POPULATION ECOLOGY OF FIDDLER CRABS (OCYPODIDAE: GELASIMINAE) IN THE MANGROVES OF PENANG ISLAND, MALAYSIA

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by

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LIST OF ABBREVIATIONS

ANOVA	Analysis of Variance
b	Growth coefficient
CCA	Canonical Correspondence Analysis
cm	Centimeter
DNA	Deoxyribonucleic Acid
g	gram
GLM	Genral Linear Model
Kn	Relative condition factor
KSP	Kuala Sungai Pinang
LOI	Loss on Ignition
PB	Pulau Betong
PPT	Parts per thousand
OTU	Operational Taxonomic Unit
PERMANOVA	Permutational Multivariate Analysis of Variance
PRIMER v7	Plymouth Routines In Multivariate Ecological Research Version 7
SIMPER	Similarity Percentage
SPSS	Statistical Package for the Social Sciences
TT	Teluk Tempoyak
m	Meters

LIST OF APPENDICES

EKOLOGI POPULASI KETAM REBAB (OCYPODIDAE: GELASIMINAE) DI PAYA BAKAU PULAU PINANG, MALAYSIA

ABSTRAK

Sedikit yang diketahui tentang ekologi populasi ketam rebab di utara Semenanjung Malaysia, oleh itu, satu kajian mengenai ekologi populasi spesies ini telah dijalankan di kawasan paya bakau Pulau Pinang selama 12 bulan dari Mac 2017 hingga Februari 2018. Persampelan bulanan telah dilakukan untuk mengumpul data ketam rebab, sampel sedimen, dan parameter fisikokimia. Kajian ini meneliti kelimpahan, corak pertumbuhan, dan tabiat pemakanan ketam rebab di ekosistem paya bakau Pulau Pinang, dengan memberi tumpuan kepada pengaruh parameter persekitaran, variasi mikrohabitat, dan perubahan musim. Taburan dan kelimpahan ketam rebab dianalisis berkaitan dengan faktor persekitaran. *Austruca annulipes* dan *Gelasimus vocans* banyak dijumpai di habitat dataran lumpur yang lebih kasar dan terang, manakala *Tubuca rosea*, *Tubuca forcipata*, dan Sp. 1 berkaitan dengan sedimen yang lebih halus dan kaya dengan bahan organik. Variasi musim memainkan peranan penting, dengan spesies seperti *T. rosea* mengekalkan keutamaan yang kuat terhadap sedimen halus merentasi musim. Interaksi tiga arah yang signifikan antara tapak, mikrohabitat, dan musim menyerlahkan dinamik kompleks yang mempengaruhi habitat ketam rebab, seperti *A. annulipes* yang menunjukkan kelimpahan lebih tinggi di dataran lumpur Pulau Betong semasa musim hujan. Penemuan ini menekankan kepentingan variabel fisikokimia, seperti komposisi sedimen dan intensiti cahaya, dalam membentuk populasi ketam rebab. Hubungan lebar karapas-berat dan faktor keadaan relatif (*Kn*) *A. annulipes*, *A. bengali*, dan *T. rosea* telah dinilai. Ketiga-tiga spesies menunjukkan pertumbuhan allometrik positif, dengan kadar pertumbuhan

jantan lebih tinggi berbanding betina. Ketiga-tiga spesies ketam rebab menunjukkan pertumbuhan allometrik sepanjang musim kering dan hujan, dengan *A. bengali* menunjukkan variasi musim yang ketara, mengalami pertumbuhan yang lebih perlahan semasa musim kering, manakala *A. annulipes* dan *T. rosea* mengekalkan pertumbuhan yang konsisten dengan sedikit peningkatan semasa musim hujan, mungkin disebabkan oleh keadaan persekitaran yang bertambah baik dan ketersediaan makanan. Corak pertumbuhan juga berbeza mengikut tapak, mencerminkan pengaruh kualiti habitat dan ketersediaan makanan. Hasil ini menekankan kepentingan ekologi spesies ini dan menggariskan keperluan untuk usaha pemuliharaan yang spesifik terhadap habitat. Analisis kandungan perut yang komprehensif menggunakan DNA metabarcoding, mikroskopi, dan analisis isotop stabil mendedahkan keutamaan pemakanan yang berbeza di kalangan ketiga-tiga spesies ketam rebab. *A. annulipes* terutamanya memakan alga dan organisma meiobentik di dataran lumpur, *A. bengali* memakan mikroalga bentik di anak sungai pasang surut, dan *T. rosea* bergantung pada detritus yang berasal dari bakau di kawasan hutan dalaman. Walaupun semua spesies berada pada tahap trofik yang serupa sebagai detritivor, perbezaan ketara dalam sumber karbon mereka diperhatikan, mencadangkan pengkhususan diet yang dikaitkan dengan habitat masing-masing. Secara keseluruhan, tesis ini memberikan pemahaman yang terperinci tentang bagaimana keadaan mikrohabitat, dinamik pertumbuhan, dan pengkhususan diet mempengaruhi populasi ketam rebab di ekosistem paya bakau, dengan implikasi penting untuk pemuliharaan dan pengurusan habitat.

