human activities are undoubtedly and strongly affecting the
distribution and existence of fiddler crabs. Crane’s observations and the findings
from this current study show that surprisingly, the result of human activities can
increase fiddler crab biodiversity.
Chapter 5

An investigation into the spatial distribution and home ranges of sympatric fiddler crabs.

5.1 Introduction

The pattern of distribution of a population of plants or animals is a fundamental characteristic of that population, yet it is an aspect that is extremely difficult to describe (Clark & Evans, 1954). The distributions displayed by populations in their natural environments could include countless patterns and can depend on a multitude of factors. Due to the fact that it is generally necessary to study organisms using samples rather than entire populations to assess distribution, estimates of population parameters can be biased or inaccurate (Clark & Evans, 1954). Aggregation patterns of organisms have often been calculated through a reciprocal of densities within quadrats (Iwao, 1972; McGregor, 2005), which can give inaccurate estimates of spatial distribution.

Fiddler crabs live around a central burrow and, from a human perspective, do not venture far from them. This makes them a suitable study organism for nearest neighbour analysis and the burrow gives a good indication of the ‘centre of origin’ of each crab territory (Crane, 1975). Fiddler crabs are governed by their intertidal existence; unlike many intertidal organisms, fiddler crabs are semi-terrestrial and are active at low tide, returning to their burrows for the high tide period. Their surface activities are confined to the low tide period which can change on a daily basis. Their lives are habitually centred around their burrows, which act as a refuge at high tide and during low tide provide shelter from desiccation and predation and are a source for water. Most individuals during low-tide periods defend a particular burrow, they feed in the surrounding area, excavate and maintain the burrow then retire into it as the tide rises, closing the burrow entrance with a plug of sediment (Bertness & Miller, 1984; Crane, 1975; Zeil & Hemmi, 2006). They defend these burrows against any intruders, both conspecifics and heterospecifics, with the mouth of the burrow being the centre of a small guarded territory (Zeil & Hemmi, 2006).
Burrows are the crabs’ mating grounds, with mating happening either on the surface or in the burrow itself. Male burrows are often the breeding spot for many species (Crane, 1975). Typically, females leave the protection of their own burrows and wander into the territories of courting males, ultimately choosing a mate. If a female is without a burrow and courts with a male it is likely that the male will give up its burrow for use by the female to incubate the eggs. Some species mate on the surface of the substrate (Crane, 1975, Christy, 1987) which has been observed at Ambeua in both *A. mjoeborgi*, *A. triangularis*, *A. perplexa*, *P. crassipes* and *G. jocelynae*.

When a male crab is ready for courtship he increases the defence of his territory, primarily through visual displays (Crane, 1975). Acoustic signals on the surface, as well as underground, can also play a part in territorial defence. During courtship, males will use their claw in a variety of movements and signals to attract a mate. The displays are usually species specific (Crane, 1975, Hyatt, 1977) and consist of movements of the major claw, often paired with movements of the minor claw, legs and body (Crane, 1975). These displays could serve the dual purpose of attracting a mate and repelling rivals (McGregor, 2005).

The spatial organization of individuals and the use they make of a particular area has been a principal area of study within ecology. When studying individual animals, a territory is the social and geographical area that an individual defends, with the concept being applied widely to both vertebrates and invertebrates (Ito, 1980). Two suggested, and still widely used definitions of a territory are "any defended area" (Noble, 1939) and "a fixed exclusive area with the presence of defence that keeps out rivals" (Brown & Orians, 1970), both which suggest that a territory appears to be an exclusive area which is inhabited and defended by an individual. The idea of a territory is, to some extent, inadequate for use in discussing fiddler crabs. Although fiddler crabs do defend the area in which they live, it would be rare to find a fiddler crab living in an ‘exclusive’ area, where there is no overlap with other individuals. A more accepted phrase is the ‘home range’, defined as the area inhabited and used by an organism during its normal activities, such as feeding, mating, and caring for young (Burt 1943). This phrase applies well to the basic state of fiddler crab communities, where in any one low-tide period, when crabs are on the sediment surface; most individuals spend their time feeding and do not necessarily exhibit any territorial behaviour.
The area in which a fiddler crab feeds is not necessarily the same area that it will defend, in other words its territory. The area that a crab defends will likely be smaller than the area where it feeds (its home range); this is due to the energy costs of defending even a small area around a burrow, so any territory is likely to be included within the home range (Yamaguchi & Tabata, 2004). For the sake of this project, the area under study will be the ‘home range’, this will be the crabs’ maximum foraging distance from its burrow, which will therefore include all the neighbouring crabs that the individual under study may encounter (Yamaguchi & Tabata, 2004). Determining the distance crabs are willing to move away from their burrows will give an indication of the size of their home range.

Combat between courting males can occur in any part of the home range, but will typically occur close to the burrow’s mouth, whilst encounters between neighbours often take place on the borders. Conspecific females are attracted to the defended area where, they are encouraged to mate by elaborate waving and acoustic signals (Christy, 1987; Crane, 1975; Salmo & Atsai, 2015). Adult females, especially when carrying eggs, have also been observed defending burrows on the surface by means of threat postures (Crane, 1975). The size of the defended ground around the burrow can vary widely depending on the species, the size of the individual and the abundance of crabs in the surrounding area.

5.1.1 Interindividual spacing

Fiddler crab populations are typically described as dense, but this human perception may be partly a product of our excellent visual ability and large size in relation to these small crabs (Pope, 2005). Population densities tend to vary both inter- and intraspesifically (Crane, 1975, Clark & Backwell, 2017) and larger crabs tend to be less densely populated than smaller crabs (de Rivera & Vehrencamp, 2001). The density of crabs in a given area can be used to estimate interindividual distances (McGregor, 2005), where a reciprocal of the density is used to yield the average distance between two individuals. This however does not consider spatial differences between individual crabs. Within the same quadrat at Ambeua, a variety of species, sexes and life stages of crab can be found so a more thorough methodology will be needed to assess the spatial distribution of crabs.
Animals alter their communication signals according to distance from conspecifics and intruders, amount of environmental noise and risk of predation (How et al., 2008). Fiddler crabs have been shown to modulate communication signals depending on the perceived distance of signal receivers (Crane, 1975; von Hagen, 1983). The distance between individual crabs will affect communication networks. Research has shown that males will increase the frequency of their waving when females approach, meaning they are able to judge the distance of conspecifics (Detto, 2007, How et al., 2007). This distance perception is also used in territorial defence since displaying males defend their territory more energetically as an intruding crab gets closer (Zucker, 1974).

Fiddler crab actions appear to act as communication networks but how these networks are affected by high alpha diversities has not yet been studied. This chapter looks to find out how high species diversity affects crab spatial distribution, species assemblages and crab home range size.

