Floral Characteristics and Pollination of *Sonneratia* spp. (Lythraceae) in Southern Thailand

Christine Ely Nuevo Diego

A Thesis Submitted in Partial Fulfillment of the Requirements for the
Degree of Master of Science in Ecology (International Program)
Prince of Songkla University
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I hereby certify that this work has not been accepted in substance for any degree, and is not being currently submitted in candidature for any degree.

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วิทยานิพนธ์ ลักษณะดอกและการถ่ายเรณูของพืชสกุล *Sonneratia* ในจังหวัดสตูล ในภาคใต้ของประเทศไทย

ผู้เขียน Christine Ely Nuevo Diego

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บทคัดย่อ

ป่าชายเลนมีส่วนช่วยอย่างมากในการบริการทางระบบนิเวศ แม้ว่าจะมีความสำคัญแต่ยังคงมีการลดลงของป่าชายเลนอย่างต่อเนื่อง ในการอนุรักษ์ชนิดพันธุ์ของสิ่งมีชีวิตนั้นจำเป็นอย่างยิ่งที่จะต้องมีความรู้เกี่ยวกับสิ่งมีชีวิตเหล่านั้น การศึกษาในครั้งนี้ถูกวิจัยขึ้นเพื่อศึกษาเกี่ยวกับชีววิทยาการสืบพันธุ์ของลำแพน (*Sonneratia ovata*) และลำแพนหิน (*S. griffithii*) โดยศึกษาการแสดงออกทางชีววิทยาของดอก รวมไปถึงลักษณะสัณฐานวิทยา การบาน การผลิตน้ำต้อย และอัตราส่วนละอองเรณูต่อออวุลของประชากรของลำแพนจำนวน 1 กลุ่มประชากรและลำแพนหิน 2 กลุ่มประชากรในจังหวัดสตูล ดอกลำแพนและลำแพนหินถูกนำมาใช้ในการทดลองเกี่ยวกับการถ่ายเรณู โดยมี 5 ชุดการทดลอง (การถ่ายเรณูแบบเปิด การถ่ายเรณูโดยแมลง การถ่ายเรณูด้วยมือระหว่างดอก การถ่ายเรณูด้วยมือภายในดอกเดียวกันและการถ่ายเรณูตามธรรมชาติ) นอกจากนี้ยังทำการสำรวจผู้ผสมเกสรโดยใช้กล้องดักถ่ายสัตว์ด้วย โดยผลการศึกษาชี้ให้เห็นว่าทั้งลำแพนและลำแพนหินมีแสดงลักษณะการถ่ายเรณูข้ามด้วยการมีระยะห่างระหว่างอับเรณูและยอดเกสรเพศเมียและการที่ยอดเกสรเพศเมียพร้อมรับการผสมก่อนอับเรณูแตก อัตราส่วนระหว่างเรณูต่อออวุลของลำแพนแสดงลักษณะระบบผสมพันธุ์แบบ facultative xenogamy จากผลการทดลองการถ่ายเรณูพบว่าลำแพนมีการผสมเกสรภายในตัวเองค่อนข้างสูง แต่ลำแพนหินแสดงการไม่มีการผสมเกสรภายในตัวเองค่อนข้างสูง มากไปกว่านั้นจากการทดลองการถ่ายเรณูชี้ให้เห็นว่าผู้ผสมเกสรนั้นมีความสำคัญอย่างมากในการสืบพันธุ์ของลำแพนและลำแพนหิน อีกทั้งในฤดูแล้งไม่พบการติดผลของลำแพนซึ่งชี้ให้เห็นถึงความแห้งแล้งในช่วงเอลนีโญ ปี พ.ศ. 2559 ทำให้เกิดข้อจำกัดด้านทรัพยากรอย่างรุนแรง ส่วนลำแพนหินมีการติดผลต่ำ เนื่องจากลมแรงที่เกิดจากการสัญจรของยานพาหนะในพื้นที่ศึกษา ค้างคาวหน้ายาวเล็กเป็นค้างคาวชนิดเดียวที่ถูกจับด้วยตาข่ายใกล้กับดอกและดูเหมือนจะเป็นสัตว์ที่ช่วยผสมเกสรที่สำคัญที่สุดของลำแพน ในทางตรงกันข้ามค้างคาวหน้ายาวเล็กและค้างคาวเล็บกุดถูกจับด้วยตาข่ายใกล้กับดอกลำแพนหิน อย่างไรก็ตาม จากการสังเกตค้างคาวพบว่าค้างคาวจะหลีกเลี่ยงบริเวณที่มีแสงส่อง ผลการศึกษานี้แสดงให้เห็นว่าสัตว์ที่ช่วยถ่าย

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ABSTRACT

Mangrove forests provide numerous ecosystem services. Despite their importance, they continue to be lost. Conserving a species requires knowledge about them. This study was conducted to find out about the reproductive biology of *Sonneratia ovata* and *S. griffithii*. Floral biology traits, including morphology, anthesis, nectar production, and pollen-ovule ratio, were determined from three *S. ovata* populations in southern Thailand and two *S. griffithii* populations in Satun Province. Flowers were used in a pollination experiment with five treatments (open pollination, insect pollination, hand-cross pollination, hand-self pollination, and spontaneous autogamy). Pollinators were also observed using camera traps. Results show that both species display xenogamous characteristics through herkogamy and protogyny. Their pollen-ovule ratio both indicate facultative xenogamy. Results from the pollination experiment showed that *S. ovata* is highly self-compatible but *S. griffithii* is largely self-incompatible. Moreover, the pollination experiments indicate that pollinators are critical for the reproduction of both species. Additionally, the lack of fruit set during the dry season of the El Niño year of 2016 appeared to indicate severe resource limitation for *S. ovata*. For *S. griffithii*, fruit set in the study site with strong vehicle-generated wind was lower. *Macroglossus minimus* was the only bat species netted near the *S. ovata* flowers, and is likely its most important pollinator. By contrast, *M. minimus* and *Eonycteris spelaea* were netted near *S. griffithii* flowers. However, bats were observed to avoid illuminated flowers. My findings reveal that pollinators increase the reproductive success of both mangrove species, indicating that conservation efforts should be directed towards both this mangrove species and its pollinators.

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Christine Ely Nuevo Diego

CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1

INTRODUCTION

1.1 General Introduction

The mangrove forest community occupies most tropical shorelines — including those of sheltered bays, estuaries, and inlets — as well as along riverbanks that are still within reach of ocean tides (Tomlinson 1986). Mangrove forests provide an estimated minimum of 1.6 billion USD yearly in ecosystem services (Polidoro *et al.* 2010). In addition to providing fuel wood, timber, food, and medicine, these forests help maintain fisheries by providing breeding habitats for offshore fish species (Barbier *et al.* 2011, Sathirathai & Barbier 2001). They also provide natural ‘‘coastal storm barriers’’ against wind and wave action during storm surge events, such as coastal floods, typhoons, and tsunamis (Barbier *et al.* 2011, Sathirathai & Barbier 2001). Additionally, mangroves have the ability to control erosion, which in Thailand, the government spends an estimated USD 3,679 per ha per year constructing artificial barriers for (Sathirathai & Barbier 2001). Lastly, mangrove forests purify water and sequester carbon, as well as provide opportunities for recreation, tourism, research, and education (Barbier *et al.* 2011, Sathirathai & Barbier 2001).

Mangrove forests presumably covered about a third of the world’s coastlines in the 1970s (Barbier *et al.* 2011). Yet despite their importance, at least 35% of these forests are now lost and continue to decline at a yearly rate of about 1-2% (Barbier *et al.* 2011). In Thailand, mangrove destruction is primarily due to the expansion of the shrimp farming industry (Sathirathai & Barbier 2001), which also contributes to about 38% of worldwide mangrove loss (Polidoro *et al.* 2010). As of 2000, Giri *et al.* (2011) estimated that there are only 137,760 km^2 of mangrove areas remaining in the world. Furthermore, about 26% of remaining mangroves are degraded due to overharvesting of timber and fuelwood (Polidoro *et al.* 2010). Although mangrove restoration projects are being implemented in many areas, successful projects are typically limited to monocultures of either *Rhizophora* or *Avicenna* species

(Polidoro *et al.* 2010). In such instances, although forest cover can be restored, the natural mangrove ecosystem and its associated ecosystem services are often deficient (Polidoro *et al.* 2010).

Sonneratia ovata Backer 1920 and *S. griffithii* Kurz 1871 are true mangrove species regarded as at risk of becoming endangered (Duke *et al.* 2010, Salmo III *et al.* 2010, Polidoro *et al.* 2010). Although *S. ovata* is widespread, only a few populations now exist, with a small number of individuals in each (Zhou *et al.* 2010). More importantly, Zhou *et al.* (2010) found that this species is genetically depauperate, which is further compounded by the observation of Nor Zalipah (2014) that these plants have low fruit set. Of even greater concern is *S. griffithii*, which is rare, no longer found in many parts of its range, and continues to decline in number (Duke *et al.* 2010).

Information about the ecology and reproduction of these two species is needed to successfully conserve this species. Although the reproductive biology of other *Sonneratia* species have been studied before, particularly in *S. alba* Sm. and *S. caseolaris* (L.) Engl. (Coupland *et al.* 2005, Nor Zalipah & Adzemi 2017, Pandit & Choudhury 2001, Primack *et al.* 1981), no such studies have been published about *S. ovata* and *S. griffithii*.

1.2 Literature Review

1.2.1 Floral Characteristics

Studying floral biology can provide insight to the pollination ecology of plants. For example, the length of the corolla or the shape of the flower can exclude possible pollinators having the wrong body shape and size (Kearns & Inouye 1993); and the position of the anthers can put pollen on different parts of the body of a pollinator, thereby minimizing the mixing of non-conspecific pollen on a pollinator’s body to prevent pollen clogging and reduce seed sets (Kearns & Inouye 1993, Stewart & Dudash 2016). The presence of herkogamy often signifies that flowers are under selection to reduce self-pollination (Willmer 2011). Furthermore, the morphology and behavior of flowers tend to be correlated with their pollinators, such that authors, including Faegri & Pijl (1979), have described several pollination syndromes: *e.g.* phalaeonophily (pollination by moths) and chiropterophily (bats).

Flowers pollinated by moths are usually tubular, commonly with small openings; are very light colored or white; are rarely inflorescences but may occur as a clump of a few flowers; have anthesis at dusk or night; often close during the day; and tend to have strong and heavy-sweet odor at night (Faegri & Pijl 1979, Willmer 2011). These phalaenophilous flowers are often pendent or horizontal with a medium amount of nectar hidden deep within the blossom (Faegri & Pijl 1979, Willmer 2011). Nectar guides are usually absent (Willmer 2011).

Flowers pollinated by bats have some similar characteristics to those pollinated by moths. These flowers also have similar colors, have no nectar guides, and have anthesis at dusk or night (Willmer 2011). However, unlike phalaenophilous plants, chiropterophilous plants have robust single flowers or inflorescences that are positioned outside the foliage, have abundant pollen in large or numerous anthers, have a fermenting odor, and have large amounts of nectar that are usually easily accessible (Faegri & Pijl 1979, Willmer 2011).

1.2.2 Plant Breeding Systems

The pollinators of any zoophilous plants can often be inferred based on the morphology of their flowers given that pollination by an animal that does not fit the flower morphologically or behaviorally is inefficient (Tschapka 2003). Although floral morphology is helpful in providing clues about a plant's pollination syndrome, Cruden (1977) suggested that the pollen-ovule (P/O) ratio is a good indicator of a plant's breeding system. P/O ratios are computed by estimating the number of pollen grains produced by a flower and dividing this by the number of ovules (Cruden 1977). Plants should be under selection to produce the number of pollen grains that best maximizes seed set (Cruden 1977). Therefore, the more efficient a breeding system is, the fewer pollen grains the plant needs to produce (Cruden 1977). Table 1 shows the results of Cruden's study on the P/O ratios of different plants and their breeding systems.

Table 1. Breeding systems and mean pollen-ovule ratios of some grass angiosperm species. (Table adapted from Cruden 1977).

Breeding Systems	N	P/O ± SE
Cleistogamy	6	4.7 ± 0.7
Obligate autogamy	7	27.7 ± 3.1
Facultative autogamy	20	168.5 ± 22.1
Facultative xenogamy	38	796.6 ± 87.7
Xenogamy	25	5,859.2 ± 936.5

1.2.3 *Sonneratia* spp.

Sonneratia flowers are hermaphroditic, having both the female (one pistil) and male (numerous stamens) reproductive parts in each flower (Tomlinson 1986). In Malaysia, *Sonneratia alba, S. caseolaris*, and *S. ovata* are known to flower year-round, with varying peak flowering months (Mohamed 2014).

There are 4 *Sonneratia* species found in Thailand: *S. alba, S. caseolaris, S. ovata,* and *S. griffithii* (Department of Marine and Coastal Resources 2012). The first two are listed as Least Concern (Kathiresan *et al.* 2010a, 2010b), *S. ovata* is listed as Near Threatened (Salmo III *et al.* 2010), and *S. griffithii* is listed as Critically Endangered (Duke *et al.* 2010) by the IUCN Redlist. According to a report by the Department of Marine and Coastal Resources (2012), *S. ovata* flowers year-round and *S. griffithii* flowers from August to November in Thailand.

1.2.4 *Sonneratia ovata* Backer 1920

In Thailand this species is called “Lamphaen” (Smitinand and Larsen 1992; Department of Marine and Coastal Resources 2012). It can be found on firm mud far away from the shore but still within salt-water influence (Tomlinson 1986; Salmo III *et al.* 2010). It can live in clay soil and can tolerate occasionally being submerged partially in water with low salinity (Department of Marine and Coastal Resources 2012).

Sonneratia ovata is primarily differentiated from the other *Sonneratia* spp. by its calyx. These wrap around the base of fruits and have fine warts (Tomlinson 1986). Their pnuematophores are also knotty, unlike those of the other *Sonneratia* spp. (Department of Marine and Coastal Resources 2012).

Sonneratia ovata has solitary flowers, but usually form inflorescences of up to four flowers, with only one or two flowers blooming at a time (Mohamed 2014). These flowers have no petals (Tomlinson 1986). Each flower only has one style although it has approximately 300 stamens (Nor Zalipah 2014). The gap between the tip of the style and the tip of the stamens is about 4 mm (Nor Zalipah 2014).

1.2.5 *Sonneratia griffithii* Kurz 1871

This species has a limited distribution and can only be found along the shores of the Andaman Sea (Tomlinson 1986; Duke *et al.* 2010). Because of its restricted distribution and the fact that it is now rare or locally extinct in parts of its range, this species has been classified as critically endangered by the International Union for the Conservation of Nature (IUCN) Redlist (Duke *et al.* 2010). In Thailand, its range spans the entire western shore and as well as islands in the Andaman Sea (Duke *et al.* 2010). It is locally called “Lamphaen hin” (Smitinand and Larsen 1992; Department of Marine and Coastal Resources 2012).

The flowers of *S. griffithii* are solitary or occur in inflorescences of up to 3 flowers found at the ends of branches. Each flower has 6-8 sepals and petals that easily fall. Its fruits are hard and dark green with a persistent calyx, like the other *Sonneratia* fruits, but the calyx of *S. griffithii* spreads outwards and the tip of each sepal curves slightly inwards instead of away from it as with *S. alba* (Department of Marine and Coastal Resources 2012).

1.3 Research Questions

1.3.1 What are the floral characteristics of *Sonneratia* spp. in southern Thailand?

1.3.2 What are the breeding systems of the *Sonneratia* spp. in Satun, Thailand based on their pollen-ovule ratio (P/O)?

1.3.3 For *Sonneratia* spp., is there a difference between the fruit and seed set produced from the following treatments: open pollination, insect pollination, hand-cross pollination, hand-self pollination, and spontaneous autogamy

1.3.4 What are the flower visitors of *Sonneratia* spp. in southern Thailand?

1.4 Research Objectives

The objective of this research was to investigate the floral and pollination biology of *Sonneratia* spp., particularly *Sonneratia ovata* and *S. griffithii*, in southern Thailand. Specifically, the study aimed to:

i) Observe floral morphological traits that indicate xenogamous or autogamous mating systems;
ii) Conduct a pollination experiment to determine if the species is self-compatible, as well as to investigate the contribution of pollinators to its reproductive success; and
iii) Observe flower visitors that potentially pollinate these mangrove species

1.5 Research Hypotheses

1.3.5 *S. ovata* has floral characteristics similar to *S. alba* and *S. caseolaris*. When Nor Zalipah (2014) studied *S. alba*, *S. caseolaris* and (some characteristics of) *S. ovata*, she discovered that there were many similarities but with clear minor differences among the three species, allowing them to be distinct from each other. I expect my findings on *S. ovata* to be similar to those of Nor Zalipah (2014) and that *S. griffithii*, being part of the genera, to be similar as well.