POPULATION ECOLOGY OF FIDDLER CRABS (OCYPODIDAE: GELASIMINAE) IN THE MANGROVES OF PENANG ISLAND, MALAYSIA

ABSTRACT

Little is known about the population ecology of fiddler crab in northern Peninsular Malaysia, and as such, a study on the population ecology of this species was conducted in mangroves of Penang Island for 12 months from March 2017 to February 2018. Monthly sampling was conducted to collect the fiddler crab, sediment sample and physicochemical parameter data. This study investigates the abundance, growth patterns, and feeding habits of fiddler crabs in the mangrove ecosystems of Penang Island, focusing on the influence of environmental parameters, microhabitat variations, and seasonal changes. The distribution and abundance of fiddler crabs were analysed in relation to environmental factors. *Austruca annulipes* and *Gelasimus vocans* were predominantly found in coarser, well-lit mudflat habitats, while *Tubuca rosea*, *Tubuca forcipata*, and Sp. 1 were associated with finer, organic-rich sediments. Seasonal variations played a significant role, with species like *T. rosea* maintaining a strong preference for finer sediments across seasons. The significant three-way interaction between site, microhabitat, and season highlights the complex dynamics influencing fiddler crab habitats as such for *A. annulipes* that show significantly higher abundance in Pulau betong mudflat during wet season. These findings highlight the importance of physicochemical variables, such as sediment composition and light intensity, in shaping fiddler crab populations. The carapace width-weight relationship and relative condition factor (*Kn*) of *A. annulipes*, *A. bengali*, and *T. rosea* were assessed. All three species exhibited positive allometric growth, with males generally showing higher growth rates than females. The three fiddler crab species exhibited an allometric growth across both dry and wet seasons, with *Austruca bengali* showing notable seasonal variation, experiencing slower growth during the dry season, while *Austruca annulipes* and *Tubuca rosea* maintained consistent growth, with slight increases during the wet season likely due to improved environmental conditions and food availability. Growth patterns varied across different sites, reflecting the influence of habitat quality and food availability. These results emphasize the ecological significance of these species and underline the need for habitat-specific conservation efforts. A comprehensive gut content analysis using DNA metabarcoding, microscopy, and stable isotope analysis revealed distinct dietary preferences among the three fiddler crab species. *Austruca annulipes* primarily consumed algae and meiobenthic organisms in mudflats, *Austruca bengali* fed on benthic microalgae in tidal creeks, and *T. rosea* relied on mangrove-derived detritus in interior forests. While all species shared a similar trophic level as detritivores, significant differences in their carbon sources were observed, suggesting dietary specialization linked to their respective habitats. Overall, this thesis provides a detailed understanding of how microhabitat conditions, growth dynamics, and dietary specialization influence fiddler crab populations in mangrove ecosystems, with important implications for conservation and habitat management.

CHAPTER 1

INTRODUCTION

1.1 Background Study

Mangroves are unique coastal ecosystems comprised of salt-tolerant trees and shrubs that thrive in intertidal areas of the tropics and subtropics (Crawford, 2019). There are approximately 50 to 80 true mangrove species and associated plants worldwide, all of which possess specialized osmoregulatory capabilities that enable them to survive the challenging environmental conditions of intertidal environment (Gabb, 2021). Mangroves play a crucial role in coastal ecosystems, providing organic detritus that supports high productivity levels in adjacent waters, sediments, and intertidal mudflats (Dewiyanti et al., 2019). The decomposition of mangrove litter contributes to the nutrient cycle, supporting a diverse range of marine and terrestrial fauna, particularly benthic communities such as crustaceans, bivalves, gastropods, and polychaetes, which inhabit different microhabitats within the mangrove ecosystem (Nagelkerken et al., 2008; Lee, 2008).

Benthic fauna plays a vital role in mangrove ecosystems by facilitating energy transfer between primary producers (mangrove detritus and microalgae) and higher trophic levels (Meijer et al., 2021). They provide a food source for fish, crustaceans, and molluscs during high tide, as well as for shorebirds, mammals, and reptiles during low tide (Kathiresan & Bingham, 2001). Additionally, benthic fauna promotes nutrient cycling through bioturbation activities, which modify sediment structure and facilitate the redistribution of material, fluids, and gases within sediment layers (Kathiresan & Bingham, 2001).

Among the benthic fauna in mangrove ecosystems, brachyuran crabs, particularly those from the families Ocypodidae and Grapsidae, are among the most abundant and diverse groups (Lee, 2008). In Peninsular Malaysia, 17 species of Ocypodidae and 36 species of Grapsidae have been reported in tropical mangroves (Tan & Ng, 1994). These crabs are recognized as ecosystem engineers. Through their burrowing and foraging activities, mangrove crabs influence sediment properties, nutrient dynamics, and overall ecosystem functioning (Kristensen, 2008).

Fiddler crabs (genus *Uca*) are a prominent group of benthic fauna within mangrove ecosystems, particularly in Peninsular Malaysia, where 17 species of Ocypodidae, including fiddler crabs, have been documented (Tan & Ng, 1994). These crabs are regarded as ecosystem engineers due to their burrowing and foraging activities, which alter sediment structure, enhance nutrient availability, and improve soil drainage and aeration (Botto & Iribarne, 2000; Sarker et al., 2021). Fiddler crabs inhabit specific microhabitats such as mudflats, tidal creeks, and interior forests, and their activities have significant ecological implications for these intertidal zones (Koch et al., 2005).