The size of individual home ranges can differ intra- and interspecifically (Clark & Backwell, 2017). When using a reciprocal of the study area, Leptuca beebei is found at high densities of, on average, 49 active individuals per 1 m² which calculates to a distance of 16 cm between individuals (Pope, 2005). The larger species Afruca tangeri is more widely spaced, at an average of 4.6 active individuals per 1 m², which calculates to a distance of 53 cm between individuals (Pope, 2005). These densities were estimated from areas of high crab activity, but crab distribution can be irregular, therefore they were regarded as ‘optimal conditions for communication networks in these species’. Other species in a similar size class as L. beebei were found at lower densities: L. terpsichores had an average of 17.5 individuals per 1 m², which calculates to a distance of 27 cm between individuals (Pope, 2005). When studying sympatric populations, due to interspecific size and behavioural differences, it is not appropriate to simply divide the study area by the number of individual crabs present. This will not give an accurate estimation of individual home range size, it could however, work with monospecific populations as home range sizes are less likely to differ intraspecifically.

Here, we assess species assemblages at a highly sympatric site to investigate population densities, crab home range sizes and interindividual distances. The aim is to answer the following questions: what happens when multiple species are living
sympatrically, what do species assemblages look like and do individuals have multiple species as neighbours?

What if established neighbours are heterospecific? Sympatric species with similar resource use often have separate ranges (Cody, 1969). Home ranges can include both conspecific and heterospecific neighbours and there may be both benefits and costs for individuals to live next to either (Fayed et al., 2008; Teal, 1958). One benefit of having a heterospecific neighbour is that they do not compete in mate attraction. Some individuals have been observed assisting smaller heterospecific neighbours in territorial defence against conspecifics (Clark and Backwell, 2017). However, heterospecifics can have detrimental impacts; for example, crabs may suffer a reduction in home range size if heterospecific neighbours are larger and more aggressive (Booksmythe et al., 2010).

The wandering of a crab depends on both the individual's courtship phase and on the richness of the substrate in providing food close to the burrow (Crane, 1975). If the substrate has a low organic matter content, it is likely that a crab will travel further from its burrow for nourishment. In the dotillid genus *Potamocypoda* (Tweedie, 1954; Crane, 1975), large burrows are occasionally communal. Individuals of this genus are smaller than most fiddler crabs and inhabit wet, muddy environments, which most likely have more abundant food supplies than the habitats of many fiddler crabs. Single occupancy of burrows in fiddler crabs very likely involves efficient distribution for undisturbed feeding over an adequate-sized patch of terrain.

In abundant populations, boundaries of individuals often overlap and home ranges and territories are smaller (Pope, 2005). Heterospecifics are often accepted within the home range, providing that the given intruder is not closely similar in size and shape to the burrow-holder. With regard to male home ranges, females and juveniles, whether wandering or feeding are often tolerated within the area, but when in close proximity, can be cautioned with mild threat postures or waves (Crane, 1975). Males will often utilise threat postures or waves much sooner if it is a male conspecific that enters the home range. With regard to female and juvenile home ranges, intruders are tolerated within the area, regardless of gender and low intensity threat motions are used only when intruders are within close range (Crane, 1975). Very young crabs rarely fight or defend a home range and many have been observed simply
submerging themselves in the substrate as the tide floods, rather than maintaining a burrow (Crane, 1975).

Adults females in some species, particularly those where mating takes place on the surface (like that of many in the Indo-Pacific), occupy burrows close to those of waving males (Christy, 1987; Crane, 1975; Salmon & Atsaises, 1968). Other females will move actively through the displacing part of the environment. Often in *Gelasimus vocans* and other species living on the lower parts of the shore, burrows can be changed freely between low and high tides of the same day, and territories may only be held for part of a single low-tide.

**Objectives:** In the present study, there are two main objectives. Firstly, to quantitatively assess the spatial distribution and the size of fiddler crab home ranges. Secondly, to determine if there are inter- and intraspecific differences in home ranges.

### 5.2 Methods

#### 5.2.1 Species assemblages

Counts of surface active individuals can underestimate actual population densities, whereas burrow counts tend to cause and overestimate. To overcome this problem and to attempt to get an accurate representation of density and species within an area, the following method was developed. In order to identify the burrows of individual crabs, ten quadrats across the five Ambeua transects were randomly selected. Coloured markers that represent the different species were made, each indicating the sex and life stage of the identified crab, markings were either MA (male adult), FA (female adult), MJ (male juvenile) or FJ (female juvenile) which were written on the markers in permanent pen. Each quadrat was drawn into the sediment (as in Chapter three) and crabs were identified ten minutes after marking the quadrats. Five crabs and their burrows were identified at a time and the corresponding markers were placed adjacent to (on the landward side) their burrow. Evidently, this process caused a disturbance and all the crabs retreated to their burrows. Once the crabs had re-emerged the process was repeated until all occupied burrows had been designated a marker. Measuring tapes were then placed along the perimeter of the quadrat to be
used as a reference in later analysis and photos of each quadrat were taken from a bird’s eye view (Figure 5.1).

![Figure 5.1](image)

**Figure 5.1** Quadrat example, showing counters next to each burrow containing a fiddler crab. Red dots represent individuals of *G. jocelynae* and green dots represent individuals of *A. perplexa*. The apparent segregation of the species shown here is not typical at Ambeau.

To determine the variation of foraging distance within a species and per gender, thirty male adults and thirty female adults of *A. perplexa* were monitored for foraging distance. Initial observations revealed that few crabs took longer than thirty minutes to reach their maximum foraging distance. Estimates were made from a distance, to avoid disturbing crabs. Estimation accuracy was tested by first marking the furthest point that a crab would forage from its burrow (after ten, twenty and thirty minutes), writing down an estimation of distance and then measuring to check precision. Foraging distance was measured at different times to determine the minimum time frame for the crab to travel its maximum distance from the burrow. For males and females, only 30% reached the maximum distance after ten minutes, 95% after twenty minutes and 98% after thirty minutes. Consequently, for this study, maximum foraging distance was classed as the maximum observed distance travelled by a crab, from its burrow in twenty minutes.
Due to low abundances of several species, twenty individuals (five male adults, female adults, male juveniles and female juveniles) of each species were chosen to determine foraging distances. Also, due to low abundances of species, some foraging distances were assessed outside of the transect areas. The twenty crabs of each species were chosen from the quadrats assessed for species assemblages and the foraging distances were measured for each individual crab over the space of twenty minutes. It should be noted that crabs were chosen whose entire maximum foraging distance was within the quadrat area (used as a radius distance encircling the burrow) to allow for analysis to take place.