1.3.6 Nor Zalipah (2014) found that the P/O ratio of *S. alba* is 63.99 ± 9.07 and that of *S. ovata* is 53.71 ± 17.73. According to Cruden (1977), this puts the P/O ratios of *S. alba* and *S. ovata* P/O ratio between obligate autogamy and facultative autogamy. Nor Zalipah classified both as being obligately autogamous, but concluded that cross pollination results in better reproductive success. Therefore, I expect the *Sonneratia* spp. in southern Thailand to have similar results as those found by Nor Zalipah and that both *S. ovata* and *S. griffithii* are facultatively autogamous plants.

1.3.7 Pandit & Choudhury (2001) found that open pollination produced significantly higher fruit set than the all-bagged treatment (which excluded all animals from the flowers) in *S. caseolaris* in India. I also expect the same result for my two study species in southern Thailand. In the Malaysian study by Nor Zalipah (2014), she found that insect pollination in *S. caseolaris* produced only half the fruit set and seed set compared to that of bat pollination. I also expect the insect and open pollination treatments of the *Sonneratia* spp. in southern Thailand to produce results similar to Nor Zalipah's since both areas (Terengganu, Malaysia and Satun, Thailand) have many bats visiting the *Sonneratia* flowers, which are predicted to be more effective pollinators.

1.3.8 Pandit & Choudhury (2001) found that *S. caseolaris* in India blooms both day and night for 56 hours and are mainly outcrossed. They found that moths and butterflies (Order Lepidoptera); wasps (Order Hymenoptera); flies (Order Diptera); birds (Order Passeriformes); squirrels and rats (Order Rodentia); and rhesus macaques (Order Primates) visit the flowers. I expect the flowers of *Sonneratia* spp. in southern Thailand to have similar flower visitors, with the addition of bats which are known to be in the area as found by Stewart *et al.* (2014).

CHAPTER 2

Pollinators increase reproductive success of a self-compatible mangrove, *Sonneratia ovata*, in southern Thailand

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Pollinators increase reproductive success of a self-compatible mangrove, *Sonneratia ovata*, in southern Thailand

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ABSTRACT

Sonneratia ovata is a pioneer mangrove tree species classified as Near Threatened. To protect it, more information about its ecology and reproduction is required. This study was conducted to find out about the reproductive biology of *S. ovata*. Floral biology traits, including morphology, anthesis, nectar production, and pollen-ovule ratio, were determined from three populations in southern Thailand. Flowers were used in a pollination experiment with five treatments (open pollination, insect pollination, hand-cross pollination, hand-self pollination, and spontaneous autogamy). Pollinators were also observed using camera traps. Results show that *S. ovata* displays xenogamous characteristics through herkogamy and protogyny. Its pollen-ovule ratio indicates facultative xenogamy. Results from the pollination experiment showed that *S. ovata* is highly self-compatible. However, in the wet season, pollination success was significantly higher for open pollination than for spontaneous autogamy, indicating that pollinators are critical for *S. ovata* reproduction. Pollination treatments produced no fruit set in the dry season, suggesting that drought during the El Niño year of 2016 posed severe resource limitation. *Macroglossus minimus* was the only bat species netted near the flowers, and likely the most important pollinator. Our findings reveal that pollinators increase the reproductive success of *S. ovata*, indicating that conservation efforts should be directed towards both this mangrove species and its pollinators.

บทคัดย่อ

ลำแพน เป็นไม้ยืนต้นป่าชายเลนเบิกนำที่ถูกจัดสถานะว่าเป็นชนิดที่เกือบอยู่ในข่ายเสี่ยงต่อการสูญพันธุ์ การศึกษาเกี่ยวกับนิเวศวิทยาและการสืบพันธุ์จึงมีความจำเป็นในการอนุรักษ์พืชชนิดนี้ การศึกษาครั้งนี้จึงศึกษาเกี่ยวกับชีววิทยาการสืบพันธุ์ของลำแพน ลักษณะทางชีววิทยาของดอกอันประกอบไปด้วยลักษณะทางสัณฐานวิทยา การบาน การผลิตน้ำต้อย และอัตราส่วนของเรณูต่อออวุลถูกศึกษาจากกลุ่มประชากร 3 กลุ่ม ในภาคใต้ของประเทศไทย ดอกลำแพนถูกใช้ในการทดลองการถ่ายเรณูซึ่งประกอบด้วย 4 ชุดการทดลอง (การถ่ายเรณูแบบเปิด การถ่ายเรณูโดยแมลง การถ่ายเรณูข้ามด้วยมือ การถ่ายเรณูภายในดอกเดียวกันด้วยมือ การถ่ายเรณูภายในดอกเดียวกันด้วยตนเอง) นอกจากนี้ สัตว์ที่ช่วยในการถ่ายเรณูถูกสังเกตด้วยกล้องดักถ่ายสัตว์ ผลการศึกษาพบว่าลำแพนแสดงลักษณะการถ่ายเรณูข้ามด้วยการมีระยะห่างระหว่างอับเรณูและยอดเกสรเพศเมียและการที่ยอดเกสรเพศเมียพร้อมรับการผสมก่อนอับเรณูแตก อัตราส่วนระหว่างเรณูต่อออวุลของลำแพนแสดงลักษณะระบบผสมพันธุ์แบบ facultative xenogamy จากผลการทดลองการถ่ายเรณูพบว่าลำแพนมีการผสมเกสรภายในตัวเองค่อนข้างสูง อย่างไรก็ตาม ในช่วงฤดูฝนความสำเร็จในการถ่ายเรณูแบบเปิดสูงกว่าการถ่ายเรณูภายในดอกเดียวกันด้วยตนเองอย่างมีนัยสำคัญ สัตว์ที่ช่วยในการถ่ายเรณูจึงมีความสำคัญต่อการสืบพันธุ์ของลำแพน ในฤดูแล้งไม่พบการติดผลของลำแพนซึ่งชี้ให้เห็นถึงความแห้งแล้งในช่วงเอลนีโญ ปี พ.ศ. 2559 ทำให้เกิดข้อจำกัดด้านทรัพยากรอย่างรุนแรง ค้างคาวหน้ายาวเล็กเป็นค้างคาวชนิดเดียวที่ถูกจับด้วยตาข่ายใกล้กับดอกและดูเหมือนจะเป็นสัตว์ที่ช่วยผสมเกสรที่สำคัญที่สุดของลำแพน ผลการศึกษานี้แสดงให้เห็นว่าสัตว์ที่ช่วยถ่ายเรณูเพิ่มความสำเร็จในการสืบพันธุ์ของลำแพน ความพยายามในการอนุรักษ์จึงควรเน้นทั้งลำแพนและสัตว์ที่ช่วยผสมเกสรของพืชชนิดนี้

Keywords:

Chiropterophily, old world tropics, pioneer mangrove species, resource limitation

2.1 Introduction

The mangrove forest community occupies most tropical shorelines — including those of sheltered bays, estuaries, and inlets — as well as along riverbanks that are still within reach of ocean tides (Tomlinson 1986). Mangrove forests provide an estimated minimum of 1.6 billion USD yearly in ecosystem services (Polidoro *et al.* 2010). In addition to providing fuel wood, timber, food, and medicine, these forests help maintain fisheries by providing breeding habitats for offshore fish species (Barbier *et al.* 2011, Sathirathai & Barbier 2001). They also provide natural ‘‘coastal storm barriers’’ against wind and wave action during storm surge events, such as coastal floods, typhoons, and tsunamis (Barbier *et al.* 2011, Sathirathai & Barbier 2001). Additionally, mangroves have the ability to control erosion, which in Thailand, the government spends an estimated USD 3,679 per ha per year constructing artificial barriers for (Sathirathai & Barbier 2001). Lastly, mangrove forests purify water and sequester carbon, as well as provide opportunities for recreation, tourism, research, and education (Barbier *et al.* 2011, Sathirathai & Barbier 2001).

Mangrove forests presumably covered about a third of the world’s coastlines in the 1970s (Barbier *et al.* 2011). Yet despite their importance, at least 35% of these forests are now lost and continue to decline at a yearly rate of about 1-2% (Barbier *et al.* 2011). In Thailand, mangrove destruction is primarily due to the expansion of the shrimp farming industry (Sathirathai & Barbier 2001), which also contributes to about 38% of worldwide mangrove loss (Polidoro *et al.* 2010). As of 2000, Giri *et al.* (2011) estimated that there are only 137,760 km^2 of mangrove areas remaining in the world. Furthermore, about 26% of remaining mangroves are degraded due to overharvesting of timber and fuelwood (Polidoro *et al.* 2010). Although mangrove restoration projects are being implemented in many areas, successful projects are typically limited to monocultures of either *Rhizophora* or *Avicenna* species

(Polidoro *et al.* 2010). In such instances, although forest cover can be restored, the natural mangrove ecosystem and its associated ecosystem services are often deficient (Polidoro *et al.* 2010).

Sonneratia ovata Backer 1920, or mangrove apple, is a true mangrove species regarded as at risk of becoming endangered (Polidoro *et al.* 2010, Salmo III *et al.* 2010, Tomlinson 1986). Although this species is widespread, only a few populations now exist, with few individuals in each (Zhou *et al.* 2010). More importantly, Zhou *et al.* (2010) found that this species is genetically depauperate, which is further compounded by the observation of Nor Zalipah (2014) that these plants have low fruit set. Information about the ecology and reproduction of *S. ovata* is needed to successfully conserve this species. Although the reproductive biology of other *Sonneratia* species have been studied before, particularly in *S. alba* Sm. and *S. caseolaris* (L.) Engl. (Coupland *et al.* 2005, Nor Zalipah & Adzemi 2017, Pandit & Choudhury 2001, Primack *et al.* 1981), no such studies have been published about *S. ovata.*

To find out about the reproductive biology of *S. ovata,* we observed floral morphological traits that indicate xenogamous or autogamous mating systems; conducted a pollination experiment to determine if the species is self-compatible, as well as to investigate the contribution of pollinators to its reproductive success; and observed flower visitors that potentially pollinate this mangrove species. We hypothesized that *S. ovata* is self-compatible, yet experiences increased reproductive success when animal pollinators (specifically, bats) promote outcrossing, as was found with *S. alba* and *S. caseolaris* (Nor Zalipah 2014, Nor Zalipah & Adzemi 2017).

2.2 Methods

2.2.1 Study sites

We conducted this study at three sites in southern Thailand: (1) Khlong Kae and (2) Hua Tang in Satun province on the western side of the peninsula, bordering Malaysia, and (3) Koh Yor in Songkhla province on the eastern side of the peninsula.

Khlong Kae (6°32'46.5"N 100°03'46.1"E) is a tiny village very close to a small river and is surrounded by a mangrove reforestation site that was planted primarily with *S. ovata* and *Rhizophora* trees (Nuevo-Diego, pers. obs.). Some of the trees used in this study were planted during reforestation efforts, but three occur naturally. The trees at this site are on soft, muddy ground that dries up when there is no rain. Unlike the reforested trees, the naturally occurring individuals have some roots that extend into the main river or its stream offshoots.

Hua Tang (6°37'09.9"N 100°04'48.7"E) is approximately 8 km from Khlong Kae. This area used to be an extensive mangrove forest but has now mostly been developed into an urban area. The trees used in this study are part of the remnants of that forest, and occur along a small brook (containing water year-round) next to a local road.

In contrast, Koh Yor is an island that sits within Songkhla Lake. The study site (7°09'20.3" to 7°09'26.7"N 100°32'04.7" to 100°32'07.9"E) is located on the western side of the island, away from the influx of sea water that enters Songkhla Lake from the Gulf of Thailand. Pornpinatepong *et al.* (2011) categorized this area as a lake-water stagnation zone. The trees used in this study are submerged in low-salinity brackish water (Pornpinatepong *et al.* 2011) that is approximately 0.1-1.5 meters deep throughout the year (Nuevo-Diego, pers. obs.).

2.2.2 Study species

Sonneratia ovata is a pioneer mangrove tree that occupies firm mud along downstream estuaries at high intertidal zones, but still within salt-water influence (Polidoro *et al.* 2010, Salmo III *et al.* 2010). It can survive in clay soil and can tolerate submersion in low-salinity water (Department of Marine and Coastal Resources 2012). This species is distributed in SE Asia, China (Hainan Island), Palau, northeastern Australia, and southern Papua New Guinea (Duke & Jackes 1987, Salmo III *et al.* 2010). It is fairly common within its range but has experienced extensive losses at the margins of its distribution (Polidoro *et al.* 2010). Hence, the IUCN Redlist classifies *S. ovata* as Near Threatened and recommends local area protection (Salmo III *et al.* 2010).

Sonneratia ovata has solitary, hermaphroditic brush flowers that form inflorescences (Nor Zalipah 2014, Tomlinson 1986). The flowers have no petals (Duke & Jackes 1987, Nor Zalipah 2014, Tomlinson 1986). Each flower has a single style surrounded by numerous shorter stamens (Duke & Jackes 1987, Nor Zalipah 2014, Tomlinson 1986). This species is known to be pollinated by bats and possibly hawk moths (Tomlinson 1986, Watzke 2006).

2.2.3 Floral biology

We observed *S. ovata* flowers (n = 14 trees) from April 2016-March 2017, noting the number of flowers per inflorescence, as well as the timing of blooming, anthesis, and anther abscission.

To determine anther-stigma distance (ASD), we used a Vernier caliper to measure the distance from the stigma to the tip of the nearest anther in 15 randomly chosen flowers from five trees in the dry season, as well as 39 randomly chosen flowers from 10 trees in the wet season.

We observed the stigma receptivity of 22 flowers from 12 trees. Prior to full bloom, we emasculated and bagged the flowers to exclude all pollinators. We tested stigma receptivity using the hydrogen peroxide test (with 3% H_2O_2) following Kearns & Inouye (1993). With the aid of a magnifying glass, we checked each stigma for the presence of bubbles, which indicates receptivity. We tested stigma receptivity at approximately 17h00, 19h00, 21h00, and 23h00 on the first night of blooming; at 05h00 the following morning; and at 19h00 and 07h00 each day thereafter until the stigma was unreceptive or the flower abscised.

We checked for pollen availability by brushing a glass capillary tube against the anthers and checking it for the presence of any pollen grains. Data were collected from 56 randomly-chosen flowers (15 flowers from three trees in Khlong Kae during the dry season, as well as 41 flowers from two trees in Khlong Kae, four trees in Hua Tang, and five trees in Koh Yor during the wet season).

To test pollen viability, we randomly collected 20 flowers from nine trees in the afternoon before anthesis and allowed them to bloom under room conditions in the laboratory, following a modified version of Kearns & Inouye (1993). We placed a petri dish under each flower to collect falling pollen. Between 20h00 and 21h00, we collected pollen and mixed it into a drop of 15% sucrose solution on a microscope slide, then covered each drop with a cover slip before placing the slides upside down inside a humid, dark chamber. After 12 hours, we added a drop of basic fuchsin to the sample (which dyed pollen grains to increase visibility) and observed the slides under a microscope at 100x magnification. We calculated the percentage of germinated pollen grains at 10 non-overlapping fields of view per slide, each field of view containing 50-100 pollen grains. We repeated this method every 12 hours for five days, in one to three replicates per flower, and averaged the results for each tree.

We collected nectar from the same flowers used to examine pollen availability (see above). We followed a modified version of the methods used by Bumrungsri *et al.* (2009), Kearns & Inouye (1993), and Nor Zalipah (2014). To prevent visitors from gaining access to the flowers, we enclosed them in nylon mesh bags before anthesis and kept them bagged throughout nectar collection. Using 75-µL microcapillary tubes, we collected nectar at 17h00, 19h00, 21h00, 23h00, 04h00, and 07h00 during anthesis. We used an Atago N-1α hand refractometer (Tokyo, Japan) to measure sugar content. We estimated nectar volume by using a Vernier caliper to measure the length of nectar within the microcapillary tube, and then converted the length to µL, where 1 mm equals 1 µL of nectar.

2.2.4 Breeding system determination using pollen-ovule ratio

We determined the pollen-ovule (P/O) ratio of *S. ovata* following Cruden (1977), Kearns & Inouye (1993), and Nor Zalipah (2014). For both pollen and ovule counts, we collected 27 random flowers from 10 trees in the afternoon prior to full bloom and kept them under room conditions in the laboratory.

To estimate the total number of pollen grains per flower, we collected 10 anthers from each flower and placed them in 1 mL of 75% ethyl alcohol. For each sample, we then vigorously mixed the solution, removed three 20-μL aliquots, and counted all pollen grains under a light microscope. We then multiplied the average number of pollen grains by the dilution factor and then by the average number of anthers per flower.

To estimate the total number of ovules per flower, we removed part of the ovary cover to reveal at least one entire locule under a stereomicroscope, and counted all ovules within the locule. We next split the ovary cross-wise and counted the number of locules. Finally, we multiplied the number of ovaries in one locule with the number of locules.