Previous studies have highlighted the role of mangrove microhabitat in influencing benthic faunal assemblages (Dissanayake & Chandrasekara, 2014). However, there is limited research on the small-scale distribution and abundance of fiddler crabs among different microhabitats in tropical mangroves, especially in Penang Island, Malaysia. This study aims to fill this gap by examining the abundance, diversity, and environmental preferences of fiddler crabs across different microhabitats in Penang Island's mangroves and determining how environmental physicochemical properties such as sediment grain size, porewater salinity, and temperature influence these patterns. Understanding these interactions is vital for assessing the ecological roles of fiddler crabs in mangrove ecosystems, particularly in the context of increasing anthropogenic pressures on these habitats.

Beyond the ecological assessment, understanding the morphological and physiological aspects of fiddler crabs can provide deeper insights into their adaptation to mangrove environments. The carapace width-weight relationship and relative condition factor are important parameters for assessing the growth, health, and overall fitness of fiddler crab species. These metrics can reveal how environmental factors and habitat conditions affect individual crab populations, which is critical for understanding their role in ecosystem dynamics and resilience.

Feeding habits further complements our understanding of the ecological roles of fiddler crabs. By integrating microscopy, DNA metabarcoding (16S and 18S rRNA genes), and stable isotope analyses, it is possible to elucidate their dietary habits and trophic positions within the mangrove food web. These approaches provide detailed information on the composition of their diets, including the assimilation of different carbon and nitrogen sources, which can explain their distribution and habitat preferences across different microhabitats.

1.2 Problem statement

The ecology of fiddler crabs in mangrove ecosystems remains underexplored, particularly in Penang Island, where rapid urbanization and coastal development have posed significant threats to mangrove habitats. The need to understand the population dynamics, habitat preferences, and ecological roles of fiddler crabs in such vulnerable environments is increasingly critical. Fiddler crabs play a significant role in sediment bioturbation, nutrient cycling, and energy transfer within mangroves. However, their distribution and abundance patterns across different microhabitats (mudflats, tidal creeks, and interior forests) in Penang Island's mangroves have not been comprehensively studied. Additionally, there is limited understanding of how environmental factors influence their distribution and what this implies for the mangrove ecosystem's functioning and health.

Moreover, while the carapace width-weight relationship and relative condition factor have been commonly used to assess crab health and growth, their applicability in understanding species adaptation to distinct mangrove microhabitats remains unexplored. Understanding the feeding habits of fiddler crabs is equally crucial, as it links their trophic roles with their distribution and population dynamics. There is a need to integrate modern molecular techniques, such as DNA metabarcoding and stable isotope analysis, to provide a comprehensive view of their dietary preferences and ecological niches.

1.3 Research Questions

1. What is the spatial and temporal distribution of fiddler crab assemblages across different microhabitats (mudflat, tidal creek, and interior forest) in the mangroves of Penang Island?
2. How do environmental factors (e.g., sediment grain size, porewater salinity, temperature) influence the abundance and distribution of fiddler crabs in these microhabitats?
3. What is the relationship between the carapace width-weight relationship, relative condition factor, and the microhabitat preferences of dominant fiddler crab species (*Austruca annulipes*, *Austruca bengali*, and *Tubuca rosea*)?
4. How do the feeding habits of fiddler crabs, as determined by microscopy, DNA metabarcoding, and stable isotope analyses, vary among species and microhabitats, and how do these dietary patterns relate to their distribution and abundance?

1.4 Objectives

This study aims to explore the population dynamics, morphological adaptations, and feeding habits of fiddler crabs in the mangroves of Penang Island. The specific objectives are:

I. To examine the influence of spatial (microhabitat differences) and temporal (seasonal) factors on fiddler crab abundance and diversity in Penang Island's mangroves, and to relate these patterns to environmental physicochemical variables. (Addressed in Chapter 3)

II. To analyze the carapace width-weight relationship and relative condition factor of *Austruca annulipes*, *Austruca bengali*, and *Tubuca rosea* in different microhabitats and assess their implications for crab growth, and habitat adaptation. (Addressed in Chapter 4)

III. To elucidate the diet composition and trophic positions of the dominant fiddler crab species using an integrated approach involving microscopy, DNA metabarcoding, and stable isotope analysis, and to relate these findings to their distribution and abundance patterns. (Addressed in Chapter 5)

This integrative study will provide a comprehensive understanding of the ecological roles and adaptive strategies of fiddler crabs in Penang Island's mangroves, informing conservation and management efforts facing ongoing habitat loss and degradation.

CHAPTER 2

LITERATURE REVIEW

2.1 General overview of fiddler crabs

Fiddler crabs, belonging to the family *Ocypodidae* under the subfamily *Ucinae*, are widely distributed across tropical and subtropical regions globally (Shih et al., 2016). Currently, 105 species are recognized, spanning 11 genera. Their distribution includes 47 species along the Indo-Pacific coast, 36 species in the Eastern Pacific, 21 species in the Western Atlantic, and 1 species in the Eastern Atlantic (Rosenberg, 2014).

The taxonomic history of fiddler crabs, particularly the genus *Uca*, dates back to the 1700s. However, early descriptions were inconsistent, with numerous synonyms in use. The genus *Uca* was originally described from illustrations of *Cancer uka una* by Seba (1758), which served as the basis for subsequent nomenclature (Rosenberg, 2014). To resolve taxonomic contradictions, the International Commission on Zoological Nomenclature declared *Cancer vocans major* as the official type specimen for the genus *Uca* (Rosenberg, 2014).