5.2.2 Image Analysis

The quadrat photos were uploaded into ImageJ software. Photos were edited to highlight each marker (Figure 5.1). After working out the home range diameter for each individual studied, ImageJ software was used to analyse the photos by setting a scale and creating a circle around the burrow that had the correct diameter of its home range (Figure 5.2). This circle gave the estimated area of each individual’s home range. A circle was chosen so as to incorporate the maximum foraging distance from the burrow in any direction; this would then include all possible neighbouring crabs. Crabs do not always forage on all sides of their burrows but analysing the whole area will allow for comparisons to be made. All crabs within each home range and the distance from the resident’s burrow were recorded, along with the species, gender and life stage of each crab.

5.3 Data analysis

For the home range data set, residual plots were analysed, and diagnostics indicated that untransformed data was not normally distributed when examined in groups of adults and juveniles. A Box-Cox plot, showing the residual plots, allowed data to be analysed for the best transformation. The output is a value of lambda: 1 indicating no transformation, 0.5 indicating square root, 0 indicating log and minus one indicating a reciprocal. The Box-Cox output gave a value of 0.27 and so this option was used to calculate the transformed data.

Regression analysis was used to estimate relationships among crab size, home range size and the number of other crabs living within the home ranges. Analysis of Variance (ANOVA) was used to determine differences in home range sizes between
species, sexes and life stages. ANOVA was also used to test for differences in nearest neighbours to investigated crabs.

![Figure 5.2 Burrow of A. perplexa male adult, represented by central green dot, with estimated home range, represented by black circle. Red dots represent the burrows of G. jocelynae, yellow dot represents the burrow of T. demani.](image)

5.4 Results

5.4.1 Home range size

In all species, carapace width was larger in males compared to females, for both adults and juveniles (Figure 5.3). *T. dussumieri* was the largest species, followed (in descending order) by: *T. demani, G. tetragonon, T. coarctata, P. crassipes, G. jocelynae, A. perplexa, A. cryptica, A. triangularis* and *A. mjoebergi.*
The mean carapace width (mm) for female adults (n = 5), male adults (n = 5), female juveniles (n = 5) and male juveniles (n = 5) of each species.

For males and females, and adults and juveniles of all species, the size of the home range increased with body size, with the correlation being significant (Figure 5.4, regression analysis: Home range area (m²) versus CW (mm), $F = 101.09$, DF = 1, $p < 0.0001$), yet it was a low correlation coefficient ($r^2 = 0.34$).

The correlation between carapace width (mm) and home range area (m²), with an $r$-squared value of 0.34.
Home range size significantly differs between species (one-way ANOVA, $F = 7.60$, $DF = 9$, $p < 0.0001$), with the home range size increasing with carapace width, yet the largest crabs did not always have the largest home ranges (Table 5.1). On average, $T.\ dussumieri$ had the largest home range and $A.\ triangularis$ had the smallest. Even though $A.\ perplexa$ was, on average, one of the smaller species, it had, on average, the second largest home range. Just as $T.\ coarctata$ was the third largest species and had one of the smaller home ranges (Table 5.1). The Tukey’s pairwise comparison shows that $T.\ dussumieri$ and $A.\ perplexa$ are significantly different from the home range sizes of the other species, and $A.\ perplexa$ and $G.\ tetragonon$ are also significantly different from the other species.

**Table 5.1** Mean home range size (m²) and mean carapace width (mm) of each species. $n = 20$.

<table>
<thead>
<tr>
<th>Species</th>
<th>Mean Home Range Size (m²)</th>
<th>Mean Carapace Width (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$T.\ dussumieri$</td>
<td>0.222</td>
<td>18.45</td>
</tr>
<tr>
<td>$A.\ perplexa$</td>
<td>0.205</td>
<td>10.41</td>
</tr>
<tr>
<td>$G.\ tetragonon$</td>
<td>0.111</td>
<td>18.32</td>
</tr>
<tr>
<td>$G.\ jocelynae$</td>
<td>0.096</td>
<td>10.78</td>
</tr>
<tr>
<td>$T.\ demani$</td>
<td>0.095</td>
<td>15.93</td>
</tr>
<tr>
<td>$A.\ cryptica$</td>
<td>0.075</td>
<td>10.61</td>
</tr>
<tr>
<td>$A.\ mjoebergi$</td>
<td>0.065</td>
<td>8.59</td>
</tr>
<tr>
<td>$T.\ coarctata$</td>
<td>0.065</td>
<td>12.90</td>
</tr>
<tr>
<td>$P.\ crassipes$</td>
<td>0.051</td>
<td>10.90</td>
</tr>
<tr>
<td>$A.\ triangularis$</td>
<td>0.042</td>
<td>8.77</td>
</tr>
</tbody>
</table>

Home range size differed significantly between the adults of all species (One-way ANOVA: Av. home range size (m²) versus Species – Adults, $F = 4.02$, $DF = 9$, $p = 0.038$). The home range size also differed significantly between juveniles of all species (One-way ANOVA: Av. Home range size (m²) versus Species – Juveniles, $F = 6.34$, $DF = 9$, $p = 0.004$). Home range size did not differ significantly between males and females of adult crabs in any species (One-way ANOVA: Av. Home range size (m²) versus F/M Adults, $p = 0.153$), neither did it differ significantly between males and females of juvenile crabs in any species (One-way ANOVA: Av. Home range size (m²) versus F/M Juveniles, $p = 0.784$). Home range size was
significantly larger in adults than juveniles in all species (One-way ANOVA: Av. Home range size (m²) versus Adult/Juvenile, p < 0.001). Because home range size was significantly larger in adults than juveniles, this appeared to be correlated with crab size, the data were further analysed by a one-way ANCOVA, using size as a covariate, showing that, even when tested against carapace width, home range size is significantly larger in adults than juveniles (One-way ANOVA: Av. Home range size (m²) versus Adult/Juvenile with CW as covariate, F = 4.02, DF = 1, p = 0.009).

For all species, the number of crabs that had burrows within a home range increased with the size of the home range (Figure 5.5), regression analysis: the number of crabs living within an individual home range was tested against the home range area (m²) (p < 0.0001). For the individuals of all species, there is no linear relationship between the number of crabs in the surrounding quadrat and the number of crabs in the home range (Regression Analysis: No. of crabs in home range versus No. of crabs in quadrat, F = 2.06, DF = 1, p = 0.153).

For an individual crab, the number of other crabs, of any species, living within its home range was measured. This was done for twenty individuals of all ten species. This number of crabs living within the home range significantly varied between species (One-way ANOVA: No. of crabs in home range versus Species, p < 0.0001). *A. perplexa* had the highest number of crabs living within the home range (Table

![Figure 5.5](image-url) Graph showing the correlation between home range area and the number of crabs within it, with an r-squared value of 0.55
having, on average, double or more, the number of crabs living in the home range of all other species. *P. crassipes* had the lowest number of crabs living within the home range (Table 5.2).