2.2.5 Pollination experiment

Following a modified version of Bumrungsri *et al.* (2009) and Nor Zalipah (2014), we performed a pollination experiment with five treatments: (1) open pollination, which allowed all pollinators to visit the flowers; (2) insect pollination, in which flowers were placed in baskets with a mesh size of 3x3 cm; (3) hand-self pollination, in which flowers were pollinated by hand using pollen from the same flower, (4) hand-cross pollination, in which flowers were emasculated before anthesis and then pollinated by hand using pollen from flowers of other *S. ovata* trees; and (5) spontaneous autogamy, in which flowers were bagged before anthesis. To protect hand-cross pollination, hand-self pollination, and spontaneous autogamy flowers from all visitors, we enclosed them in nylon mesh bags (mesh size of 1x1 mm) throughout each flower's entire blooming period. These bags were supported by a light frame to prevent the bags from contacting the flowers. Flowers in the hand-cross and hand-self pollination treatments were pollinated once between 20h00 and 21h00. We randomly chose an average of three flowers per treatment per tree throughout each season, using six trees in the dry season (75 flowers) and 16 trees in the wet season (193 flowers).

To protect the flowers from insect and small animal damage, we enclosed all experimental flowers in the same mesh bags described above (without the internal frame to keep the bags light) three days after the night of anthesis. We calculated pollination success as the proportion of flowers surviving for at least 14 days, since most floral abortions were observed to occur within the first two weeks following anthesis.

We checked all experimental flowers weekly for abortions and maturity. Fruits are mature after eight to 11 weeks, when they are easy to remove from their calyces, have a sour smell, and softer flesh. We collected, dried, and counted the seeds from all mature fruits. All fruits with seed damage were excluded from seed counting.

2.2.6 Floral visitors

We observed bat visitation rates for 31 camera trap nights using M-1100i (trigger speed: 0.5 s, recovery time: 5.5 s) and D55 IRxt (trigger speed: 1.7 s, recovery time: 8 s) Moultrie camera traps (Alabaster, USA). We set up the camera traps before sunset 1.5-3 m away from *S. ovata* blossoms and removed the camera traps early in the morning. To identify bats visiting the flowers, we set up mistnets (2.6 m x 9 m) as close as possible to *S. ovata* flowers for two nights from 17h30 until midnight in Khlong Kae. We checked the nets at least once every half an hour and assumed that all bats caught in the nets were visiting *S. ovata* flowers. We identified the bats using Francis (2008). We observed insects in person and caught those visiting flowers by hand.

2.2.7 Data analyses

We used two measures of reproductive success: pollination success and fruit set. We calculated pollination success as the number of flowers lasting two weeks divided by the total number of flowers subjected to treatments. On the other hand, we calculated fruit set as the number of mature fruits divided by the number of flowers that survived more than two weeks (which were considered to be successfully pollinated). Thus, pollination success reflects the proportion of flowers receiving sufficient pollen to fertilize ovules, while fruit set also incorporates any fruit abortion that occurred (*e.g.*, due to poor pollen quality or resource limitation).

We used R statistical environment 3.4.2 (R Core Team 2017) for all analyses. We present all descriptive results as mean ± SE. We performed generalized linear mixed modelling (GLMM) using the package "lme4" (Bates *et al.* 2015) to determine which predictors significantly affected each of our dependent variables (pollination success, fruit set, and seed count). Pollination success and fruit set were analyzed with binomial distributions, and seed count was analyzed with a Poisson distribution. The fixed factors for each analysis were treatment, season, location, and pairwise interactions, while tree individual was included as a random factor. Nested likelihood ratio tests were used to determine which model best fit the data, and model selection was verified by Akaike Information Criterion (AIC) scores. We ran Tukey tests using the package "Lsmeans" (Lenth 2016) for post-hoc testing.

2.3 Results

2.3.1 Floral biology

While individual *S. ovata* trees did not flower continuously, at the population level they almost continuously had flowers from April 2016-March 2017. However, we observed that *S. ovata* trees had few or no flowers during continuous days of rain or strong heat, and tended to flower more after the end of a rainy period.

Sonneratia ovata formed cymes and occasionally compound cymes composed of one to five flowers each (1.92 ± 0.11, n=93 flowers from 11 trees). These white flowers bloom only for a single night, with one to two flowers per cyme blooming at a time. Only once did we observe all four flowers of a cyme bloom in the same night. Full bloom occurs around 18h30 during the wet season and around 19h30 in the dry season. Stamens start falling around midnight and have generally completely abscised by noon the following day.

Sonneratia ovata has a white androecium with the style located in the center. The shortest stamens are closest to the style, while the longest stamens are around the perimeter and point outwards away from the style. The mean length of the style is 35.05 ± 0.64 mm, while those of the stamens are 20.28 ± 0.55 mm (short stamens) to 31.07 ± 0.50 mm (long stamens). The average ASD is 14.77 ± 0.82 mm (n=54 flowers from 15 trees). The model that best explained ASD included season (GLMM, χ_1^2=7.1951, p=0.00731), but not location (GLMM, χ_2^2=2.6061, p=0.2717). The interaction between season and location could not be tested, as we only had data for both seasons at a single location. Stigmata are significantly closer to the nearest stamens in the dry season (ASD 8.48 ± 0.95, n=15 flowers from five trees) compared to wet season (ASD 17.19 ± 0.78, n=39 flowers from 10 trees) (Tukey's test, t=-2.575, p<0.05). This is because the center stamens (which are closest to the stigma) were longer (dry: 23.108±0.78 mm; wet: 19.19±0.62 mm) and the styles were shorter (dry: 31.59±0.74; wet: 36.38±0.74) in the dry season.

We observed that the stigmata of *S. ovata* were already receptive upon exsertion from the flower (around 16h30), approximately 2 hours before anthers begin to unfold. We did not test receptivity before exsertion. After exsertion, the stigmata started browning at varying rates starting near the edges, before the entire surface turned brown. Brown areas no longer responded to the hydrogen peroxide test, indicating that these areas were no longer receptive. Unpollinated flowers remained receptive or partially receptive until the flowers fell on the second or third day.

Anthers dehisced around 19h30 to 20h30, approximately an hour after the flowers were completely open, when it was already dark. Pollen remained available on attached stamens until midmorning the following day.

A small proportion of pollen grains remained viable for up to 5 days in the laboratory, although percent germination started to decline rapidly following 12 h (Figure 1). The highest percent germination observed for a single flower was 83.95%, from pollen collected at anther dehiscence. On average, percent germination remained close to 50% during the first 12 h and decreased steadily thereafter, with only about 10% germination for pollen collected 48 h after anther dehiscence.

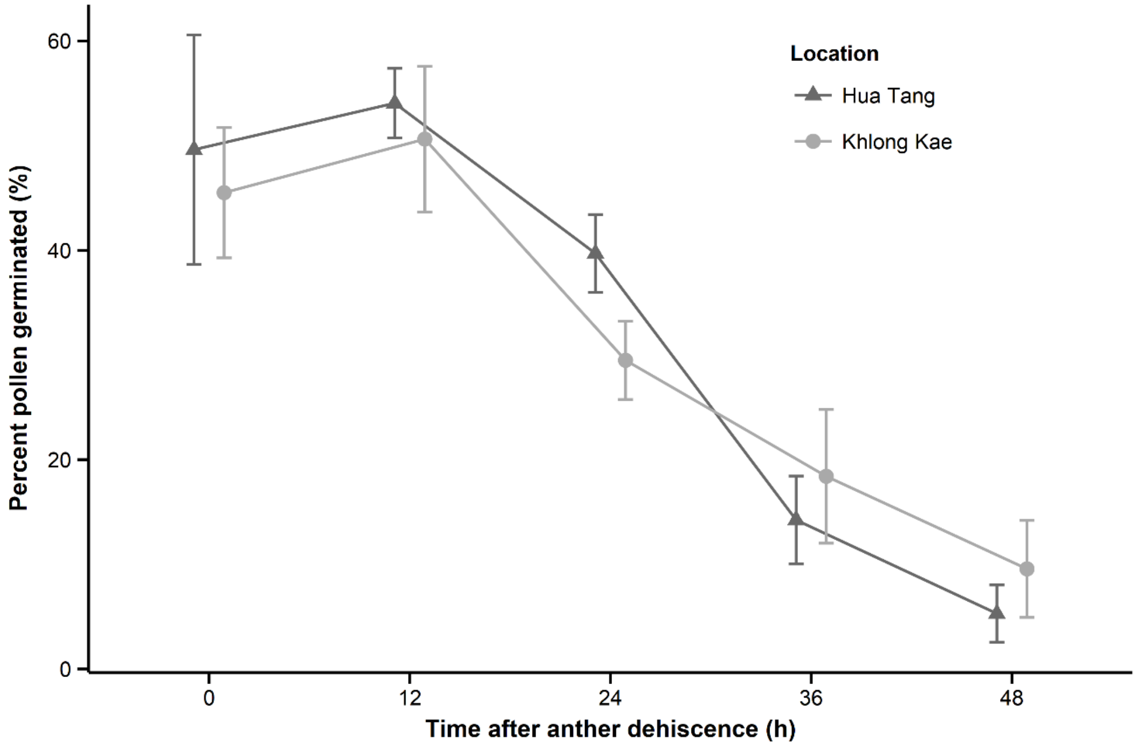

Figure 1. The percentage of *Sonneratia ovata* pollen that germinated (mean ± SE) in 15% sucrose solution when collected from zero to 48 hours after anther dehiscence (n = 20 flowers from nine trees).

Flowers generally began secreting nectar (Figure 2) before they had completed blooming (n=49 out of 52 flowers from 15 trees observed). The model that best explained the total amount of nectar produced included both season (GLMM, χ^2_1=16.514, p<0.001) and location (GLMM, χ^2_2=10.022, p<0.01). In Khlong Kae (the only site where nectar was collected in both seasons), the total amount of nectar produced during the dry season (316.95 ± 45.37 µL) was significantly lower (Tukey's test, z=-5.128, p<0.0001) than in the wet season (616.38 ± 23.57 µL). Post-hoc analysis of differences across locations revealed that Hua Tang flowers produced significantly more nectar (695.88 ± 40.21 µL) than did Koh Yor flowers (537.72 ± 21.11 µL) (Tukey's test, t=2.780, p<0.05). Additionally, wet season nectar production peaked at around 21h00 while there was no substantial peak during the dry season (Figure 2).

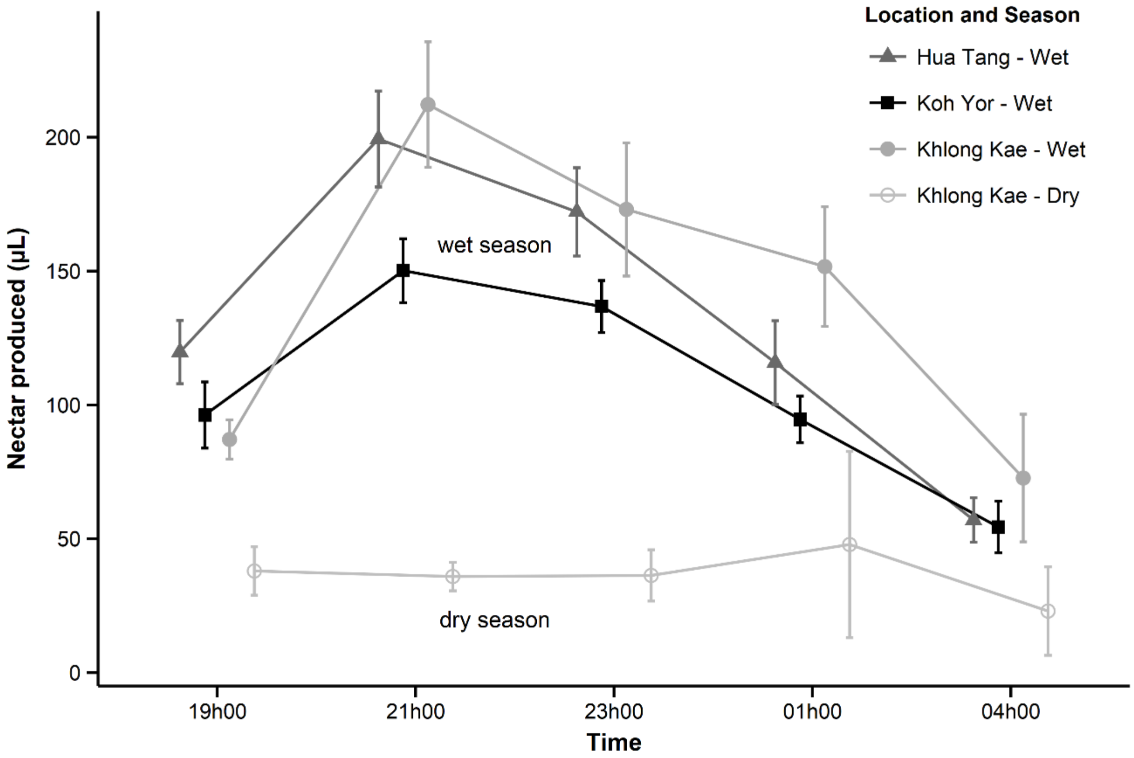

Figure 2. Average amount of nectar per *Sonneratia ovata* flower (mean ± SE, n = 20 flowers from nine trees) collected at different times during anthesis; flowers from three locations were used during the wet season while only one location was examined during the dry season.

A comparison of nectar sugar concentration in Khlong Kae revealed no significant differences between seasons (GLMM, χ^2_1=0.0013, p=0.9716), we therefore pooled data across seasons. Nectar sugar concentration peaked around 21h00 (approximately 20% Brix) and slowly decreased throughout the night (Figure 3). Further examination of the effect of location on nectar sugar concentration revealed this factor was significant (GLMM, χ^2_2=15.526, p<0.01), with the average nectar sugar concentration from Koh Yor flowers (18.36 ± 0.13) being significantly higher than from the flowers of both Khlong Kae (17.42 ± 0.28) (Tukey's test, z=-3.241, p<0.01) and Hua Tang (16.81 ± 0.20) (Tukey's test, z=-4.802, p<0.01).

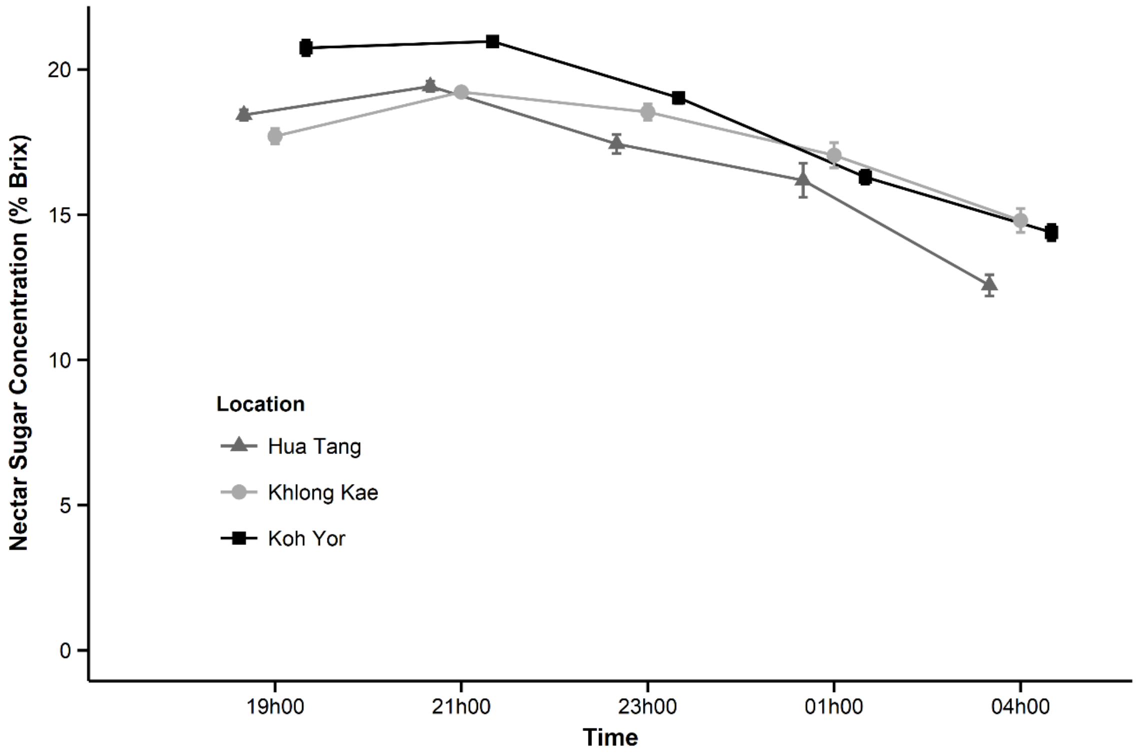

Figure 3. Average sugar concentration (mean ± SE) of *Sonneratia ovata* nectar collected at different times during anthesis (n = 20 flowers from nine trees). Season did not significantly affect sugar concentration, while location did. Koh Yor flowers had higher concentrations than both Khlong Kae flowers (Tukey's test, z=-3.241, p<0.01) and Hua Tang flowers (Tukey's test, z=-4.802, p<0.01).