In 1954, Bott introduced a classification method based on the front width of the carapace between the eyestalks, splitting the genus *Uca* into broad-fronted *Minuca* and narrow-fronted *Uca* species (Rosenberg, 2014). Later, in 1973, Bott further divided the genus into 10 genera based on gonopod structure (Rosenberg, 2014; Tan & Ng, 1994). Crane (1975) provided a comprehensive revision, recognizing 62 species within the single genus *Uca*, which he subdivided into nine subgenera. His classification considered features such as front width, gonopod structure, and major cheliped morphology. Crane's detailed descriptions significantly contributed to the discovery of new fiddler crab species.

Recent revisions by Shih et al. (2016) have promoted the subgenera to full genera. Today, 11 genera of fiddler crabs are recognized: *Afruca*, *Uca*, *Gelasimus*, *Austruca*, *Cranuca*, *Leptuca*, *Minuca*, *Paraleptuca*, *Petruca*, *Tubuca*, and *Xeruca* (Rosenberg, 2014). A total of 105 species are now identified worldwide (http://www.fiddlercrab.info/uca_species.html).

A distinguishing feature of fiddler crabs is their pronounced sexual dimorphism. Males possess one greatly enlarged claw, which can constitute more than half their body weight (Crane, 1975; Rosenberg, 2014), whereas females have two small chelae, resembling the male's minor claw. The minor claw plays a crucial role in feeding, as crabs use it to scoop sediment into their mouthparts (Crane, 1975). The enlarged claw in males serves as a display tool during mating rituals and territorial defence (Crane, 1975).

Fiddler crabs are colonial and often inhabit areas in large numbers (Crane, 1975). They live in burrows, which are vital for survival, serving as refuges from predators and desiccation during low tide (Crane, 1975). For males, burrows are also key to mating and territory defense, while females use them for incubating eggs (Christy & Salmon, 1984). Male crabs must either excavate new burrows, find unoccupied ones, or engage in combat to secure an existing burrow when their original is taken by a female for incubation (Mautz et al., 2011).

Female fiddler crabs carry fertilized eggs beneath their abdomen, and, like other intertidal crabs, release them into the water when they are ready to hatch (Christy & Salmon, 1984). The hatched larvae, or zoea, live as plankton for several weeks, undergoing multiple moulting stages before developing into megalopae (Christy & Salmon, 1984). These megalopae eventually molt into immature crabs and settle on the benthic substrate (Simith et al., 2010). Incubation takes about two weeks, while the larval stages can last from weeks to months, depending on the species (Rosenberg, 2014; Yamaguchi, 2002). For example, *Tubuca arcuata* can live up to five years, reaching a body size of approximately 4 mm in captivity (Otani et al., 1997), while *Leptuca uruguayensis* can live up to two years in the wild (Spivak et al., 1991).

Fiddler crabs typically inhabit intertidal zones, including sandy or muddy flats, riverbanks, salt marshes, and mangroves (Crane, 1975). In tropical mangroves, their distribution has been closely studied in relation to habitat characteristics (Tan & Ng, 1994; Mokhtari et al., 2015). Although multiple species can coexist in these areas, they often exhibit distinct habitat preferences (Bezerra et al., 2006; Mokhtari et al., 2015; Nobbs, 2003). Their distribution is influenced by both spatial and temporal factors (Zolkhiflee et al., 2021). For instance, Koch et al. (2005) observed zonation patterns in species such as *Uca maracoani* in lower intertidal zones, with *Leptuca cumulanta* and other species inhabiting higher zones.

Similar patterns were observed during preliminary sampling for this study. On sandy beach flats, *Austruca annulipes* was dominant, while *Gelasimus vocans* was found in drier areas, and *Tubuca paradussumieri* occupied muddier zones near mangroves. *Austruca bengali* was most abundant in tidal creeks with pebbles, coexisting with *Tubuca forcipata*, *Tubuca dussumieri*, *Uca* sp. 1, and other ocypodid and grapsid crabs. Deeper in the mangroves, *Tubuca rosea* inhabited muddier substrates.

The distribution of fiddler crabs, particularly in mangrove ecosystems, is influenced by several environmental factors, including sediment texture, vegetation cover, and physicochemical parameters like temperature, salinity, and pH (Colpo &

Lopez-Greco, 2017; Creek et al., 2015; Shock et al., 2009). These factors can affect their biological processes, such as metabolism and reproduction. Burrow activity, feeding behaviour, courtship displays, and interspecies interactions are also important in maintaining species territories (Weis & Weis, 2004).

Although significant advances have been made in understanding the biology and ecology of fiddler crabs, particularly regarding their distribution and habitat preferences, some aspects remain understudied. Species-specific microhabitat preferences and the impact of environmental parameters on their behaviour and survival warrant further investigation.

2.2 Environmental factors affecting fiddler crab distribution

The distributional patterns of mangrove crabs are strongly influenced by physical factors, particularly sediment characteristics, as these crabs rely on sediment to sustain their biological and physical functions, including feeding and reproduction (Mokhtari et al., 2015; Bezerra et al., 2006). Sediment characteristics, encompassing chemical, physical, and biological properties, can vary both spatially and temporally within the mangrove ecosystem. Biological properties of sediment, such as microbial biomass, bioturbation activity and organic detritus, play an important role in nutrient cycling and support various biological functions of mangrove crabs (Kristensen et al., 2008; Kristensen, 2000). Key components such as sediment particle size, organic matter content, water content, conductivity, porewater salinity, temperature, pH, and light intensity significantly impact the biology and physical processes of mangrove crabs. These factors can restrict certain species to specific microhabitats within the intertidal zone (Sen & Homechaudhuri, 2015).