<table>
<thead>
<tr>
<th>Species</th>
<th>Mean no. of crabs in home range</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>A. perplexa</em></td>
<td>6.20</td>
</tr>
<tr>
<td><em>T. dussumieri</em></td>
<td>3.75</td>
</tr>
<tr>
<td><em>G. tetragonon</em></td>
<td>3.40</td>
</tr>
<tr>
<td><em>G. jocelynae</em></td>
<td>2.40</td>
</tr>
<tr>
<td><em>A. mjoebergi</em></td>
<td>2.35</td>
</tr>
<tr>
<td><em>T. coarctata</em></td>
<td>1.90</td>
</tr>
<tr>
<td><em>A. triangularis</em></td>
<td>1.85</td>
</tr>
<tr>
<td><em>T. demani</em></td>
<td>1.70</td>
</tr>
<tr>
<td><em>A. cryptica</em></td>
<td>1.58</td>
</tr>
<tr>
<td><em>P. crassipes</em></td>
<td>1.00</td>
</tr>
</tbody>
</table>

There was no significant difference in the number of crabs within the home range between males and females of all species (One-way ANOVA: No. of crabs in area versus Sex, *p* = 0.096). There were significantly more crabs living in the home ranges of adult crabs compared to juveniles (One-way ANOVA: No. of crabs in area versus Adult/Juvenile, *p* < 0.001), this corresponds with the fact that the home range size of adults was larger than juveniles, which is likely due to adult crabs naturally being larger than juveniles.

### 5.4.2 Nearest neighbour analysis

In terms of the nearest neighbours that were conspecifics, the percentages for each species were as follows: *A. perplexa* 90%, *G. jocelynae* 87.5%, *A. triangularis* 81.25%, *P. crassipes* 71.43%, *A. cryptica* 58.83%, *G. tetragonon* 52.64%, *T. dussumieri* 47.37%, *T. coarctata* 43.75%, *T. demani* 41.18% and *A. mjoebergi* 36.84%. None of the species had all nearest neighbours as conspecifics and only six species had more than half of the nearest neighbours as conspecifics. Eight of the species investigated had three or more species as nearest neighbours (Figure 5.6) *T.*
coarctata, T. demani, T. dussumieri and A. mjoebegi all had five or more different species as nearest neighbours (Figure 5.6).

![Figure 5.6](image_url)

**Figure 5.6** Observed species of nearest neighbour for the ten species of fiddler crab at Ambeua. The species of the nearest neighbour for each species of crab at Ambeua, shown as a percent of the total for all individuals of each species.

The average percentage of crabs within each home range that were conspecifics were as follows for each species: *A. perplexa* 87.10%, *A. triangularis* 86.49%, *G. jocelynae* 83.33%, *P. crassipes* 55.00%, *A. cryptica* 42.42%, *T. coarctata* 39.47%, *G. tetragonon* 30.88%, *A. mjoebegi* 23.40%, *T. dussumieri* 22.08%, *T. demani* 20.00%.

When studying male to female ratios of nearest neighbours (Figure 5.7), there was no significant difference between species (One-way ANOVA: Sex of nearest neighbour versus Sex p = 0.198). Although there was no significant difference, there were slightly more male crabs living closest to other males (60%), than female crabs living closest to other females (51%). When comparing all species, it is evident there is no consistent pattern of males living next to males and females next to females. Yet for some species the ratios are quite high. For individuals of *P. crassipes*, 85%
of males live nearest to another male (not necessarily of the same species). In terms of nearest neighbours to female crabs, for individuals of *A. triangularis*, 85% of females live closest to male crabs (again, not necessarily the same species). In males and females of *T. coarctata*, the two sexes lived next to equal numbers of both sexes. For individuals of *T. dussumieri*, only 20% of females lived next to other females.

When studying male to female ratios of nearest neighbours in relation to life stage, there was no significant difference between adults and juveniles (One-way ANOVA: Sex of nearest neighbour versus Life Stage \( p = 0.092 \)). In both female adults and female juveniles, 50% lived closest to females. Whereas, in male adults and male juveniles, 52% of male adults lived next to males, and 70% of male juveniles lived next to males. When studying adult to juvenile ratios of nearest neighbours, there was a significant difference between the life stages (One-way ANOVA: Life stage of nearest neighbour versus Adult/Juvenile, \( p = 0.046 \)), with 60% of adults living next to adults and only 31% of juveniles living next to juveniles.
Figure 5.7 Percentages of nearest neighbours shown in terms of whether they were male or female; a) females of each species, b) males of each species

The distance of the nearest neighbour to the investigated crabs differs significantly between species (One-way ANOVA: Distance Away (m) of nearest neighbour versus Species, p = 0.001), with individuals of *G. tetragonon*, on average, living furthest from their nearest neighbours that any other species (Table 5.3) and *A. triangularis* living closest.
Table 5.3 Mean distance of the nearest neighbour in the home range of each species. (n = 20)

<table>
<thead>
<tr>
<th>Species</th>
<th>Mean distance of nearest neighbour (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>G. tetragonon</em></td>
<td>10.48</td>
</tr>
<tr>
<td><em>T. demani</em></td>
<td>9.48</td>
</tr>
<tr>
<td><em>T. dussumieri</em></td>
<td>9.34</td>
</tr>
<tr>
<td><em>A. perplexa</em></td>
<td>8.98</td>
</tr>
<tr>
<td><em>A. cryptica</em></td>
<td>8.70</td>
</tr>
<tr>
<td><em>G. jocelynae</em></td>
<td>8.14</td>
</tr>
<tr>
<td><em>A. mjoebergi</em></td>
<td>8.11</td>
</tr>
<tr>
<td><em>T. coarctata</em></td>
<td>6.70</td>
</tr>
<tr>
<td><em>P. crassipes</em></td>
<td>5.88</td>
</tr>
<tr>
<td><em>A. triangularis</em></td>
<td>4.85</td>
</tr>
</tbody>
</table>

5.5 Discussion

Fiddler crab distribution can be complex. In some locations, species rarely overlap in distribution as each species is adapted to a particular habitat (Teal, 1958; Nobbs, 2003). In other locations, many species can coexist at one time including the species in this study (Barnes, 2010; Icely & Jones, 1978; Crane, 1975; Lim, 2005). The overlap currently observed provided an ideal opportunity to study interspecific interactions.