2.3.2 Breeding system determination using pollen-ovule ratio

The mean number of stamens per flower was 338.59 ± 5.90 (n=27 flowers from 10 trees). The average estimated number of pollen grains per flower was 2,771,868.33 ± 188,289.47. The average estimated number of ovules per flower was 3,337.11 ± 140.83. Therefore, the mean P/O ratio is 850:1 (± 65.14 pollen grains). Based on Cruden (1977), this ratio indicates that *S. ovata* is facultatively xenogamous, *i.e.*, obligately self-pollinating and optionally cross-pollinating.

2.3.3 Pollination experiment

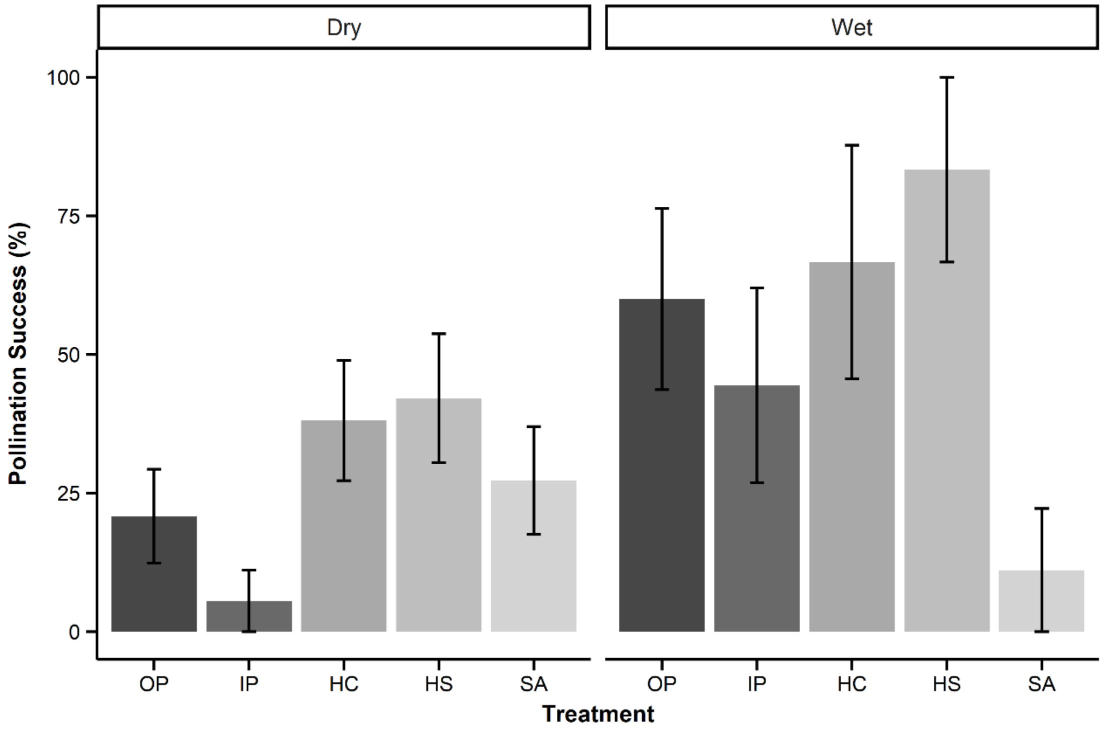

Figure 4. Pollination success of *Sonneratia ovata* in Khlong Kae during the dry and wet seasons, where pollination success is the percentage of fruits retained two weeks after floral anthesis (n = 75 flowers from six trees in the dry season and 193 flowers from 16 trees in the wet season). The treatments were: OP = open pollination, IP = insect pollination, HC = hand-cross pollination, HS = hand-self pollination, and SA = spontaneous autogamy. Overall wet season pollination success was significantly higher than in the dry season. Moreover, the pollination success of the hand-self pollination treatment was borderline significantly greater than that of the insect pollination treatment; no other treatments were significantly different.

The model that best explained pollination success in Khlong Kae included both treatment (GLMM, χ^2_4=12.259, $p<0.05$) and season (GLMM, χ^2_1=8.0366, $p<0.01$), but not their interaction (GLMM, χ^2_4=6.1728, p=0.1866). Wet season pollination success was significantly higher than that of the dry season (Tukey's test, z=-2.681, p<0.01) (Figure 4). Moreover, the hand-self pollination treatment was borderline significantly greater than the insect pollination treatment (Tukey's test, z=2.727, p=0.0502), but no other treatments were significantly different (Figure 4).

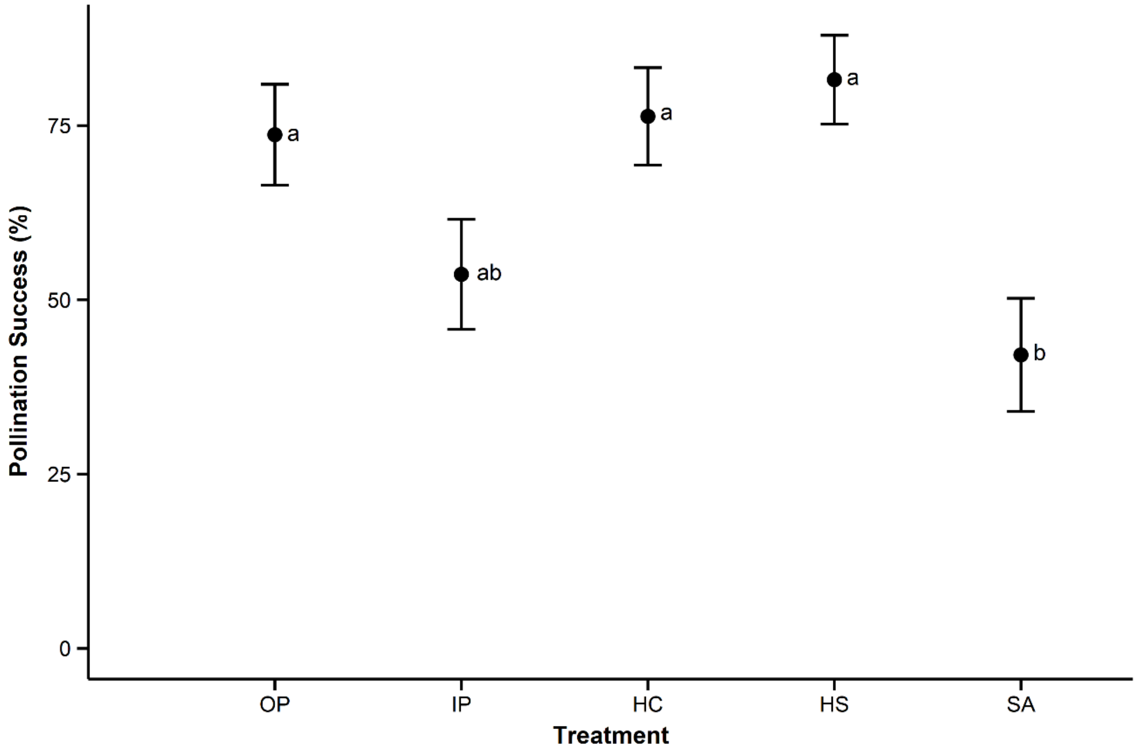

Figure 5. Pollination success of *Sonneratia ovata* flowers during the wet season, where pollination success is the percentage of fruits retained two weeks after floral anthesis. Data were pooled across all three study sites: Khlong Kae and Hua Tang (Satun Province), and Koh Yor (Songkhla Province). The treatments were: OP = open pollination, IP = insect pollination, HC = hand-cross pollination, HS = hand-self pollination, and SA = spontaneous autogamy. Different letters denote significant differences (Tukey's test, P<0.05).

Examining pollination success for all three sites during the wet season revealed that the best model included treatment (GLMM, χ^2_4=19.538, p<0.001), but not location (GLMM, χ^2_2=5.5668, p=0.06183) nor their interaction (GLMM, χ^2_8=9.7059, p=0.2863). A post-hoc test revealed that open pollination (73.68 ± 7.24%), hand-crossed (76.31 ± 6.99%), and hand-selfed (81.57 ± 6.37%) treatments had significantly higher pollination success than spontaneous autogamy (42.11 ± 8.12%) (Tukey's test; z=-2.892 p<0.05, z=-3.017 p<0.05, and z=-3.417 p<0.01, respectively) (Figure 5).

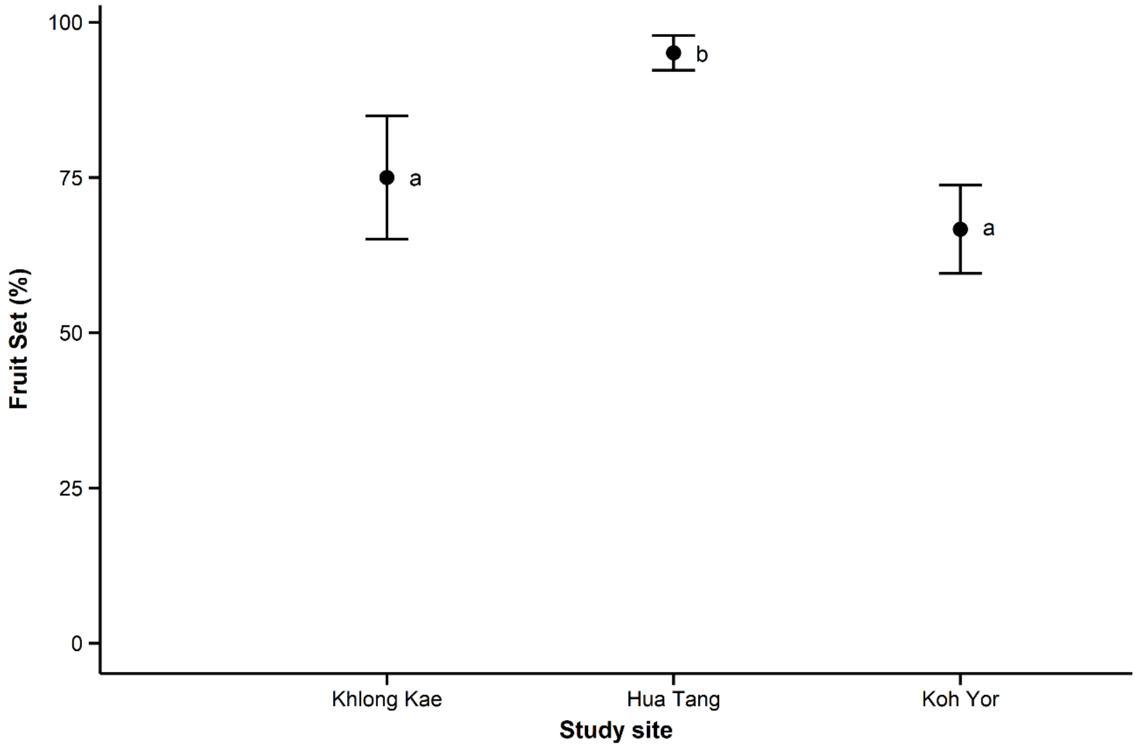

Figure 6. Fruit set of *Sonneratia ovata* flowers during the wet season, where fruit set is the percentage of mature fruits (8-12 weeks old) collected out of all the flowers that were successfully pollinated (n = 126 flowers from 17 trees). Data were pooled across all three study sites: Khlong Kae and Hua Tang (Satun Province), and Koh Yor (Songkhla Province). Different letters denote significant differences (Tukey's test, P<0.05).

Fruit set was only found in the wet season. The model that best described fruit set included location (GLMM, χ^2_2=10.008, p<0.01), but not treatment (GLMM, χ^2_4=0.725, p=0.9482) nor their interaction (GLMM, χ^2_8=13.425, p=0.09803); where the fruit set in Hua Tang (95.08 ± 0.02.79%) was significantly higher than in Khlong Kae (75.00 ± 9.93%) and in Koh Yor (66.67 ± 7.11%) (Tukey's test; z=--2.362 $p<0.05$, z=-3.314 $p<0.01$, respectively) (Figure 6).

For all mature fruits, the mean total seed count per fruit was 355.39 ± 26.90 (n=63 fruits from 13 trees). The model that best explained seed count included treatment (GLMM, χ^2_4=1017.7, $p < 0.001$) and the treatment-by-location interaction (GLMM, χ^2_8=270.15, $p < 0.001$), but not location alone (GLMM, χ^2_2=5.3414, p=0.0692). A pairwise comparison of treatments within each location revealed that most treatments in Khlong Kae (except hand-cross pollination with both hand-self pollination and open pollination) and Hua Tang (except hand-cross pollination with insect pollination and hand-self pollination with open pollination) were significantly different from each other. In Koh Yor, five out of 10 pairwise comparisons among treatments were significantly different; comparisons that were not significantly different consisted of hand-cross pollination with both open pollination and spontaneous autogamy, insect pollination with open pollination and spontaneous autogamy, and open pollination with spontaneous autogamy (Figure 7).

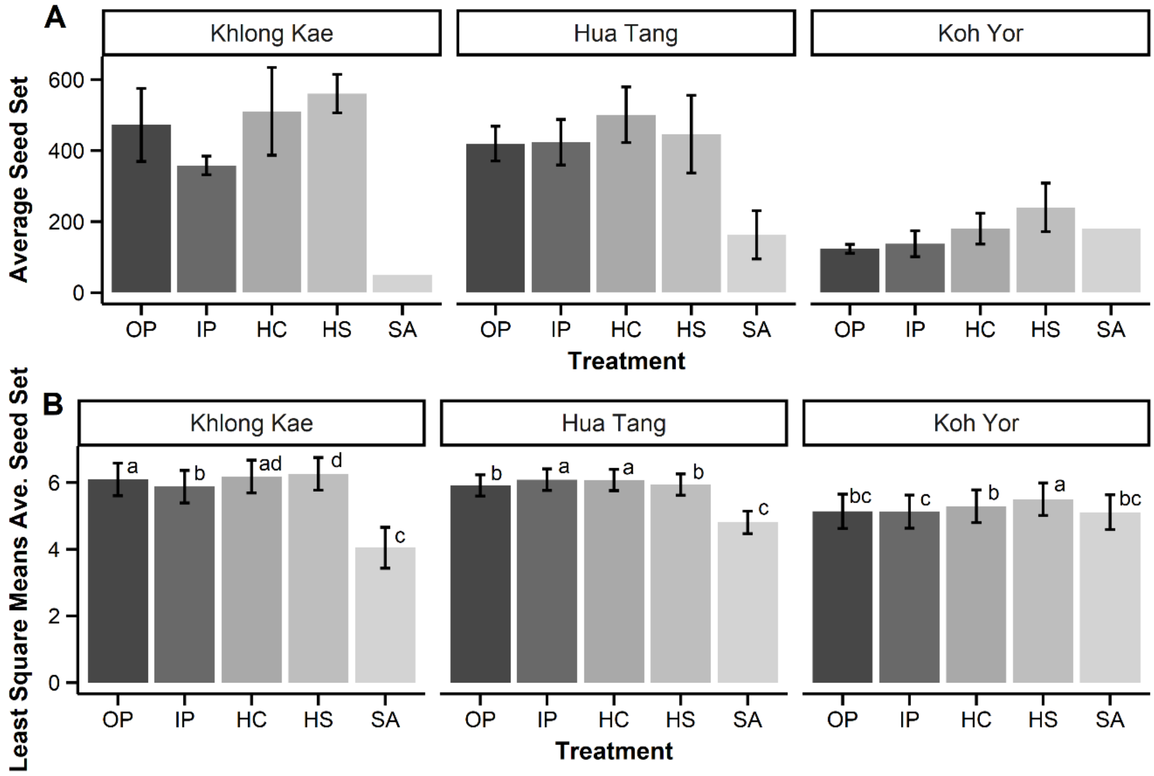

Figure 7. Average seed count per *Sonneratia ovata* fruit (mean ± SE) for the five treatments: OP = open pollination, IP=insect pollination, HC=hand-cross pollination, HS=hand-self pollination, and SA=spontaneous autogamy conducted at 3 locations (n = 64 fruits from 14 trees) (A). Least square means of average seed count per fruit (from generalized linear mixed model predictions) for the five treatments. Seed set was significantly affected by treatment as well as the interaction between treatment and location (GLMM). Pairwise comparisons were performed among treatments within each location. Different letters denote significant differences (Tukey's test, P<0.05) (B).