Previous studies have demonstrated the crucial role of sediment grain size in influencing fiddler crab distribution (Bezerra et al., 2006; Koch et al., 2005; Mokhtari et al., 2015). Fiddler crabs are surface deposit feeders that graze on the mangrove floor, utilizing their minor chelipeds to scoop sand particles into their buccal cavities, where setae on the merus of the first and second maxillipeds separate detritus from the sand particles (Crane, 1975). Species morphology was different to suit the sediment in their respective habitats, with variations in setae aiding in the efficient removal of particles of different sizes. This leads to species-specific preferences for either sandy or muddy microhabitats, as evidenced by differences in setae structure (Bezerra et al., 2006; Mokhtari et al., 2015; Yamaguchi & Ogata, 2000). Sediment is also vital for constructing burrows, which fiddler crabs use for refuge during high tide, desiccation avoidance during low tide, and mating (Crane, 1975). For example, Chen et al. (2017) noted that burrow depth varies depending on soil grain size, with differences observed in species like *Xeruca formensis*.

Light penetration in mangrove forests is closely related to canopy density, with significant effects on sediment surface temperature and the distribution of fiddler crabs. Some species, such as *Tubuca elegans*, prefer open mudflats avoid of vegetation, as these open areas provide unobstructed space for visual mating displays and territorial behavior (Nobbs, 2003). Conversely, species like *Tubuca flammula* and *Tubuca signata* are more commonly found in vegetated areas where shade reduces desiccation risk by lowering sediment temperature (Nobbs, 2003). The presence or absence of canopy cover can thus strongly influence habitat selection by different species.

Fiddler crabs are particularly vulnerable to heat and desiccation due to tidal fluctuations that cause variations in sediment temperature and water content between the low and high intertidal zones (Nobbs & Blamires, 2017). Temperature affects key biological processes such as reproduction, metabolism, and survival rates. For instance, the reproductive process of *Leptuca uruguayensis* is temperature-dependent, with higher temperatures (24°C to 28°C) accelerating regeneration processes and improving survival rates in some species compared to cooler temperatures (16°C to 20°C) (Saher & Qureshi, 2012). Temperature-related impacts thus directly influence the distribution and reproductive success of various fiddler crab species, contributing to species-specific habitat preferences.

Salinity is a key environmental factor influencing the distribution and physiological responses of mangrove crabs. In tropical regions, salinity plays a significant role in structuring mangrove crab assemblages (Frusher et al., 1994; Ravichandran et al., 2007; Shock et al., 2009). Fiddler crabs (*Uca* spp.) are euryhaline and exhibit remarkable adaptability to a wide range of salinities. However, fluctuations in salinity levels may still affect their metabolism, oxygen consumption, molting process, and overall survival (Shock et al., 2009). Thurman (1998) highlighted the adaptive strategies of *Uca* species in different salinity regimes, particularly in relation to osmoregulation. These crabs maintain homeostasis through behavioural and physiological adjustments, allowing them to survive in both hypo- and hypersaline conditions.

2.3 Spatial and temporal variation in mangrove ecosystems

Mangrove ecosystems exhibit significant spatial and temporal heterogeneity, which plays a critical role in shaping the distribution and abundance of benthic fauna such as fiddler crabs. The complex interplay of microhabitats, tidal regimes, and seasonal changes creates diverse environmental gradients that influence species assemblages and their ecological functions, as demonstrated by studies on the intertidal crab *Neohelice (=Chasmagnathus) granulata*, which reveal how these factors interact to affect crab activity and distribution (Luppi et al., 2013). Understanding how spatial and temporal factors interact is essential for assessing species distributions and the ecological dynamics within mangrove ecosystems.

The spatial structure of mangrove ecosystems, including the distribution of mudflats, tidal creeks, and forest interiors, creates distinct microhabitats that support varying communities of fiddler crabs (Bezerra et al., 2006; Mokhtari et al., 2015). Each microhabitat presents unique physical and chemical conditions, such as sediment texture, organic matter content, and porewater salinity, which affect species-specific adaptations and habitat preferences. For instance, sediment grain size has been shown to influence fiddler crab morphology, feeding strategies, and burrowing behavior, with different species occupying sandy, muddy, or mixed sediments based on their physiological and ecological needs (Koch et al., 2005; Bezerra et al., 2006).

In addition, the spatial distribution of vegetation, such as mangrove canopy cover, has been linked to variations in light availability and temperature, which further shape fiddler crab distribution (Nobbs, 2003). Species that rely on visual signals for mating and territorial displays, such as *Tubuca elegans*, tend to prefer open mudflats, while species that seek protection from desiccation, such as *Tubuca signata*, are more commonly found in shaded, vegetated areas (Nobbs, 2003).