5.5.1 Quantitative Assessment of Home Ranges

It is not surprising that home range size increased with size of crab, the area around each burrow provides the crab with food, hence the larger sizes of home range in larger crabs could be explained due to food requirements. Furthermore, the radius of the home range is several times wider than the crab itself and the size of the defended area can depend on the maximum size of the display stance (Crane, 1975). Other than *A. perplexa* and *G. jocelynae*, *G. tetragonon*, *T. demani*, and *T. dussumieri* had the largest home range sizes, which is, as stated above, likely to occur because these species are largest at Ambeua. Although *P. crassipes* and *T. coarctata* had two of the smallest home range sizes, they are not two of the smallest species, this likely occurs because they live in mangrove areas, where organic content is often higher and consequently they can probably gain sufficient food from
a smaller area. It would be very interesting to know how all these parameters change when species live monospecifically.

The fact that *A. perplexa* was one of the smaller species but had the second largest home range could be due to the fact that *A. perplexa* live on intermediate sediments (see Chapter 3, section 3.5.1), which usually have a lower organic matter content, consequently crabs may have to travel further than other species to obtain enough food. *A. perplexa* was the second most abundant species on the shore (see Chapter 3, section 3.5.1), therefore with more individuals of the same species, it is likely the density of crabs is higher. With a higher density of crabs in the surrounding area, individuals may have to travel further to gather food if overlaps between home ranges are common. Furthermore, *A. perplexa* is, by far, the most active crab at Ambeua in terms of signalling. Most of the other species of crab at Ambeua have only been observed signalling when another crab is within the immediate vicinity or during courtship, whereas *A. perplexa* often exhibited additional low intensity waves during feeding, which could be due to high densities of individuals.

Although *G. jocelynae* is the most abundant species on the shore, unlike *A. perplexa*, they were observed to have the fourth largest home range size, yet fewer crabs living within their home range. Due to the fact that *G. jocelynae* is the only species to inhabit the lower shore, the individuals can live further apart from one another in these areas. *G. jocelynae* is able to live in a variety of habitats and when living on the upper shore, where more species are present, preliminary observations show that the distance to nearest neighbour is reduced. Further investigation would have to be done to quantify these observations, but it is thought that when densities and competition from other species is high, the home range of *G. jocelynae* is reduced, yet when it lives monospecifically, the home range size increases.

### 5.5.2 Individual differences in home range

The crabs appear to live in dense populations of, mixed-sex, mixed-age and mixed-species colonies, with the active space of nearly all studied individuals including multiple other crabs. The home ranges of all species studied, contained both conspecific and heterospecific neighbours, both of which can come with benefits and costs for individuals to live next to (Fayed et al., 2008; Teal, 1958). One benefit of
having a heterospecific neighbour is that they do not compete in mate attraction, if heterospecifics are smaller, it is likely that they will also compete less for space. However, having a heterospecific neighbour can be costly, crabs may suffer a reduction in home range size if heterospecific neighbours are larger (Booksmythe et al., 2010).

The data gathered here shows that residents of *A. perplexa*, *A. triangularis* and *G. jocelynae* live near to conspecifics more than any other species. *A. perplexa* and *G. jocelynae* are the most abundant species at Ambeua; due to these high densities it is likely that a neighbour will be a conspecific. The distribution of *A. triangularis* could be explained by the specific habitat of this species. *A. triangularis* lives in shaded areas with fine sediment and this species has one of the lowest abundances at Ambeua, therefore with a niche habitat and low numbers, there crabs are likely to live near to one another. For courtship reasons alone, it would be beneficial for individuals of *A. triangularis* to live adjacent to one another as finding a mate would be difficult in a wide area with multiple other species present.

*T. coarctata*, *P. crassipes* and *A. triangularis* had the lowest distances to nearest neighbours, this could be due to the fact that these crabs live in areas with fine sediments, which usually have a higher organic content, therefore crabs can likely obtain sufficient food from a small area and therefore may live closer together. *G. tetragonon*, *T. demani* and *T. dussumieri* had the highest distances to nearest neighbours, which is likely due to the fact that these species are the largest at Ambeua and have larger home range sizes to cope with the need to obtain food. Additionally, being larger species, they are likely able to negotiate larger home range boundaries with smaller neighbours (Clark & Backwell, 2017).

After territorial boundaries have been set, aggression between neighbours reduces (Fayed et al., 2008). In a study looking at *A. mjoebergi* and *Tubuca elegans*, there was a greater distance between burrows of heterospecific neighbours compared with conspecific neighbours, which was related to the sizes of both individuals. *T. elegans* was the larger species and occupied larger areas than *A. mjoebergi* (Clark & Backwell, 2017), therefore it is likely that territorial boundaries were set and maintained by individuals of *T. elegans.*
Juveniles were found to have an adult as a nearest neighbour more often than a fellow juvenile, this potentially occurs simply because there are more adult crabs on the shore. It could also be because having a larger neighbour can often be beneficial to smaller crabs. Crabs have often been observed defending smaller neighbours, which is thought to be because having a smaller neighbour, and retaining them, means more space and no need for renegotiation of home range boundaries with potentially larger new neighbours (Backwell & Jennions, 2004; Clark & Backwell, 2017).

The spatial variables were different for adult and juvenile crabs, indicating that the distribution of adult crabs may be caused by mechanisms affecting the adult crabs themselves. Further work will need to be done into the requirements of juvenile fiddler crabs and how this changes over time. Requirements such as space and sediment type will likely change over time and will undoubtedly affect their choice of habitat and the size of their home range. When tested with size as a covariate, adult crabs still had significantly larger home ranges than juveniles, which could be due to many factors, such as differences in feeding habits and territorial and mating behaviours between adults and juveniles. Juveniles did not exhibit mating behaviours and rarely exhibited defensive waving (personal observations). Following settlement, juvenile crabs may alter their habitat according to their changing habitat requirements as they grow. For example, juvenile *Leptuca pugilator* feed on muddy substrates and lack the specialized mouthpart structures needed to feed efficiently on sandy sediments like adult crabs (O’connor, 1990).

Males were more often seen living next to other males than next to females, which there could be multiple reasons for. Females often live in all female groups (Crane, 1975; Pope, 2005) and subsequently wander to male areas for courtship. Males may live alongside other males to entice more females into the area – females would have more choice of mate with more males present, which could drive higher densities of male crabs (Pope, 2005; Salmon & Atsaides, 1968).

Further work would include studies into territory size as well as home range. Males and females of *Ilyoplax pusilla* were shown to have a similar home range size, yet males had larger territory sizes, such spatial property of territory is considered to be associated with courtship (Wada, 1993). The impact of home range sizes on
individuals of each species needs further investigation to understand the spatial distribution of crabs. Such work would include studying crab signalling, for instance; in terms of the spatial distribution of species, when individuals signal, how many receivers does a signal reach and how many signals can individuals receive simultaneously? Does crab activity depend on crab density and patterns of distribution – including the consideration of which factor drives which? Male territory area may be driven by courtship display; male fiddler crabs defend a small circle with a radius several times wider than themselves, often with the crab measured in the maximum width of display posture (Crane, 1975). In some species, where the male display is vertical, the territory appears to be smaller, whereas, in species with advanced display that requires more space, the defended area is relatively much larger (Crane, 1975).