2.3.4 Floral visitors

We only caught a single bat species during two nights of mistnetting near *S. ovata* flowers during the wet season: the Dagger-toothed Long-nosed Fruit Bat (*Macroglossus minimus*). Nine individuals were caught each night; the different forearm lengths and weights of each indicate that they were all different individuals, with the exception of one that was possibly a recapture. However, camera trapping caught fewer bats than mistnetting did. Out of 31 camera trap nights, the camera traps captured only six visitations (twice each for three different camera trap nights). In the dry season the visits occurred once around 23h00, twice around 00h00, and once around 02h00. In the wet season there was a single visit each around 21h00 and 23h00. As the bats caught by the camera traps were moving very fast, it was difficult to identify them. However, the bats appear likely to be *Macroglossus* individuals, based on morphology (*e.g.*, small body size and long snout) and behavior (*e.g.*, delicate fluttering, compared to the more laborious flight of other pteropodid bat species).

We observed stingless bees (*Lepidotrigona cf. ventralis* and *L. cf. terminata*), red weaver ants (*Oecophylla smaragdina*), yellow crazy ants (*Anopolepis gracilipes),* small black ants, and sunbirds (Family Nectariniidae) visiting the flowers daily. However, we never saw any of them touch both the stigma and the anthers. Stingless bees visited only during the day (particularly in the morning until all the anthers have fallen and occasionally late in the afternoon) to collect pollen. The red weaver ants generally stayed outside the flower, seemingly more interested in creating hives and farming aphids (Family Aphidoidea). We rarely observed the yellow crazy ants but found some that were collecting nectar. The small black ants collected nectar at night from flowers that were manually emasculated, but not from non-emasculated flowers. The yellow crazy ants and the small black ants were never observed touching either the anthers or the stigmata. Additionally, sunbirds came early in the morning to forage on any remaining nectar. These birds drank nectar from one side of the flower and were never observed to touch the style. Additionally, we observed (but rarely) hawk moths (Family Sphingidae) and bees (possibly carpenter bees of Family Apidae) in the area, but did not see them landing on flowers.

2.4 Discussion

Sonneratia ovata is unique in that it is considered a pioneer species, which are typically associated with high reproductive rates (Turner 2004), yet it is at risk of becoming endangered (Duke & Jackes 1987, Polidoro *et al.* 2010) and has low genetic diversity (Zhou *et al.* 2010). Our results confirm that *S. ovata* is capable of self-pollination in addition to outcrossing. This mixed mating system is supported by a combination of both xenogamous and autogamous characteristics. With such flexibility, its survival should be assured. However, our data shows that its reproductive success is dependent on both animal pollinators and environments with sufficient water.

The mix of selfing and outcrossing traits found in *S. ovata* may be because *S. ovata* is a pioneer species (which often favors self-compatibility) and is also bat-pollinated (facilitating outcrossing) (Tomlinson 1986). Since mangroves occupy shorelines and newly-formed mud flats, particular species of mangroves are considered pioneer species (Richards 1996). Pioneer species typically have rapid growth, are self-compatible, and produce large numbers of seeds (Tomlinson 1986, Turner 2004). Self-compatibility is important for pioneer species, as it allows single individuals to colonize new areas, even without the presence of other conspecifics (Willmer 2011). Thus, the ability of *S. ovata* to self-fertilize likely facilitates its colonization of newly-formed mud flats.

Yet despite being self-compatible, *S. ovata* requires pollinators for the best quantity and quality seed set, as seen from our spontaneous autogamy treatment that produced the significantly lowest pollination success (Figure 5) and seed set (Figure 7). Pandit & Choudhury (2001) conducted exclusion experiments on *Sonneratia caseolaris* and found zero fruit set for their spontaneous autogamy treatment. However, Nor Zalipah & Adzemi (2017) found that, for *Sonneratia alba*, fruit set from the spontaneous (automatic) autogamy treatment were not significantly different from insect and open pollination treatments. The differences among these three *Sonneratia* species may be due to their varying degrees of herkogamy. For both *S. ovata* and *S. alba*, the ASD is less than 17 mm (lesser in *S. ovata* than in *S. alba*) (Nor Zalipah 2014), whereas the ASD in *S. caseolaris* is 20-32 mm (Nor Zalipah 2014, Pandit and Choudhury 2001,). Our results thus indicate that the ASD of *S. ovata* flowers (~15 mm), while not completely excluding self-pollination, does reduce the likelihood of successful fertilization when pollinators are absent.

In addition to dependence on pollinators for reproductive success, it appears that climatic variation highly affects *S. ovata* reproduction. In the El Niño year of 2016, the mean annual temperature in Thailand was 1.8 °C higher than normal during the summer months of April and May (Climatological Center, Meteorological Development Bureau 2017). Additionally, the country was drier than usual from February to May, especially in April and May (64-79% below normal rainfall) (Climatological Center, Meteorological Development Bureau 2017). This extreme climate may have imposed severe resource limitation on *S. ovata*, resulting in no fruit set in the dry season, even for flowers that were hand-pollinated and therefore received sufficient pollen. An additional explanation, which is not mutually exclusive, is that the significantly lower nectar amount produced by *S. ovata* flowers during the drought (Figure 2) may have reduced their attractiveness to pollinators, as proposed by Phillips *et al.* (2018). Optimal foraging theory (Stephens & Krebs 1986) would therefore predict that nectarivores should forage at other plant species that are perhaps more drought-tolerant and thus able to offer greater floral rewards, even under very dry conditions. Moreover, the nearby *S. alba* trees found along the seaward edge of Satun (less than two kilometers away), as well as others scattered along nearby streams, were possibly less affected by the drought, and may have been a better food source for pollinators than *S. ovata* flowers. We suggest more studies on the effect of climate change on the reproductive success of this species.

Macroglossus minimus was the only pollinator of *S. ovata* that we observed. These bats are exclusively nectarivorous (Francis 2008) and are able to fly up to 1 km from their roosts (Winkelmann *et al.* 2003) in spite of their tiny size (11-18 g; Francis 2008). *Macroglossus minimus* roost in mangrove forests and start foraging at dusk, with some continuing until dawn (Start & Marshall 1976). As specialized nectar and pollen feeders, these bats have long snouts and reduced molars (Faegri & Pijl 1979). When feeding, these bats have been observed to land directly on the flowers, making contact with both the stigmata and the anthers of *S. ovata* flowers, and leave within a few seconds (Start & Marshall 1976, Stewart & Dudash 2017). Additionally, these bats regularly visit their preferred plant species, or even specific trees, and carry abundant pollen on their bodies (Start & Marshall 1976, Stewart *et al.* 2014, Stewart & Dudash 2017). These traits make *M. minimus* very important pollinators of *S. ovata.*

The low capture rates of the camera traps may be because *M. minimus* are swift fliers, flying at an estimated speed of 10 m/s (Winkelmann *et al.* 2003), and stay on the blossom for only a few seconds (Start & Marshall 1976, Stewart & Dudash 2017) . Low capture rates could also be due to the positioning of the cameras, since we only had access to the lower and middle canopy (*i.e.*, if *M. minimus* prefer to forage near the top of the canopy), and seldom had access to flowers at the tips of canopy branches, which are more accessible to flying animals. However, the results of our pollination experiment indicate that overall pollinator visitation to *S. ovata* is sufficient, given that pollination success of the open pollination treatment was relatively high and not significantly different from the hand-pollinated treatments (*i.e.*, there is no pollen limitation; Figure 5).

The findings of this study demonstrate that the reproductive success of *S. ovata* is inexorably linked to the presence of its pollinators. Pollination success is much higher when bats have access to *S. ovata* flowers. Also, cross pollination is essential for promoting and spreading any genetic variability occurring in *S. ovata* populations. Correspondingly, *M. minimus* in Southeast Asia has only rarely been observed outside the mangrove area (Start 1974, Stewart 2016) and can be assumed to favor mangrove flowers as food sources. Protecting mangrove forests, including *S. ovata*, is important as they provide us with numerous benefits that are commonly overlooked (Sathirathai & Barbier 2001). In particular, because *S. ovata* generally grow in newly formed mudflats (Tomlinson 1986), they may be especially important as coastal storm barriers and in erosion control. Therefore, in order to protect *S. ovata* and environmentally-important mangrove forests, we should not only protect its habitat, but also its bat pollinators.

2.5 Acknowledgment

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CHAPTER 3

Pollinators required for reproductive success of largely self-incompatible critically endangered mangrove, *Sonneratia griffithii*, in Satun, Thailand

This manuscript will be submitted as a research note

Pollinators required for reproductive success of largely self-incompatible critically endangered mangrove, *Sonneratia griffithii*, in Satun, Thailand

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ABSTRACT

Mangrove forests provide numerous ecosystem services. Despite their importance, they continue to be lost. One such mangrove species negatively affected by deforestation and land conversion is the critically endangered *Sonneratia griffithii*. Conserving a species requires knowledge about them. To understand the reproductive biology of *S. griffithii*, we observed floral morphological traits that indicate autogamous or xenogamous breeding systems, conducted a pollination experiment, and observed flower visitors that potentially pollinate this mangrove species. *S. griffithii* trees flower asynchronously in the rainy season. Their flowers display both herkogamy and protandry and are highly self-incompatible. The pollen-ovule ratio suggests that this species is facultatively xenogamous. All pollination experiment treatments (open pollination, insect pollination, hand-cross pollination, hand-self pollination, and spontaneous autogamy) produced fruit set to varying degrees. Seed set was significantly highest in the hand-cross pollination treatment, followed by the insect and open pollination treatments, respectively. Two species of bats were mistnetted near the *S. griffithii* flowers: *Macroglossus minimus* and *Eonycteris spelaea.* Environmental factors appear to affect *S. griffithii* reproduction as well. In areas where there is strong vehicle-generated wind, fruit retention (until maturity) is lower. Also, bats were observed to avoid illuminated flowers. To protect this species, we recommend local area protection, preventing high-speed roads from being built through *S. griffithii* forests, and limiting light pollution.

Keywords: Chiropterophily, critically endangered species, old world tropics, light pollution

3.1 Introduction

Mangrove forests provide numerous ecosystem services (Ewel *et al.* 1998). Mangrove plants that live in the forest interior and in riverine areas are important for trapping sediments, recycling nutrients, as well as providing habitats for wild animals and plant products for humans (Ewel *et al.* 1998). Despite their importance, at least 35% of worldwide mangrove forests have been lost to land conversion and overharvesting (Barbier *et al.* 2011). Unfortunately, this trend continues at about 1-2% yearly (Barbier *et al.* 2011).

One such interior mangrove species that has been greatly harmed by deforestation and land conversion is *Sonneratia griffithii* Kurz 1871 (Department of Marine and Coastal Resources 2012). *Sonneratia griffithii* is a critically endangered species (Duke *et al.* 2010). In addition to being distributed only along the Andaman Sea from India to Indonesia (Duke *et al.* 2010), its distribution range is in an area of extensive mangrove deforestation, where 80% of all mangrove loss worldwide has occurred (Duke *et al.* 2010; Polidoro *et al.* 2010). Mangrove forests in these areas have mostly been converted to coastal developments, including rice and shrimp farms (Polidoro *et al.* 2010). Thus, *S. griffithii* is rare, locally extinct in many parts of its range, and its populations are continuously diminishing (Duke *et al.* 2010). As of the beginning of this century, less than 500 individuals are known from India (Duke *et al.* 2010; Polidoro *et al.* 2010). Additionally, Polidoro *et al.* (2010) reported that this species has low seed viability, further contributing to it declining population size. Unfortunately, not much is known about *S. griffithii* reproduction. Although the floral biology of other *Sonneratia* species have been studied before, *ie. S. alba* Sm. and *S.*

caseolaris (L.) Engl. (Coupland *et al.* 2005, Nor Zalipah & Adzemi 2017, Pandit & Choudhury 2001, Primack *et al.* 1981), no such studies have been published about *S. griffithii*.

In this study, we examined two *S. griffithii* populations in southern Thailand. We observed floral morphological traits that indicate autogamous or xenogamous breeding systems, conducted a pollination experiment to both determine if the species is self-compatible as well as to investigate the contribution of pollinators to its reproductive success, and observed flower visitors that potentially pollinate this mangrove species. We hypothesized that *S. griffithii* trees are self-compatible, yet have higher reproductive success when animals (*e.g.*, bats) visit and cross-pollinate them, as was found with *S. alba, S. caseolaris,* and *S. ovata* (Chapter 2 of this thesis, Nor Zalipah 2014, Nor Zalipah & Adzemi 2017).

3.2 Methods

3.2.1 Study sites

We conducted this study at two sites in Satun province on the western side of the Thai peninsula, bordering Malaysia: (1) Hua Tang and (2) Koh Nok. Hua Tang is east of downtown Satun City. It used to be a mangrove forest but is now mostly an urban area. The two trees used in the study area (6°37'04.7"N 100°04'56.0"E) are part of the remnants of that forest and stood along Yarttrasawaddee Street. Koh Nok is a village located south of downtown Satun City, where Highway 406 crosses a tributary of the Mak Bang River. The area we call Koh Nok in this study actually extends beyond the village but is still along Highway 406 (from the northern end at approximately at 6°35'40.8"N 100°03'42.9"E until the southern end at approximately at 6°34'07.8"N 100°03'43.2"E). The trees used in this study all border the highway.

3.2.2 Study species

Sonneratia griffithii is one of four *Sonneratia* species found in Satun province, southern Thailand (Department of Marine and Coastal Resources 2012). Of the four species, *S. griffithii* is the tallest, growing up to 25 m in height and towering over the other mangrove trees in the area (Department of Marine and Coastal Resources 2012).

Similar to other *Sonneratia* species, *S. griffithii* has solitary flowers or inflorescences composed of up to three hermaphroditic brush flowers (Tomlinson 1986, Department of Marine and Coastal Resources 2012). Each flower has a single style surrounded by numerous shorter stamens (Tomlinson 1986, Smitinand & Larsen 1992). *Sonneratia* species in Satun can be distinguished from each other by the sepals of their fruits; the sepals of *S. griffithii* extend outwards, perpendicular from the fruit (Department of Marine and Coastal Resources 2012).

Very little information has been published about the floral visitors and pollinators of *S. griffithii*, but nectarivorous bats (*Macroglossus minimus* and *Eonycteris spelaea*) have been caught at *S. griffithii* flowers in Satun, Thailand (Stewart 2016).

3.2.3 Floral biology

We observed *S. griffithii* floral traits, recording the number of flowers per inflorescence, blooming time, length of anthesis, and anther abscission from October 2016-March 2017. We also collected data on anther-stigma distance, stigma receptivity, pollen viability and nectar production.

For anther-stigma distance (ASD), we measured the distance from the stigma to the nearest anther using a Vernier caliper in 11 randomly chosen flowers from five trees.

Using the same methods described in Chapter 2 of this thesis, I observed the stigma receptivity of three flowers from one tree, tested pollen viability using eight flowers from four trees for 84 hours, as well as collected nectar to check nectar amounts and nectar sugar concentration on five flowers from two trees.

3.2.4 Breeding system determination using pollen-ovule ratio

Following the same method in Chapter 2 of this thesis, I computed the pollen-ovule (P/O) ratio of *S. griffithii* using 15 random flowers from six trees.

3.2.5 Pollination experiment

We performed a pollination experiment with the same five treatments used in Chapter 2 of this thesis using a total of 70 flowers from eight trees with an average of 1.75 flowers per tree. We calculated pollination success as the proportion of flowers surviving for at least 21 days, because most floral abortions were observed to occur within the first three weeks after anthesis. Mature fruits are easier to remove from their calyces and have softer flesh than unripe fruits. This occurs 8-10 weeks after anthesis. We collected, dried, and counted all seeds from all mature fruits, except from fruits with seed damage caused by insects. Because all fruits from the hand-selfed and spontaneous autogamy treatments experienced seed damage from insects, we could only count and compare the seed set of three treatments: open, insect, and hand-cross pollination.

3.2.6 Floral visitors

We observed bat visitation rates for six camera trap nights using the same method and equipment mentioned in Chapter 2 of this thesis. To identify the bat species visiting the flowers, we set up a mistnet (2.6 m x 9 m) as close as possible to *S. griffithii* flowers for one night from sunset until 23h00 along Highway 406. We checked the nets at least once every half an hour and assumed that all bats caught in the nets were visiting *S. griffithii* flowers. We identified the bats using Francis (2008). We observed insects visiting the flowers in person.

3.2.7 Data analysis

We calculated reproductive success following the same methods used in Chapter 2 of this thesis, except for pollination success which was calculated as the number of flowers surviving three weeks divided by the total number of experimental flowers and fruit set as the number of mature fruits divided by the number of flowers that were not aborted after the end of the third week (*i.e.*, the flowers considered to have been successfully pollinated).

For all analyses, we used the same software and packages used in Chapter 2 of this thesis. We performed generalized linear modelling (GLM) and generalized linear mixed modelling (GLMM) using the package “lme4” (Bates *et al.* 2015) to determine which predictors had significant effects on each of our dependent variables (ASD, pollination success, fruit set, and seed count). We analyzed ASD with a normal distribution, pollination success and fruit set with binomial distributions, and seed count with a Poisson distribution. For each analysis, the fixed factors were treatment, location, and pairwise interactions, whereas tree individual was included as a random factor. To determine which model best fit the data, we used nested likelihood ratio tests. Finally, we used the package “Lsmeans” (Lenth 2016) for Tukey post-hoc testing.