Seasonal variations are critical in shaping the abundance and distribution patterns of mangrove crabs (Luppi et al.,2013). In tropical mangrove ecosystems, seasonal variations in temperature, salinity, and rainfall can drive shifts in the availability of resources, habitat conditions, and physiological stressors (Ward et al., 2016). Seasonal variations have been found to affect crab activity, with more intense behaviours such as feeding, burrowing, and reproductive cycles occurring when habitats are submerged during tidal inundation (Luppi et al., 2013). For instance, during the wet season, increased rainfall and freshwater runoff can reduce salinity, alter sediment structure, and increase nutrient availability, creating favourable conditions for some fiddler crab species while constraining others with lower salinity tolerance (Thurman, 1998; Thurman, 2003). Conversely, the dry season is characterized by higher temperatures and greater exposure to desiccation, forcing crabs to modify their behaviour by seeking refuge in shaded areas or deeper burrows (Nobbs & Blamires, 2017). These seasonal dynamics can result in shifts in species composition and habitat use, with certain species thriving under specific conditions, highlighting the importance of considering temporal factors in understanding fiddler crab ecology.

The influence of spatial (microhabitat differences) and temporal (seasonal) factors on fiddler crab abundance and diversity in Penang Island's mangroves is important to understanding the ecological dynamics of the mangrove ecosystem. The spatial and temporal dimensions of this study provide a framework for assessing how habitat conditions and resource availability change over time, influencing species distribution patterns and their ecological roles within mangrove ecosystems.

2.4 Carapace-weight relationship and relative condition factor (*Kn*) in fiddler crabs

Understanding the carapace-width relationship (CWR) and relative condition factor (*Kn*) of marine organisms, including fiddler crabs, provides valuable insights into their general growth patterns, habitat suitability, and health assessments. Fiddler crabs, although not commercially harvested, play a crucial ecological role in mangrove ecosystems, acting as ecosystem engineers by sediment bioturbation activity and contributing to nutrient cycling and primary production (Kristensen, 2008). The CWR provides a general model to understand the overall growth pattern of fiddler crabs and how carapace size correlates with body mass. This method is ideal for stock assessments and comparisons across different habitats and environmental conditions (Pauly, 1984; Froese, 2006). In particular, studying the growth and condition of *Austruca annulipes*, *Austruca bengali*, and *Tubuca rosea*, species that dominate different microhabitats in Malaysian mangroves, provides valuable insights into their habitat-specific adaptations and responses to environmental conditions. In crabs, the relative growth of certain body parts, such as the male major claw, shows positive allometry—where certain traits grow at a faster rate compared to overall body size (Rosenberg, 2002). This positive allometric growth in fiddler crabs, particularly males, underscores their importance in reproductive behaviours, territory defence, and species-specific adaptations to varying environmental conditions.

The relative condition factor (*Kn*), first introduced by Le Cren (1951), is a widely used index to measure the well-being of an organism and is particularly valuable in assessing the health and reproductive status of crustaceans (Gubiani et al., 2020). *Kn* adjusts the condition factor by comparing an individual's actual weight to the expected weight for its size within a specific population, taking into account natural variations in body structure or reproductive state. This adjustment provides a clearer, more accurate picture of an organism's health compared to the population average (Froese, 2006). The expected weight (W') is typically derived from a carapace-weight regression model, developed for the species or population in question, helping to remove biases related to individual differences in body shape or reproductive condition.

Using the relative condition factor is especially useful for studying fiddler crabs (*Uca* spp.) because these species exhibit distinct growth patterns influenced by sexual dimorphism, particularly in the size of the male claw. Moreover, fiddler crabs occupy highly variable environments, including different microhabitats within mangroves, where local conditions such as salinity, sediment type, and food availability can influence their growth and health. By employing *Kn*, researchers can better assess how individual crabs are performing in specific microhabitats relative to the population's expected growth trends, providing valuable insights into habitat quality and environmental stress.

The Bhattacharya method for age cohort analysis for example can further elucidate growth patterns in *Austruca annulipes*, *Austruca bengali*, and *Tubuca rosea*. This method allows for the identification of distinct age cohorts based on size-frequency data, offering insights into how these species grow and adapt within their specific microhabitats. By linking CWR and *Kn* analyses to age cohort structures, this research aims to provide a comprehensive understanding of fiddler crab growth and their ecological significance in mangrove ecosystems.

2.5 Dietary habits of fiddler crab

Fiddler crabs are deposit feeders that primarily consume organic material from the sediment (Milner et al., 2010). They use their small chelipeds to scoop sand into their buccal cavity, where setae on their maxillipeds filter organic matter and reject indigestible particles in the form of feeding pellets or mud balls near their burrows. The diet of fiddler crabs typically includes detritus, bacteria, fungi, and benthic microalgae such as diatoms, microalgae, and dinoflagellates, which are likely major nitrogen sources for these crabs (France, 1998; Rodelli et al., 1984; Tue et al., 2017). Stable isotope studies have highlighted that fiddler crabs primarily rely on microphytobenthos as a carbon source, rather than mangrove detritus (Bouillon et al., 2004; France, 1998; Kawaida et al., 2019; Rodelli et al., 1984). However, due to the wide variety of available food sources, it is challenging to precisely determine the food selectivity of fiddler crabs.

The diet of fiddler crabs is intricately linked to their habitat preferences. This suggests that different species of fiddler crabs may exhibit dietary partitioning based on their specific microhabitat distribution within intertidal area. Stable isotope analyses have shown that fiddler crabs exhibit distinct dietary patterns based on their habitat preferences. For example, stable isotope values for crabs decrease from the seawater edge to the mangrove forest, reflecting differences in available food sources along the intertidal gradient (Tue et al., 2017). Morphological studies further support this, showing that fiddler crabs inhabiting sandy areas tend to have a higher number of spoon-tipped setae on their maxillipeds, while those in muddy areas have fewer setae, affecting their food preferences (Bezerra et al., 2006; Mokhtari et al., 2015).