Although all aspects of spatial distribution and interspecies distance have not been covered in this study, it is evident that there are many factors affecting their distribution. Substratum properties, the presence of additional environmental variables, such as shade and shore height, as well as crab density and behaviour, determine the distribution of fiddler crabs. The spatial heterogeneity in the environmental can be translated to a spatial heterogeneity in the distribution of fiddler crabs (Ribeiro et al., 2005).

The fiddler crabs at Ambeua have been studied over a four-year period, showing that although there is a high alpha diversity, which could lead to increased competition, these species have consistently been present, meaning the close habitats of multiple species may not have the negative impacts on individuals that it is thought to (Guiasu & Dunham, 1999). Niche theory suggests that in order to coexist, two species must diverge in their use of resources (Hardin, 1960). When sympatric species occupying the same or similar niches are driven to interact, one often emerges as the dominant species at the expense of the other (Bach et al., 1976). By observing the continued existence of mixed species populations, it is possible that there are various discrepancies between theoretical estimates from the classic niche and competition theories.
Chapter 6

General Discussion

Fiddler crabs live in warm climates along the mudflats of protected bays, in mangrove forests, near river mouths, and the banks of tidal streams. Both as species and individuals they can adjust to wide ranges of environmental conditions, including tidal height, temperature, moisture and salinity. Species-specific differences are apparent, not only in morphology and physiology but in social activities as well. This study investigated an area of high species coexistence and some of the species-specific differences that allow this coexistence to occur.

Partitioning of the intertidal zone has long been documented in fiddler crabs (Barnes, 2010; Crane, 1975; Icely and Jones, 1978; Thurman, 1985; Thurman et al., 2010), yet this is the first study to investigate the factors driving coexistence with such a high species abundance. The species in this study are exhibiting a level of sympathy that is rarely recorded, which provided the opportunity to study how closely related species that compete for similar resources manage to coexist. This multifaceted study has by no means covered all aspects of species coexistence and it would be difficult to ever truly state it was entirely understood how these species are coexisting.

This study set out to further understand the distribution of sympatric fiddler crab species and the diversity of form and function between coexisting species. Fundamental to addressing any of the aims was initially identifying the ten species living at Ambeua, which was achieved through studying anatomy, morphology and genetics (Chapter 2).

This study aimed to establish the distribution of the crabs across the site and to determine the factors that affect their distribution (Chapter 3). This study also aimed to compare the major differences in mouthpart morphology, specifically the second maxilliped, to determine whether differences in mouthpart morphology are linked to species habitats (Chapter 4). The distribution of individual crabs was studied to determine whether crabs are more likely to live nearest to a conspecific or an allospecific, and whether this varies inter- and intraspecifically (Chapter 5).
Through addressing these aims, this chapter will explore the results from the thesis and assess the main factors affecting the distribution of the fiddler crabs coexisting at Ambeua. The chapter will also consider the ecological processes that were not covered in this investigation and discuss future work required to further the understanding of coexistence.

6.1 Ecological preferences

Through investigating the morphology, behaviour and habitat structure of then ten species of fiddler crab at Ambeua, it was found that they occupy distinct, but overlapping niches. It is likely that the anatomical adaptations and physiological mechanisms which permit the species to occupy even marginally different habitats, allow these species to coexist. Data on crab size, shore height zonation, substrate type, number of spoon tipped setae and shading can be found in Table 6.1. This information demonstrates the differences between the species that allow for this coexistence to occur.

This study has indicated the ecological preferences for each species at Ambeua, yet for some of these species there is very little published ecological data, which makes it difficult to draw conclusions as to whether their distribution patterns are altered by the sympatry at this site. Two studies, one in the Philippines, the other in Malaysia, looked at the distribution patterns of *A. perplexa* and *A. triangularis* (Boregon & Evangelio, 2015; Mokhtari et al., 2015). Both studies found that individuals of *A. perplexa* lived in unshaded areas with sandy substrates and *A. triangularis* lived in shaded areas with finer substrates. The findings in both these studies correspond with the results of this investigation, suggesting that although there is a high number of species present at Ambeua, at least some of the species appear to be living in what might be considered their fundamental niche.

The distribution of the ten species of fiddler crab has been studied here in relation to many environmental factors. Shore height, the features of the substrate and shading appear to be the most important factors that influence the spatial distribution of the species at Ambeua and will be addressed here individually.
6.1.1 Shore height

The Wakatobi has a semi-diurnal tidal cycle with the inter-tidal zone largely exposed at low tide. Tidal amplitude is roughly two metres which, given the shallowness of the shore slope, allows for the occupancy of species with a range of emersion time requirements. The activities of fiddler crabs are closely related to tidal regimes, with the crabs remaining in their burrows throughout high tide and emerging during low tide. Species are often found across different tidal zones, adapted to varying degrees of immersion and emersion, substrate type and shading.

6.1.2 Substrate properties

Fiddler crabs utilize the substrate they live on as a food source, which their mouthparts are structured to process (Miller, 1961). Previously, a relationship has been found in fiddler crabs between the anatomy of the mouthparts and the substrate of the habitat (e.g. Crane, 1975; Lim, 2004; Lim and Kalpana, 2011; Maitland, 1990; Miller, 1961). Studies have shown that fiddler crabs feeding on fine muddy sediments have many plumose setae on the second maxilliped, and fiddler crabs feeding on coarse sand have spoon-tipped setae on the second maxilliped (Maitland, 1990). Other studies have shown that for species feeding primarily on coarse sandy sediments, there is an abundance of spoon-tipped setae on the second maxillipeds, whereas species that feed on fine silty sediments, there are much fewer spoon-tipped setae on the second maxillipeds (Bezerra et al., 2006; Lim, 2004; Lim and Kalpana, 2011). In this study, all species had both plumose and spoon-tipped setae, but in varying proportions and abundances. However, the relationship between mouthpart morphology and substrate is not fixed and can be flexible (Thurman et al., 2013) which appears to be the case in this study.