3.3 Results

3.3.1 Floral biology

In 2016-2017, as a population, *S. griffithii* started flowering from late September until January 2017. However, trees did not flower continuously nor synchronously; individual trees flowered at varying times, with each tree’s flowering period lasting only a few weeks.

Observations of 77 flowers from eight trees revealed that *S. griffithii* has 2.30 ± 0.16 flowers per cyme, where one to two flowers per cyme bloom at the same time. Flowers start to open between approximately 17h00 to 18h00, two hours before full bloom (19h00 to 21h00). We observed the flowers to have most of their anthers still attached until the second night after anthesis, but all gone the morning after (approximately 33 hours after anthesis) (n = four flowers from one tree).

Sonneratia griffithii flowers have a white androecium with the shortest stamens in the middle closest to the style which is located at the center. The longest stamens are at the edge of the androecium and point away from the style. Stamen length ranges between 19.65±1.58 mm (short stamens) to 37.33±1.08 mm (long stamens). On the other hand, the style is 45.51±1.34 mm long. Thus, the average ASD is 25.86 ± 1.27 mm (n=11 flowers from five trees). The model that best explained ASD did not include its only predictor: location (GLMM, χ^2_1= 0.1183, p= 0.7309).

We found that the stigmata of *S. griffithii* were already receptive upon exsertion from the flower (around 18h00), approximately 2 hours before anthers dehisced between 19h00 and 21h00 (n = three flowers from one tree). We did not test receptivity before exsertion. The stigmata started browning at varying rates during or after full exsertion, starting near the edges, before the entire surface turned brown (typically no earlier than 72 h). These darkened areas no longer responded to the hydrogen peroxide test, showing that they were no longer receptive.

A small proportion of pollen grains remained viable for more than five days in the laboratory, although percent germination started to decline after 36 h (Figure 8). The highest percent germination (approximately 50%) occurred from 12 to 36 h after anther dehiscence. After 132 hours, germination rate had decreased to approximately 10%.

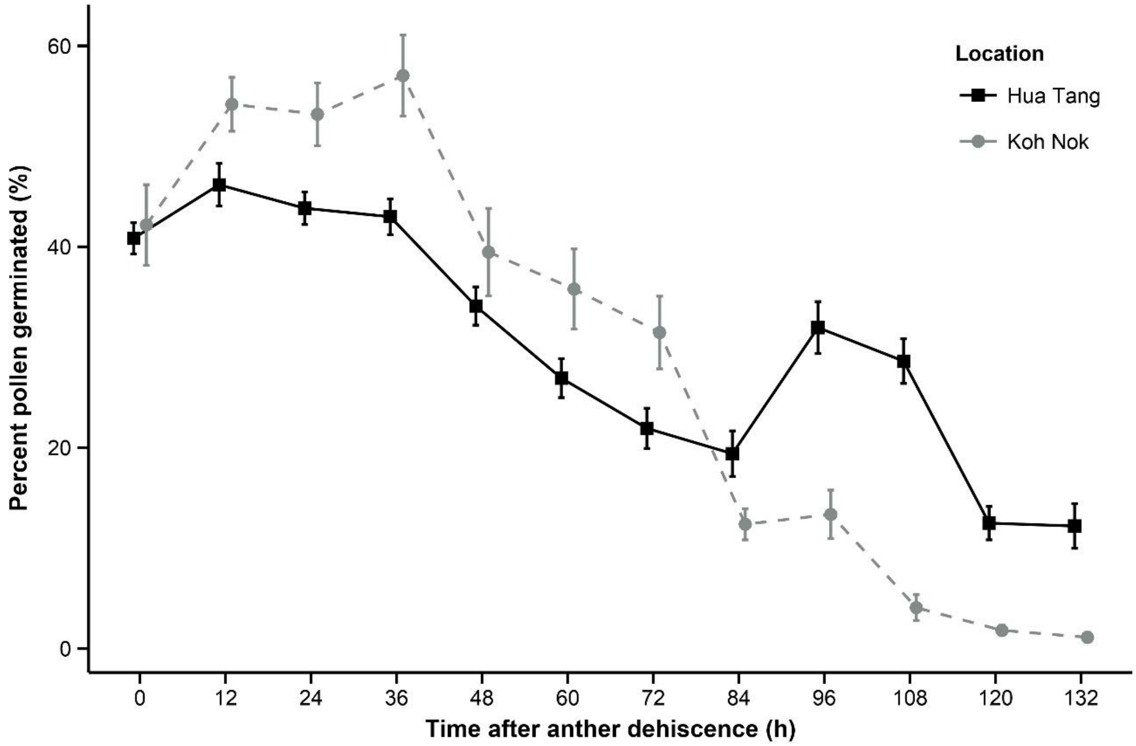

Figure 8. The percentage of *Sonneratia giffithii* pollen that germinated (mean ± SE) in 15% sucrose solution when collected from zero to 132 hours after anther dehiscence (n = eight flowers from four trees).

Nectar secretion generally occurred before anther dehiscence, peaked from 21h00 until 23h00, and decreased thereafter (n = five flowers from two trees) (Figure 9). The highest value was 389.52 µL collected at approximately 21h00 followed by 347.96 µL collected at approximately 23h00. By 07h00, there was minimal to no nectar found in the flowers.

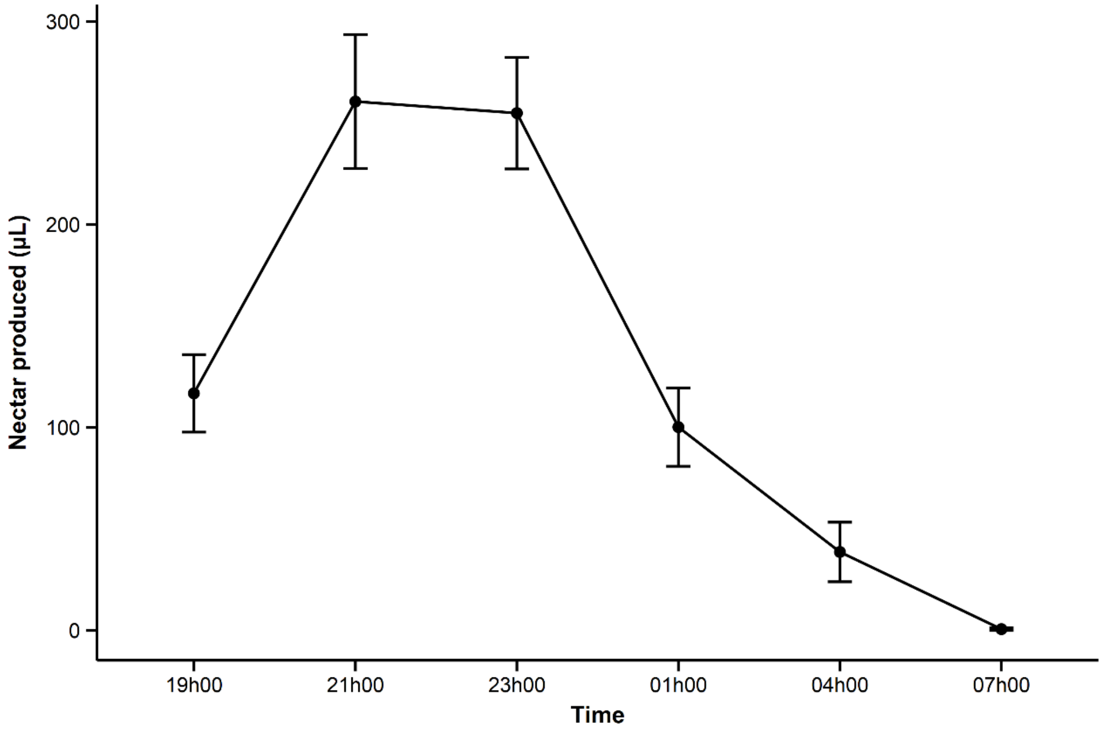

Figure 9. Average amount of nectar per *Sonneratia griffithii* flower (mean ± SE) collected at different times during anthesis (n = five flowers from two trees for all time periods except 07h00, in which only three flowers from one tree were examined).

Nectar sugar concentration partially coincided with the nectar production, where it peaked at 21h00 and gradually decreased thereafter (Figure 10). The highest recorded sugar concentration was 19.40 % Brix (at approximately 21h00) and the lowest was 11.00 % Brix (at approximately 04h00) (n = four flowers from two trees).

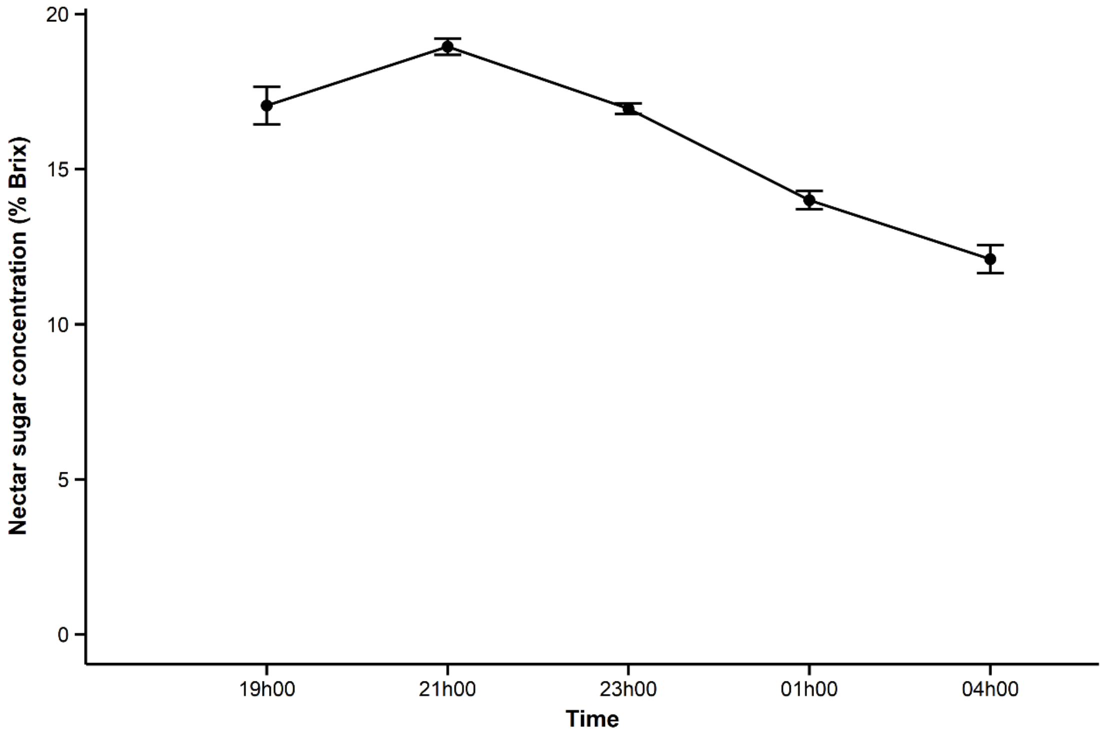

Figure 10. Average sugar concentration (mean ± SE) of *Sonneratia griffithii* nectar collected at different times during anthesis (n = four flowers from two trees).

3.3.2 Breeding system determination using pollen-ovule ratio

Sonneratia griffithii had an average of 432.95 ± 15.79 stamens and a mean estimated number of 2,309,590.53 ± 173,300.51 pollen grains per flower. The estimated number of ovules per flower was 2,901.47 ± 157.76 (n = 19 flowers from seven trees). Thus, the mean P/O ratio is 867:1 (± 109.83 pollen grains). This value indicates that *S. griffithii* is facultatively xenogamous according to Cruden (1977).

3.3.3 Pollination experiment

Although the hand-crossed treatment (55.56% ± 17.57%) produced the highest pollination success while the hand-selfed (15.38% ± 10.42%) and spontaneous autogamy (12.50% ± 8.54%) treatments produced the lowest pollination success

(Figure 11), the model that best explained pollination success for *S. griffithii* excluded all predictors: treatment (GLMM, χ^2_4=6.5254, p=0.1632), location (GLMM, χ^2_1=0.1585, p=0.6905), and their interaction (GLMM, χ^2_4=6.9418, p=0.139).

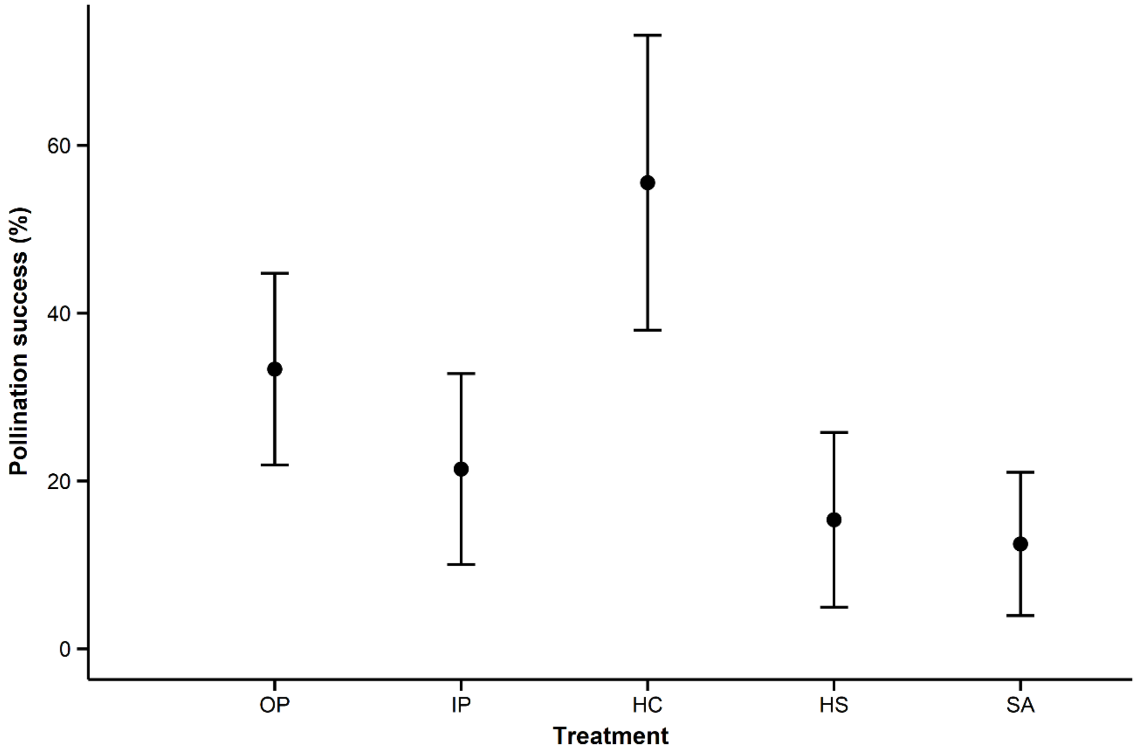

Figure 11. Pollination success *of Sonneratia griffithii*, where pollination success is the percentage of fruits retained three weeks after floral anthesis (n = 70 flowers from eight trees). The treatments were: OP = open pollination, IP = insect pollination, HC = hand-cross pollination, HS = hand-self pollination, and SA = spontaneous autogamy.

All treatments produced fruit set, and the percentages were relatively similar (open pollination: 67% ± 21%, n = six flowers from four trees; insect pollination: 67% ± 33%, n = three flowers from two trees, hand-cross pollination: 60% ± 24%, n = five flowers from three trees; hand-self pollination: 50% ± 50%, n = two flowers from two trees; and spontaneous autogamy: 50%, n = two flowers from one tree). Accordingly, the model that best described fruit set did not include treatment (GLMM,

χ_4^2=1.4927, p=0.8279) or the interaction between treatment and location (GLMM, χ_2^2 =0.00, p=1.00), however, it did include location (GLMM, χ_1^2=8.6491, p<0.01). Fruit set in Hua Tang (100% ± 0.00%, n = seven flowers from two trees) was greater than in Koh Nok (36% ± 15%, n = 11 flowers from five trees), however this difference was not statistically significant in the post-hoc test (Figure 12).