Various methods have been employed to study their feeding habits, including field observations, gut content analysis, and stable isotope analysis. Hoffman et al. (1984) who conducted the first intensive study of the feeding habits of fiddler crab combining microscopy and field experiment, reported that bacteria, algae and protozoa were observed in the gut of *Minuca pugnax* and exclusion of this species resulted an increase of meiofaunal abundance in sediment. Subsequent studies through microscopy proved that fiddler crab is an omnivorous deposit feeder with the main diet consist of detritus, diatom, algae and animal matter. However, stable isotope analysis of carbon conducted from 1984 – 2019 suggested that the major carbon source of fiddler crab was microphytobenthos with studies by Tue et al. (2012) and Tue et al. (2017), suggested fiddler crab supplement their diet with bacteria, ciliate protozoa and nematode. Previous stable isotopes studies unanimously agreed that fiddler crab do not assimilate mangrove detritus as their carbon source and their $\delta^{15}N$ was in small range ($\delta^{15}N$ of 6.1 ‰ for *Tubuca flammula* (Tue et al., 2017) to $\delta^{15}N$ of 9.0 ‰ for *T. arcuata* (Tue et al., 2012) indicating that these fiddler crab species in the same trophic level.

Disagreements between methods can be due to differences in analytical methods with different resolutions to determine the main food source. Microscopy and DNA metabarcoding measure the ingested diet items in gut over the preceding period of 6 – 48 hours, whereas stable isotope approaches estimate the assimilated items absorbed and integrated into consumer's tissue over longer period I. e muscle tissue provide assimilation of diet items for months (Nielsen et al., 2018).

The three methods used have their respective limitations and advantages. Microscopic examination is time consuming and often difficult for detail identification as most of the food item has been macerated or semi-digested and soft bodied prey item that leave no traces (Berry et al., 2015; Pompanon et al., 2012). Despite these limitation, microscopic examination is the only methods that can identifies life-stages and prey-predator size relationship (Nielsen et al., 2018). Riaz et al., (2020) who conducted microscopic examination on gut content of mesopelagic fish reported size and weighed of prey consumed have linear relationship with size and weighed of the predator fish. Microscopy also enables quantification of prey item through various methods such used in this study i.e. frequency of occurrence method (Hyslop, 1980).

DNA metabarcoding of gut contents through Illumina Miseq Sequencing was conducted to identify microbial and dietary relative abundance and taxonomical diversity using operational taxonomic units (OTU). In OTU analysis, DNA sequences are clustered together in one OTU by means of a similarity threshold (usually 97%) assigning sequences to species and higher taxonomic levels (Blaxter et al., 2005). This method able to provide very high taxa resolution and quantify the diet proportions. Berry et al. (2015) comparative study demonstrated that DNA metabarcoding revealed the presence of food items and parasite not previously recorded in marine fishes. DNA metabarcoding that described taxa relative abundance and diversity using OTU has limitations and biases that needs to be consider before executions. For example, the incomplete current public sequence reference database limits the ability to identify several prey taxa, the digestion rate of prey found in the gut might affect DNA detection and detection of secondary prey leading to overestimating of the prey taxa for an organism (Sakaguchi et al., 2017).

Stable isotope analysis provides long term data but have poor resolution on prey item. Previous study on feeding ecology of fiddler crab based on carbon stable isotope analysis only able to conclude microphytobenthos as the main food source because it has almost the same isotope value (Kawaida et., 2019; Bouillon et al., 2004; France, 1998; Rodelli et al., 1984).

Integrative analysis of microscopic identification, DNA metabarcoding to identify dietary and bacterial taxa and stable isotope analysis to precisely quantify the diet composition of the three fiddler crab species. This study highlights the thoroughness of combining multiple methods in explaining the feeding habits of invertebrate with a non-selective feeding habit. This methods combination has been used recently by researchers and has successfully elucidated the feeding ecology of several organisms and coprolites (Nakamura et al., 2020; Whitaker et al., 2019; Witt et al., 2021; Zhou et al., 2021).

Fiddler crabs play a vital role in mangrove ecosystems, acting as both prey and ecosystem engineers. Their feeding activities influence sediment structure, nutrient cycling, and benthic community composition. While traditional methods such as microscopy and stable isotope analysis have provided valuable insights into their diet, the advent of DNA metabarcoding offers new opportunities for exploring their feeding habits in greater detail. By combining these methods, future studies can deepen our understanding of the ecological processes shaping the distribution and diet of fiddler crabs in mangrove ecosystems.

CHAPTER 3

AN ASSESSSMENT OF FIDDLER CRAB ASSEMBLAGE STRUCTUREIN RELATION TO PHYSICAL HABITAT CHARACTERISTICS IN MANGROVES OF PENANG ISLAND, MALAYSIA

3.1 Introduction

3.1.1 Background study

Fiddler crabs (family Ocypodidae, subfamilies Ucinae and Gelasiminae) are among the most dominant macrobenthic organisms in intertidal habitats from tropical to warm temperate regions. Globally, these crabs are represented by 11 genera (e.g., *Uca, Tubuca, Austruca, Gelasimus*) (Rosenberg, 2014). They play crucial roles as ecosystem engineers in mangrove environments as their activities—such as burrowing, feeding, and foraging—facilitate nutrient cycling, sediment aeration, and the translocation of organic matter, thereby maintaining the ecological integrity of mangrove ecosystems (Kristensen et al., 2008; Nobbs & Blamires, 2015). Consequently, changes in the structure of fiddler crab populations can have cascading effects on mangrove ecosystem functions, which makes understanding their spatial and temporal distribution patterns essential (Checon & Costa, 2017).