The area at Ambeua where all ten species coexist at diversities as high as seven species per 2 m², does not appear to have marked gradients in substrate type, which is thought to be one of the biggest drivers of fiddler crab distribution. The morphology of the second maxillipeds differs between species, adapting them to
different substrate types, yet at Ambeua they are all living and feeding on substrate of the same particle size range. This begs the questions of why this is occurring. Similar situations have been noted in Brazil. *Leptuca uruguayensis* from southern Brazil has been found feeding on distinctly different substrates: it has been seen coexisting on sandy substrates with *Minuca rapax* and *Leptuca leptodactyla* but can live in diverse habitats when living alone - ranging from silt to mud to sand substrates (Vernberg & Vernberg, 1967). It appears that mouthpart structures, with a mixture of spoon-tipped and plumose setae, can potentially provide the flexibility to feed on various substrates under competitive pressures. There is also the possibility, that the area which all ten species are inhabiting at Ambeua could be the edge of some, or all, of their fundamental niche or ‘comfort zones’ of substrate type. When looking outside of this area of coexistence, species abundances and densities reduce, and species seem to occupy the sediment type to which their mouthparts are suited, meaning the area of coexistence at Ambeua could be where all species ‘overlap’.

### 6.1.3 Shading

As well as shore height and the relationship between mouthparts and sediment type, shade also appears to determine the distribution of the fiddler crabs at Ambeua. Some species prefer to live in vegetated areas whereas some are found in unshaded and exposed areas (Bezerra *et al*., 2006; Crane, 1975). A study in northeast Brazil found *L. leptodactyla* and *M. rapax* living at the edge of the mangrove, *Leptuca thayeri* was found in vegetated areas, while *U. maracoani* was only found on an extensive open mudflat that was exposed during low tides (Bezerra *et al*., 2006). The primary reason fiddler crabs may choose to live in vegetated/shaded areas is considered to be to avoid high temperatures which lead to desiccation (Bezerra *et al*., 2006; Crane, 1975). Some fiddler crabs may choose to avoid vegetated areas due to the possibility of it limiting their visibility. *Tubuca elegans*, when studied in an Australian mangrove, was shown to employ visual signals more often than other fiddler crab species and chose to avoid vegetated habitats, in order to maximise visibility of signals (Nobbs, 2003).

Species of fiddler crab that live in shaded areas are not well adapted to tolerate increased temperatures and desiccate much faster than those that live in unshaded
areas (Bezerra et al., 2006; Koch et al., 2005). In a north Brazilian mangrove, unshaded muddy banks were dominated by *U. maracoani*, most likely because of its ability to tolerate high temperatures, with oxygen consumption increasing up to temperatures of 35°C (Koch et al., 2005). All other species present in the Brazilian mangrove showed highly reduced oxygen consumption in temperatures above 30°C, indicating that high temperatures cause them physiological stress (Koch et al., 2005).

A study at Ambeua looking into the respiratory physiology of three fiddler crab species found that their metabolic responses differed in accordance to where they lived on the shore (Jimenez and Bennett, 2005). *G. vocans* and *G. tetragonon* live in the lower intertidal zone and experience long immersion times, whereas *P. crassipes* inhabits high intertidal zones and has short immersion periods. Oxygen consumption in water was low for all species, yet when in air *G. vocans* and *G. tetragonon* had much higher oxygen consumption rates, this is likely due to their prolonged periods spent underwater which therefore lead to a greater oxygen debt to repay (Jimenez and Bennett, 2005).
Table 6.1 Size, habitat characteristics and the number of spoon-tipped setae of fiddler crabs the Ambeua shore.

<table>
<thead>
<tr>
<th>Species</th>
<th>Mean Adult CW (mm)</th>
<th>Shore Height Range</th>
<th>Mean Substrate Type</th>
<th>Mean no. of spoon tipped setae on 2\textsuperscript{nd} maxilliped</th>
<th>Unshaded Areas</th>
<th>Shaded Areas</th>
<th>Mangrove Houses</th>
<th>% of total crabs at Ambeua</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Tubuca coarctata</em></td>
<td>12.90</td>
<td>Mid – High</td>
<td>Mud</td>
<td>78.5 ± 6.8</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>2.7</td>
</tr>
<tr>
<td><em>Tubuca demani</em></td>
<td>15.93</td>
<td>Mid – High</td>
<td>Sandy Mud – Mud</td>
<td>111.0 ± 5.4</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>2.9</td>
</tr>
<tr>
<td><em>Tubuca dussumieri</em></td>
<td>18.45</td>
<td>Mid – High</td>
<td>Sandy Mud – Mud</td>
<td>87.7 ± 9.2</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>3.5</td>
</tr>
<tr>
<td><em>Paraleptuca crassipes</em></td>
<td>10.90</td>
<td>High</td>
<td>Mud</td>
<td>79.7 ± 2.8</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>12.2</td>
</tr>
<tr>
<td><em>Gelasimus jocelynnae</em></td>
<td>10.78</td>
<td>Low – Mid</td>
<td>Sand – Muddy Sand</td>
<td>172.5 ± 10.1</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>52.2</td>
</tr>
<tr>
<td><em>Gelasimus tetragonon</em></td>
<td>18.32</td>
<td>Mid</td>
<td>Sand – Muddy Sand</td>
<td>142.2 ± 6.0</td>
<td>*</td>
<td></td>
<td></td>
<td>4.4</td>
</tr>
<tr>
<td><em>Austruca cryptica</em></td>
<td>10.61</td>
<td>Mid</td>
<td>Muddy Sand</td>
<td>966.2 ± 32.5</td>
<td>*</td>
<td></td>
<td></td>
<td>0.5</td>
</tr>
<tr>
<td><em>Austruca mjoebergi</em></td>
<td>8.59</td>
<td>Mid</td>
<td>Muddy Sand</td>
<td>770.5 ± 44.2</td>
<td>*</td>
<td></td>
<td></td>
<td>0.4</td>
</tr>
<tr>
<td><em>Austruca perplexa</em></td>
<td>10.41</td>
<td>Mid</td>
<td>Muddy Sand</td>
<td>643.7 ± 37.1</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>18.1</td>
</tr>
<tr>
<td><em>Austruca triangularis</em></td>
<td>8.77</td>
<td>Mid - High</td>
<td>Mud</td>
<td>59.7 ± 4.8</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>3.2</td>
</tr>
</tbody>
</table>
6.2 Individual spatial distribution

Fiddler crab distribution can be complex; in some locations, species rarely overlap in distribution, yet in others, many species can coexist at one time (Barnes, 2010; Icely & Jones, 1978; Crane, 1975, Lim, 2005) including the species in this study. The distribution of individual crabs was studied to determine whether crabs are more likely to live nearest to a conspecific or an allospecific. The data gathered here shows that residents of *A. perplexa*, *A. triangularis* and *G. jocelynae* live near to conspecifics more than any other species. *A. perplexa* and *G. jocelynae* are the most abundant species at Ambeua which may explain why they live next to conspecifics so frequently. Although the data shows relationships with species living next to conspecifics, definitive conclusions are hard to draw from the limited data set. Further work would be required, both at Ambeua and in areas with monospecific populations, to determine whether differences occur and if they are due to the high species abundances and densities.