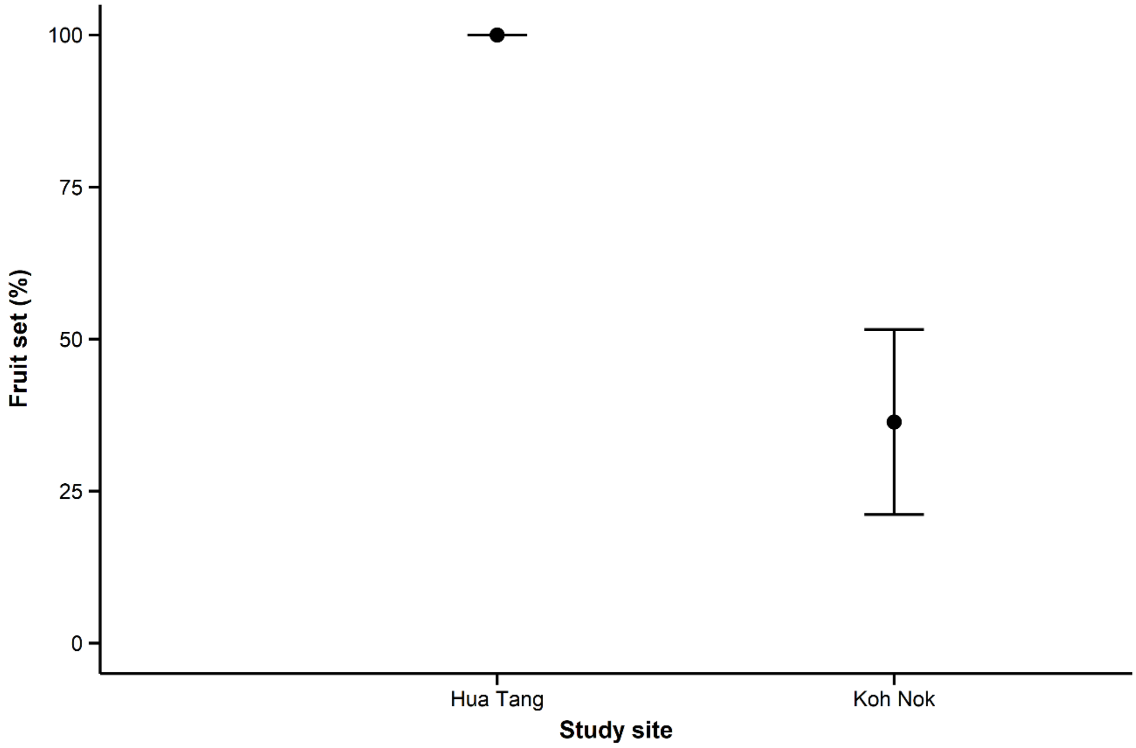

Figure 12. Fruit set of *Sonneratia griffithii*, where fruit set is the percentage of mature fruits (8-10 weeks old) collected out of all the flowers that were successfully pollinated (survived more than three weeks) (n =18 flowers from seven trees).

The average seed set per fruit was 186.14 ± 29.74 seeds (n = seven fruits from two trees). The model that best explained seed count included treatment (GLM, χ_2^2= 26.661, p < 0.001) and tree individual (GLM, χ_1^2 = 20.86, p < 0.001); the interaction between the two could not be tested since one tree only retained fruits from a single treatment (χ_0^2). A pairwise comparison revealed that all three treatments with undamaged seed sets were significantly different from each other (Tukey's test; hand-cross with insect pollination:

z = 3.314, p < 0.01; hand-cross with open pollination: z = 10.487, p < 0.0001: insect with open pollination, z = 6.902, p < 0.0001) (Figure 13).

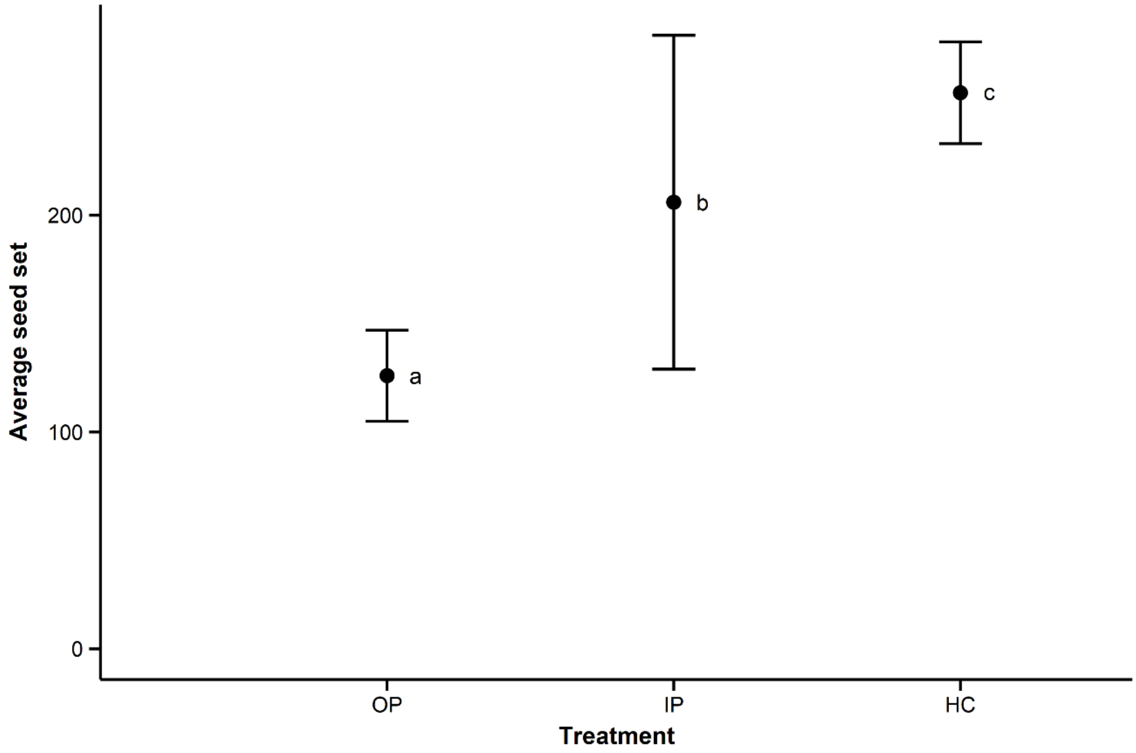

Figure 13. Average seed set per *Sonneratia griffithii* fruit (mean ± SE) for the three treatments: OP = open pollination, IP = insect pollination, and HC = hand-cross pollination. Treatment significantly affected seed set (GLMM). Pairwise comparisons were performed and different letters denote significant differences (Tukey's test, P<0.01). (n = seven fruits from six trees)

3.3.4 Floral visitors

We caught no animal visitations to the *S. griffithii* flowers using the camera traps. However, we caught two species of bats by mistnetting: the Dagger-toothed Long-nosed Fruit Bat (*Macroglossus minimus*) and the dawn bat (*Eonycteris spelaea*). Three *M. minimus* and three *E. spelaea* were caught before 20h00, in addition to one of each caught at approximately 21h00. The different forearm lengths and weights of each indicate that they were all different individuals.

We observed that stingless bees were the most common insects visiting the anthers of *S. griffithii,* during the day. We also found some small black ants (particularly when we emasculated the flowers) and some red weaver ants (*Oecophylla smaragdina*), but the latter are not as common or as aggressive in *S. griffithii* trees as they are in *Sonneratia ovata* trees (Chapter 2 of this thesis). Although these three types of insects were observed visiting or moving around the *S. griffithii* flowers, they were never observed contacting the stigmata, and only stingless bees were observed contacting the anthers.

Moreover, we encountered a swarm of giant honey bees (*Apis dorsata*) around some blooming *S. griffithii* flowers one night. However, we were not able to observe their behavior because they were attracted to our torch lights whenever we turned the lights on.

3.4 Discussion

Sonneratia griffithii is a critically endangered species (Duke *et al.* 2010, Polidoro *et al.* 2010) and its populations continue to dwindle in size and number, primarily due to human activity. Our study was conducted from October 2016 through March 2017, yet by January 2018, both Hua Tang study trees and many of the Koh Yor study trees had been cut down. Although the low sample sizes available for this study possibly prevented us from gaining more statistically conclusive results, some information can still be gleaned from this work.

Sonneratia griffithii, similar to other *Sonneratia* species, display xenogamous characteristics through herkogamy and protogyny (Chapter 2 of this thesis, Nor Zalipah 2014). Additionally, nectarivorous bats are known to visit *S. griffithii* (Stewart 2016). It is expected, therefore, that this species reproduces by cross-pollination. However, it has never before been proven if this tree species is self-compatible (Tomlinson 1986). The pollen-ovule ratio estimated in this study indicates that this species is facultatively xenogamous, and our pollination experiment results indicate that *S. griffithii* is largely self-incompatible, as there was a 3.7-fold difference in the pollination success between the hand-cross pollinated (56%) and hand-self pollinated (15%) flowers. While this difference was not significant, it may be due to a lack of statistical power from small samples sizes.

Moreover, *S. griffithii* fruit set appears to be affected by location, since all Hua Tang fruits reached maturity unlike the fruits in Koh Nok. One potential explanation for the observed difference is the amount and speed of vehicle traffic passing through each site. In both study sites, the study trees stand along the road. However, Hua Tang is a residential area. Despite having many vehicles passing through daily, these vehicles do not travel very fast because of the number of pedestrians passing by, as well as the number of vehicles entering and exiting Yarttrasawaddee Street. On the other hand, most of Highway 406 is unpopulated (except at the residential area which only occupies a little over 300 m of the highway), and most of the vehicles passing through travel 70-120 km/h. These passing vehicles, particularly speeding trucks and buses, generate wind that buffets *S. griffithii* trees growing close to the highway. This is aggravated by the fact that, in most parts of the Koh Nok study area, the highway is about two meters higher than the surrounding ground, making a larger portion of the canopy exposed to vehicle-generated wind. If the trees are close enough to the highway and unprotected by other foliage, these short bursts of wind can cause the brittle branches of *S. grifithii* to break, especially when they are carrying heavy fruits (Nuevo Diego, pers. obs.).

Camera trapping recorded no bats. This is likely partly because *S. griffithii* trees are very tall and we were only able to reach the lower canopy. Furthermore, similar to the frugivorous bat *Carollia sowelli* (Lewanzik & Voigt 2014), we observed that bats did not visit *S. griffithii* flowers that were directly lit by streetlamps. Hence, most of the bats we observed only visited the middle or upper parts of the canopy, or the part of the tree facing away from the street lights. Highway 406 does not have streetlamps except at the residential area. However, the high beam lights and speed of passing vehicles may have also affected bat visits. If such anthropogenic activity is deterring pollinator visits, it may also explain the observed pollen limitation in our study. Our pollination experiment revealed that the open pollination and insect pollination treatments had both lower percentages of pollination success (Figure 11) and lower seed sets (Figure 13) than the hand-cross pollinated treatment (although only seed set was statistically different between treatments). As there are no published studies on the effect of light pollution on nectarivorous bats, we suggest further inquiry into this topic.

Despite the camera traps not recording any bats, mistnetting caught two exclusively nectarivorous bat species: *M. minimus* and *E. spelaea*. The former is a small bat that resides in the mangrove forest (Start & Marshall 1976) while the latter roosts in caves (Acharya *et al.* 2015a) and is the biggest strictly-nectarivorous bat species in Southeast Asia (Francis 2008, Acharya *et al.* 2015b). Both bat species land on flowers to feed but only stay for a few seconds, and carry large pollen loads (Start & Marshall 1976, Stewart *et al.* 2014, Acharya *et al.* 2015b, Stewart & Dudash 2017). Moreover, *M. minimus* individuals tend to exhibit high floral constancy and visit their preferred plants regularly (Stewart & Dudash 2017). These traits make these two bat species excellent pollinators of *S. griffithii*. In fact, both bat species were recognized by Stewart & Dudash (2017) as very important bat pollinators of *Sonneratia* species in southern Thailand. While we did not observe any insect species making contact with flower stigmata, our pollination experiment results indicate that they can contribute to *S. griffithii* reproduction as well, and further work is needed to elucidate which species are likely pollinators.

The findings of this study emphasize how little we know about the critically endangered *S. griffithii* and how quickly they are disappearing. Our results confirm that pollinators are critical for *S. griffithii* reproduction, as this species is largely self-incompatible. Moreover, our results suggest that *S. griffithii* is pollen limited, which may indicate low pollinator populations at our study sites. To protect this species, one of the recommendations by the International Union for the Conservation of Nature (IUCN) is local area protection (Duke *et al.* 2010). Our findings indicate that such local protection should also include (1) preventing high-speed roads from being built through

S. griffithii forests and (2) limiting light pollution, as these factors may negatively affect both the *S. griffithii* trees as well as their pollinators.

3.5 Acknowledgment

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CHAPTER 4

GENERAL DISCUSSION

Sonneratia ovata and *S. griffithii* are threatened species – the former at risk of becoming endangered, and the latter already critically endangered (Duke *et al.* 2010, Salmo III *et al.* 2010, Polidoro *et al.* 2010). Both species are at least partially capable of self-pollination in addition to outcrossing. This mixed mating system is supported by a combination of both xenogamous and autogamous characteristics. With such flexibility (and local area protection), their survival should be assured. However, my data shows that their reproductive success is dependent on both animal pollinators and environmental conditions.

The mix of selfing and outcrossing traits found in the two species may be because both are pioneer species (favoring self-compatibility) and are also bat-pollinated (facilitating outcrossing) (Tomlinson 1986). Both species grow along riverbanks and newly-formed mud flats (Tomlinson 1986). The first plants thriving in unoccupied and unshaded areas are considered pioneer species (Richards 1996). Pioneer species typically have rapid growth, are self-compatible, and produce large numbers of seeds (Tomlinson 1986, Turner 2004). Self-compatibility is particularly important for pioneer species as it allows single individuals to colonize new areas, even without the presence of other conspecifics (Willmer 2011). Thus, the ability of *S. ovata* and *S. griffithii* to self-fertilize likely facilitates its colonization of newly-formed mud flats.

Yet despite being at least partially self-compatible, *S. ovata* and *S. griffithii* require pollinators for the best quantity and quality seed set, as seen from my spontaneous autogamy treatment that produced the significantly lowest pollination success (Figure 5) and seed set (Figure 7) for *S. ovata*, as well as the lowest pollination success for *S. griffithii* (Figure 11). Although the latter is not significantly different from the other treatment results, this may be due to the lack of statistical power. The low pollination success of spontaneous autogamy treatment in both species is partially due to the distance between the stigma and the anthers. The average ASD of *Sonneratia* *ovata* was 14.77 ± 0.82 mm, while that of *S. griffithii* was 25.86 ± 1.27 mm. Moreover, *S. ovata* exhibits greater self-compatibility (seen by the similar percentages of pollination success for the hand-crossed and hand-selfed treatments; Figure 5) than does *S. griffithii* (where hand-selfed pollination success was much lower than hand-crossed pollination success; Figure 11). Unsurprisingly, the pollination success of spontaneous autogamy in *S. ovata* was higher (42.11 ± 8.12%) than in *S. griffithii* (12.50% ± 8.53%).

Based on the P/O ratio table by Cruden (1977), the breeding systems of the two species were both facultatively xenogamous, or primarily outcrossing and secondarily self-pollinating. However, it did not show how different the actual breeding systems of these two species really are. Additionally, although my pollination success results agreed with the breeding system based on the P/O ratio, the results that Nor Zalipah (2014) found were very different. Based on the P/O ratio of *S. caseolaris*, she predicted that it is obligately autonomous. However, cross-pollination increased both fruit and seed set in the species, indicating that cross-pollination is beneficial and important. In retrospect, I believe that pollen and ovule number can still provide useful information, but using the P/O ratio to predict the breeding system is unnecessary when pollination experiments will be conducted anyway.

In comparison to *S. caseolaris* which produces 623.189 ± 60.619 seeds per fruit (Nor Zalipah 2014), *S. ovata* only produces 355.39 ± 26.90 and *S. griffithii* 186.14 ± 29.74 seeds per fruit. These relatively low seed sets may contribute to the rarity of both *S. ovata* and *S. griffithii*, particularly to the latter since it is also largely self-incompatible.

On top of their reproductive biology, it appears that environmental conditions highly affect *Sonneratia* reproduction. In the case of *S. ovata*, higher temperatures and drier weather conditions may have prevented all the experimental flowers from growing into mature fruits, even for flowers that were hand-pollinated and therefore received sufficient pollen. In the El Niño year of 2016, the mean annual temperature in Thailand was 1.8 °C higher than normal during the summer months of April and May (Climatological Center, Meteorological Development Bureau 2017). Additionally, the country was drier than usual from February to May, especially in April and May (64-79% below normal rainfall) (Climatological Center, Meteorological Development Bureau 2017). An additional explanation, which is not mutually exclusive, is that the significantly lower nectar amount produced by *S. ovata* flowers during the drought (Figure 2) may have reduced their attractiveness to pollinators, as proposed by Phillips *et al.* (2018). Optimal foraging theory (Stephens & Krebs 1986) would therefore predict that nectarivores should forage at other plant species that are perhaps more drought-tolerant and thus able to offer greater floral rewards, even under very dry conditions. Moreover, the nearby *S. alba* trees found along the seaward edge of Satun (less than two kilometers away), as well as others scattered along nearby streams, were possibly less affected by the drought, and may have been a better food source for pollinators than *S. ovata* flowers. I suggest more studies on the effect of climate change on the reproductive success of this species.