Despite their importance, there is limited research on how environmental parameters shape the distribution and abundance of fiddler crabs across different microhabitats in mangrove ecosystems, particularly in Malaysia. Existing studies in the region often focus on general descriptions of crab communities or are restricted to specific areas, such as southern Peninsular Malaysia (Sasekumar, 1974; Tan & Ng, 1994; Ashton et al., 2003). Few studies have investigated the influence of both spatial and temporal scales on fiddler crab assemblages and their relationships with environmental parameters (Mokhtari et al., 2015). This gap in knowledge is particularly relevant for Penang Island in northern Malaysia, where rapid urbanization and human activities have led to significant mangrove loss, altering habitat structures and potentially affecting the benthic communities that rely on these habitats (Ramli & Zhang, 2017; Chee et al., 2017).

Mangroves in Malaysia, covering approximately 5376.86 km^2, support a high diversity of flora and fauna. Based on the data, the mangrove covers in Peninsular Malaysia decreased from 116,746 hectares in 1990 to 114,353 hectares in 2000, and further to 110,953 hectares in 2017 which represents a reduction of approximately 2.05% between 1990 and 2000, and 2.97% from 2000 to 2017 (Omar & Misnan, 2020). Penang Island, located in northern Peninsular Malaysia, covers approximately 0.19% of the mangroves in Peninsular Malaysia but faces a rapid rate of habitat loss due to urban development and human activities (Ramli & Zhang, 2017; Chee et al., 2017). This loss reduces spatial complexity in mangrove habitats, which can affect species distribution, resource partitioning, and interspecific interactions within these ecosystems (Carugati et al., 2018). Therefore, understanding how fiddler crabs utilize different microhabitats—namely, mudflats, tidal creeks, and interior forests—can provide insights into how environmental changes impact these communities.

Understanding how these microhabitats affect the distribution of fiddler crabs is essential, as changes in habitat conditions—whether due to natural variability or human impact—can lead to shifts in species composition and ecological processes (Sousa & Dangremond, 2012). For instance, sediment grain size has been found to influence the spatial distribution of fiddler crabs significantly, with species exhibiting morphological adaptations that allow them to thrive in specific sediment types (Kalpana & Lim, 2011; Mokhtari et al., 2015). Additionally, other environmental factors like light penetration, soil temperature, and salinity can also affect habitat suitability and species abundance (Nobbs & Blamires, 2015; Murai & Backwell, 2006; Shock et al., 2009).

In addition to spatial variability, seasonal changes play a critical role in influencing fiddler crab abundance and behaviour. Tropical regions like Penang Island experience distinct wet and dry seasons, which affect environmental conditions such as temperature, humidity, and sediment moisture (Meteorological Department of Malaysia, n.d.). Previous studies have shown that crab activity and abundance can fluctuate significantly between seasons, with certain species becoming more active or abundant during wetter periods due to increased nutrient availability and favourable habitat conditions (Crane, 1975; Nobbs & Blamires, 2015). Therefore, both spatial heterogeneity across microhabitats and temporal variations due to seasonal changes are key to understanding the dynamics of fiddler crab populations.

By focusing on these specific microhabitats and their environmental characteristics, this study provides valuable insights into the ecology of fiddler crabs and the potential impacts of environmental changes on mangrove ecosystems. The findings will be crucial for informing conservation and management strategies aimed at preserving the biodiversity and ecosystem functions of mangroves, which are increasingly threatened by anthropogenic pressures and climate change.

3.1.2 Objectives

This study aims to fill the knowledge gap by examining the influence of spatial and temporal variability on fiddler crab abundance and distribution in Penang Island's mangroves. Specifically, the objectives are:

1. To identify the influence of microhabitats and seasonal variations on fiddler crab abundance.
2. To determine the relationships between fiddler crab abundance and physicochemical variables in mangrove ecosystems.

3.2 Materials and methods

3.2.1 Study area

Sampling was conducted at three mangrove sites on Penang Island, located on the northwest coast of Peninsular Malaysia (Figure 3.1). The study sites are Teluk Tempoyak (TT) (05′ 15.930′ N, 100′ 11.54′ E), Pulau Betong (PB) (05′ 18.432′ N, 100′ 11.663′ E), and Kuala Sungai Pinang (KSP) (05′ 23.473′ N, 100′ 11.622′ E) (Figure 3.1-3.4). The range of intertidal heights (mean low water spring and mean high water spring) in Penang Island is 0.72–2.69 m (National Hydrographic Center, 2017). Within each site, three microhabitats approximately 50 m apart were selected across the tide line, beginning from the uppermost edge of mangrove vegetation (mean high water spring) towards seawater (mean low water spring). Microhabitats within each mangrove were affected by different levels of tidal inundation (Table 3.1). Descriptions for each site and microhabitat were observed and recorded in Table 3.1.