6.3 Ecological considerations

There are many other factors that could contribute to species coexistence that were not investigated in this study. Some recognised factors being: organic matter content of the substrate, differences in body size between the species, mouthpart variation between conspecific adults and juveniles, larval recruitment, predation and parasitism.

An important point that was not under investigation in this study is that the Ambeuan fiddler crab species show a wide spectrum of body size (also noted by Barnes, 2010), which could assist coexistence (Abrams, 1983). Sizes range from the large *T. dussumieri, T. demani* and *G. tetragonon* (>15 mm carapace width), through *G. jocelynae, T. coarctata, P. crassipes, A. perplexa* and *A. cryptica* (usually around 10 mm) to the small *A. mjoebergi* and *A. triangularis* (<10 mm). Further analysis would need to be done to determine whether this contributes to the coexistence of these species. It would be interesting to note whether the size of individuals at this site matches the size of crabs of the same species elsewhere, or whether some sort of character displacement may be occurring.
Further to the species under study here, four species of sentinel crabs (*Macrophthalmus convexus* Stimpson 1858, *Macrophthalmus crinitus* Rathbun 1913, *Macrophthalmus definitus* Adams & White 1849 and *Macrophthalmus latreillei* Desmarest 1822) were also present at Ambeua, within some of the same quadrats as the fiddler crabs. *Macrophthalmus* spp. are ecologically similar to fiddler crabs, in that they are also deposit feeders and characteristically make their burrows in the low intertidal zone (Schuwerack *et al*., 2006), which consequently makes this site of coexistence even more remarkable.

Understanding how individuals of the sympatric species arrived at Ambeua is an important topic of investigation and would need further study before it could be understood. Larval migration is a complex topic to study as it requires in depth knowledge about tides and currents. The currents in the Wakatobi region are highly variable (Clifton, 2010): during the north-westerly monsoon season (usually November to April) the currents run anti-clockwise around Sulawesi, yet during the rest of the year no clear pattern is apparent. Considering that the megalopae of developing crabs use the flood tide to reach a shore and settle into the adult habitat (Simith *et al*., 2010; Thurman *et al*., 2013), complex and variable currents could contribute to such high species richness. The relationship between larval recruitment and local current regimes could be a major factor influencing the geographical distribution of many fiddler crab species (Grantham & Shanks, 2003).

Further analysis would need to be done to determine whether the observed coexistence at Ambeua is stable or unstable. In conditions of stable coexistence, there are often long-term trends, where species densities may vary, but overall remain stable. In situations of unstable coexistence, numbers can drop and there will not be a trend towards recovery, meaning species are not maintained over long time periods (Chesson, 2000). The type of coexistence and resource partitioning at Ambeua is a stabilising mechanism; whereby interspecific competition is reduced because species compete for different resources. This stabilising mechanism is supported by the lack of interspecific competition, showing minimal agnostic interactions between individuals of different species, despite their territory overlaps.
6.4 Understanding coexistence and future work

Understanding the coexistence of closely related species is an important topic when furthering conservation. Intertidal zones are being rapidly degraded on a global scale (Short et al., 2011), with large areas of mangrove being cleared around Hoga and Kaledupa over the last 30 years for timber, fuel, and to accommodate the increasing populations. These regions require protection, especially in areas of high species richness.

The results of this study indicated that species abundance increased both in the mangrove and underneath the housing. Human activities are undoubtedly affecting the distribution and existence of fiddler crabs and surprisingly, with Ambeua as one example, these affects are increasing diversity. Together with an example of sixteen species near to a sewage outlet in the Panama Canal (Crane, 1975), the evidence at Ambeua suggests that anthropogenic factors are directly altering the ecosystem, which appears to be allowing crabs to dwell in places otherwise uninhabitable.

Conclusions from this study contribute to the ecological understanding of sympatric fiddler crabs and the factors that affect their distribution. It is evident from this thesis that fiddler crabs are able to coexist and partition resources in high species abundances. This is not only one of the largest number of fiddler crab species reported to occur within such a small area, but is possibly a record diversity for the number of species of co-existing deposit-feeding crabs. The reasons why so many species are able to coexist here is not entirely understood, but it is evident that they are capable of maintaining high levels of alpha diversities. The data from this thesis, as well as the fact that Ambeua has, to date, the only known population of A. cryptica, should be used to aid the local management of the possible impacts of habitat loss in such an important ecosystem.
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Thurman, C. L., II (1982). On the distinctness of the fiddler crabs *Uca minax* (LeConte) and *Uca longsignalis* Salmon & Atsaides in their region of sympatry (Decapoda Brachyura, Ocyopodidae). *Crustaceana*, 43(1), 37–50.


### Appendix

**Table 1.** Enumeration of all spoon tipped (ST) setae on the dorsal surface of both the left and right second maxilliped.

<table>
<thead>
<tr>
<th>Species</th>
<th>No. of ST Setae (Left)</th>
<th>No. of ST setae (Right)</th>
<th>No. of P setae (Left)</th>
<th>No. of P setae (Right)</th>
<th>Total Left</th>
<th>Total Right</th>
</tr>
</thead>
<tbody>
<tr>
<td>Austruca mjoebergi</td>
<td>744</td>
<td>784</td>
<td>348</td>
<td>370</td>
<td>1092</td>
<td>1154</td>
</tr>
<tr>
<td>Austruca perplexa</td>
<td>673</td>
<td>712</td>
<td>446</td>
<td>411</td>
<td>1119</td>
<td>1123</td>
</tr>
<tr>
<td>Austruca triangularis</td>
<td>55</td>
<td>69</td>
<td>320</td>
<td>348</td>
<td>375</td>
<td>417</td>
</tr>
<tr>
<td>Austruca cryptica</td>
<td>886</td>
<td>943</td>
<td>407</td>
<td>392</td>
<td>1293</td>
<td>1335</td>
</tr>
<tr>
<td>Paraleptuca crassipes</td>
<td>87</td>
<td>78</td>
<td>437</td>
<td>459</td>
<td>524</td>
<td>537</td>
</tr>
<tr>
<td>Gelasimus tetragono</td>
<td>147</td>
<td>138</td>
<td>407</td>
<td>424</td>
<td>554</td>
<td>562</td>
</tr>
<tr>
<td>Gelasimus jocelynae</td>
<td>154</td>
<td>181</td>
<td>318</td>
<td>332</td>
<td>472</td>
<td>513</td>
</tr>
<tr>
<td>Tubuca dussumieri</td>
<td>91</td>
<td>109</td>
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