In the case of *S. griffithii*, strong wind and the presence of light may have reduced its reproductive success, since all Hua Tang fruits reached maturity unlike the fruits in Koh Nok. One potential explanation for the observed difference is the amount and speed of vehicle traffic passing through each site. In both study sites, the study trees stand along the road. However, Hua Tang is a residential area. Despite having many vehicles passing through daily, these vehicles do not travel very fast because of the number of pedestrians passing by, as well as the number of vehicles entering and exiting Yarttrasawaddee Street. On the other hand, most of Highway 406 is unpopulated (except at the residential area which only occupies a little over 300 m of the highway), and most of the vehicles passing through travel 70-120 km/h. These passing vehicles, particularly speeding trucks and buses, generate wind that can cause the exposed brittle branches of *S. grifithii* to break, especially when they are carrying heavy fruits.

In addition to strong wind, the presence of light appears to discourage nectarivorous bats from visiting *S. griffithii* flowers. Similar to the behavior of frugivorous bat *Carollia sowelli* (Lewanzik & Voigt 2014), I observed that bats did not visit *S. griffithii* flowers that were directly lit by streetlamps. Hence, most of the bats I observed only visited the middle or upper parts of the canopy, or the part of the tree facing away from the street lights. Highway 406 does not have streetlamps except at the residential area. However, the high beam lights and speed of passing vehicles may have also affected bat visits. If such anthropogenic activity is deterring pollinator visits, it may also explain the observed pollen limitation in this study. The pollination experiment revealed that the open pollination and insect pollination treatments had both lower percentages of pollination success (Figure 11) and lower seed sets (Figure 13) than the hand-cross pollinated treatment (although only seed set was statistically different between treatments). As there are no published studies on the effect of light pollution on nectarivorous bats, I suggest further inquiry into this topic.

Macroglossus minimus was the only pollinator of *S. ovata* observed. Whereas for *S. griffithii*, *M. minimus* and *E. spelaea* were both observed. The former is the strictly-nectarivorous bat species that resides in the mangrove forest (Start & Marshall 1976, Francis 2008) while the latter roosts in caves (Acharya *et al.* 2015a) and is the biggest strictly-nectarivorous bat species in Southeast Asia (Francis 2008, Acharya *et al.* 2015b). Both bat species land on flowers to feed but only stay for a few seconds, and carry large pollen loads (Start & Marshall 1976, Stewart *et al.* 2014, Acharya *et al.* 2015b, Stewart & Dudash 2017). Moreover, *M. minimus* individuals tend to exhibit high floral constancy and visit their preferred plants regularly (Stewart & Dudash 2017). These traits make these two bat species excellent pollinators of *S. ovata* and *S. griffithii*. In fact, both bat species were recognized by Stewart & Dudash (2017) as very important bat pollinators of *Sonneratia* species in southern Thailand. While I did not observe any insect species making contact with *S. griffithii* flower stigmata, the pollination experiment results indicate that they can contribute to *S. griffithii* reproduction as well, and further work is needed to elucidate which species are likely pollinators.

The low and zero capture rates of the camera traps (for *S. ovata* and *S. griffithii*, respectively) may be because of the positioning of the cameras. I only had access to the lower and middle canopy (*i.e.*, if *M. minimus* prefer to forage near the top of the canopy), and seldom had access to flowers at the tips of canopy branches, which are more accessible to flying animals. Additionally, in the case of *S. griffithii*, lower branches were exposed to light, repelling the bats from visiting flowers in these locations. However, the results of the pollination experiment indicate that overall pollinator visitation to *S. ovata* is sufficient, given that pollination success of the open pollination treatment was relatively high and not significantly different from the hand-pollinated treatments (Figure 5). By contrast, pollination experiment on *S. griffithii* flowers revealed that the open pollination and insect pollination treatments had both lower percentages of pollination success (Figure 11) and lower seed sets (Figure 13) than the hand-cross pollinated treatment (although only seed set was statistically different between treatments).

CHAPTER 5

CONCLUSION AND RECOMMENDATIONS

My results confirm that pollinators are critical for *S. griffithii* reproduction, as this species is largely self-incompatible. Moreover, it suggests that *S. griffithii* is pollen limited, which may indicate low pollinator populations at my study sites. Similarly, my results also demonstrate that the reproductive success of *S. ovata* is inexorably linked to the presence of its pollinators. Pollination success is much higher when bats have access to *S. ovata* flowers. Also, cross pollination is essential for promoting and spreading any genetic variability occurring in *S. ovata* populations. Correspondingly, *M. minimus* in Southeast Asia has only rarely been observed outside the mangrove area (Start 1974, Stewart 2016) and can be assumed to favor mangrove flowers as food sources.

The reproductive biology of both *S. ovata* and *S. griffithii* may be the cause for their rarity, particularly for the latter species. However, it is the anthropogenic factors, which are rapidly reducing their available habitat, that makes them endangered. To protect these mangrove species, one of the recommendations by the International Union for the Conservation of Nature (IUCN) is local area protection (Duke *et al.* 2010, Salmo III *et al.* 2010). My findings indicate that such local protection should also include (1) preventing high-speed roads from being built through *Sonneratia* forests and (2) limiting light pollution that repels pollinators and reduces pollination success in these mangrove species.

I recommend further studies on the seed germination of fruits resulting from pollination experiments, the effect of light on nectarivorous bats, and the effect of drought on the reproductive success of *Sonneratia.*

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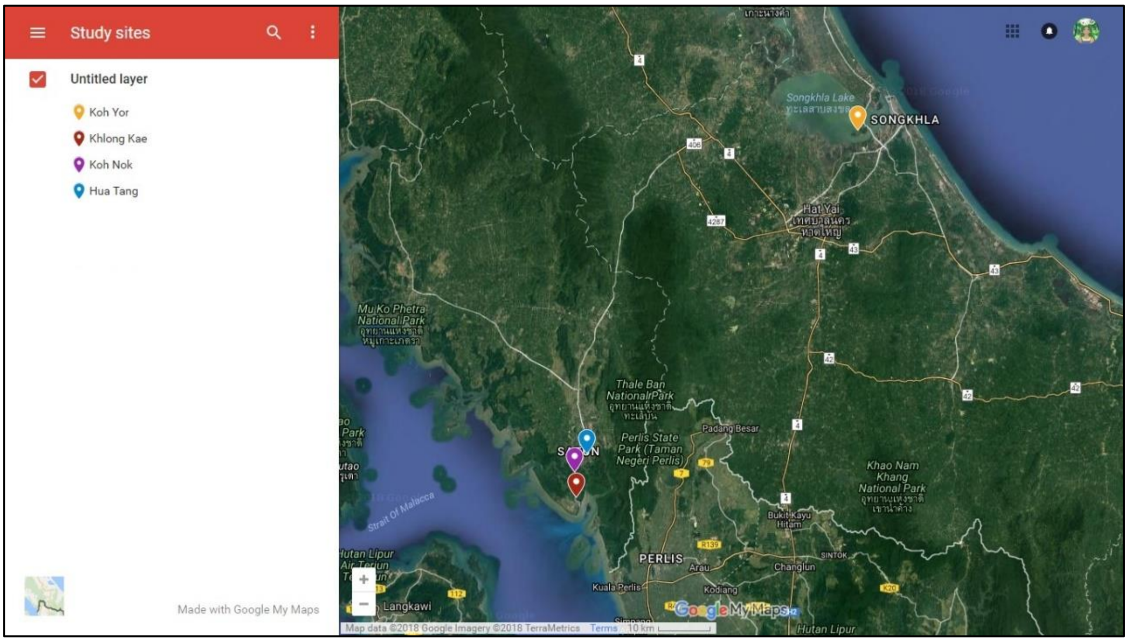
APPENDICES

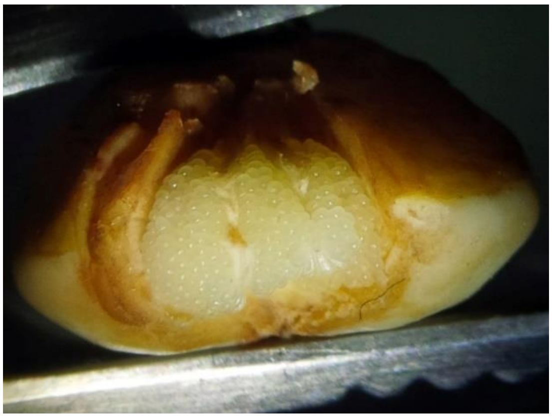
Appendix 1: Location of study sites.



Appendix 2: Photos of *Sonneratia ovata* and *S. griffithii* flowers, flower parts, fruits, and seeds.

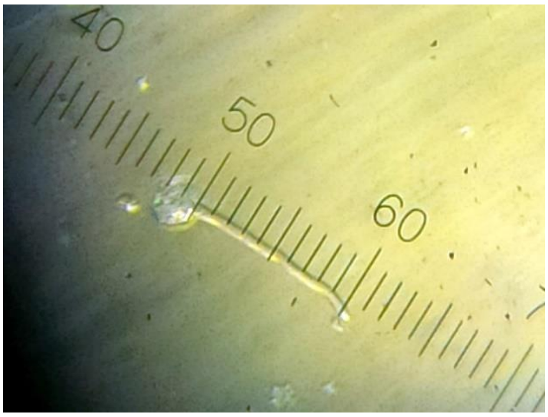
Sonneratia ovata flower

Sonneratia griffithii flower



Sonneratia ovata ovules

Sonneratia griffthii ovules



Unstained *Sonneratia ovata* pollen and pollen tube

Sonneratia griffithii pollen with pollen tube growth stained with basic fuchsin

Sonneratia ovata fruits

Sonneratia griffithii fruits

Sonneratia ovata seeds

Sonneratia griffithii seeds

Appendix 3: Photos of some flower visitors of *Sonneratia ovata* and *S. griffithii*

Lepidotrigona cf. ventralis

Lepidotrigona cf. terminata

Brown-throated sunbird *(Anthreptes malacensis)*

Macroglossus minimus

Eonycteris spelaea

Appendix 4: Number of flowers per *Sonneratia ovata* cyme.

Tree ID	KY01	KY02	KY03	KY04	KY05	KY06	SoHT01	SoHT02	SoHT04	SoHT05	SoHT08
Cyme 1	4	3	1	2	1	2	1	3	2	1	2
Cyme 2	3	2	1	3	1	3	1	3	3	3	1
Cyme 3	2	1	2	2	3	1	1	2	3	1	NA
Cyme 4	2	3	2	2	1	4	1	2	4	5	NA
Cyme 5	2	2	1	2	1	4	2	1	1	3	NA
Cyme 6	2	1	1	3	1	1	3	1	1	3	NA
Cyme 7	3	3	3	1	3	1	1	NA	1	1	NA
Cyme 8	3	1	1	1	1	2	1	NA	NA	1	NA
Cyme 9	2	1	1	1	1	1	3	NA	NA	NA	NA
Cyme 10	5	1	2	2	3	1	1	NA	NA	NA	NA

Appendix 5: Number of flowers per *Sonneratia griffithii* cyme.

Tree ID	Sg495A	Sg448A	Sg448B	Sg448C	Sg441A	SgKN	SgHT01	SgHT02
Cyme 1	6	3	1	1	3	4	3	3
Cyme 2	5	2	1	1	2	3	6	3
Cyme 3	2	1	1	1	2	4	3	3
Cyme 4	2	2	1	1	3	2	3	4
Cyme 5	2	6	1	2	1	1	3	3
Cyme 6	1	3	1	1	2	1	3	2
Cyme 7	2	2	1	1	2	3	3	3
Cyme 8	3	1	NA	1	2	1	4	2
Cyme 9	2	2	NA	1	1	1	8	1
Cyme 10	1	2	NA	2	2	1	5	2

Appendix 6: Length of the style, anthers closest to the style, and Anther-Stigma Distance (ASD) of *Sonneratia ovata* flowers.

Tree	Season	Location	Style length (mm)	Stamen length (mm)	ASD (mm)
So01	Dry	Khlong Kae	28.68	19.63	9.05
So01	Dry	Khlong Kae	31.98	25.05	6.93
So01	Dry	Khlong Kae	28.21	24.27	3.94
So02	Dry	Khlong Kae	33.87	21.70	12.17
So02	Dry	Khlong Kae	33.08	19.14	13.94
So03	Dry	Khlong Kae	28.78	19.77	9.01
So03	Dry	Khlong Kae	29.14	17.77	11.37
So03	Dry	Khlong Kae	31.99	21.41	10.58
So11	Dry	Khlong Kae	32.02	26.68	5.34
So12	Dry	Khlong Kae	30.86	26.88	3.98
So12	Dry	Khlong Kae	26.60	25.47	1.13
So12	Dry	Khlong Kae	35.95	26.91	9.04
So12	Dry	Khlong Kae	32.22	25.03	7.19
So12	Dry	Khlong Kae	35.99	23.18	12.81
So12	Dry	Khlong Kae	34.43	23.73	10.70
KY02	Wet	Koh Yor	40.48	20.74	19.74
KY02	Wet	Koh Yor	42.88	21.24	21.64
KY02	Wet	Koh Yor	38.00	21.56	16.44

Tree	Season	Location	Style length (mm)	Stamen length (mm)	ASD (mm)
KY02	Wet	Koh Yor	39.72	16.80	22.92
KY02	Wet	Koh Yor	38.62	15.20	23.42
KY02	Wet	Koh Yor	38.60	19.54	19.06
KY02	Wet	Koh Yor	38.64	20.98	17.66
KY02	Wet	Koh Yor	41.68	15.10	26.58
KY05	Wet	Koh Yor	35.12	16.48	18.64
KY05	Wet	Koh Yor	21.68	15.00	6.68
KY05	Wet	Koh Yor	35.00	17.96	17.04
KY05	Wet	Koh Yor	31.60	16.18	15.42
So10	Wet	Khlong Kae	30.16	19.26	10.90
So10	Wet	Khlong Kae	31.00	19.70	11.30
So10	Wet	Khlong Kae	33.60	11.30	22.30
So14	Wet	Khlong Kae	34.02	19.08	14.94
So14	Wet	Khlong Kae	31.08	20.84	10.24
So14	Wet	Khlong Kae	31.88	12.76	19.12
SoHT01	Wet	Hua Tang	35.63	20.85	14.78
SoHT01	Wet	Hua Tang	36.35	23.14	13.21
SoHT01	Wet	Hua Tang	36.98	27.86	9.120
SoHT02	Wet	Hua Tang	40.64	12.60	28.04

Tree	Season	Location	Style length (mm)	Stamen length (mm)	ASD (mm)
SoHT02	Wet	Hua Tang	28.90	17.56	11.34
SoHT02	Wet	Hua Tang	28.70	14.30	14.40
SoHT03	Wet	Hua Tang	41.58	15.50	26.08
SoHT03	Wet	Hua Tang	35.56	23.44	12.12
SoHT03	Wet	Hua Tang	36.68	19.22	17.46
SoHT03	Wet	Hua Tang	38.60	21.84	16.76
SoHT03	Wet	Hua Tang	32.76	18.36	14.40
SoHT04	Wet	Hua Tang	36.06	20.20	15.86
SoHT04	Wet	Hua Tang	41.32	18.5	22.82
SoHT04	Wet	Hua Tang	40.40	26.00	14.40
SoHT07	Wet	Hua Tang	39.56	23.42	16.14
SoHT07	Wet	Hua Tang	38.54	17.24	21.30
SoHT07	Wet	Hua Tang	33.72	18.92	14.80
SoHT08	Wet	Hua Tang	38.00	17.40	20.60
SoHT08	Wet	Hua Tang	42.74	27.52	15.22
SoHT08	Wet	Hua Tang	42.46	22.82	19.64
SoHT08	Wet	Hua Tang	39.88	22.14	17.74

Appendix 7: Length of the style and anthers closest to the style, as well as the Anther-Stigma Distance (ASD) of *Sonneratia griffithii* flowers.

Flower ID	Location	Style length (mm)	Stamen length (mm)	ASD (mm)
Sg446	Koh Nok	40.98	13.46	27.52
Sg446	Koh Nok	46.88	17.20	29.68
Sg448A	Koh Nok	39.26	16.86	22.40
Sg448A	Koh Nok	45.20	20.64	24.56
Sg448A	Koh Nok	44.92	27.60	17.32
Sg448A	Koh Nok	50.70	22.88	27.82
Sg448B	Koh Nok	46.00	15.60	30.40
Sg495A	Koh Nok	41.74	12.90	28.84
SgHT02	Hua Tang	45.26	25.00	20.26
SgHT02	Hua Tang	44.62	17.26	27.36
SgHT02	Hua Tang	55.10	26.78	28.32

Appendix 8: The average percentage of *Sonneratia ovata* pollen that germinated in 15% sucrose solution when collected every 12 hours (h) after anther dehiscence.

Tree ID	Location	0 h	12 h	24 h	36 h	48 h	60 h	72 h	84 h	96 h	108 h	120 h	132 h
So01	Khlong Kae	31.51	42.46	31.12	29.31	20.80	NA	NA	NA	NA	NA	NA	NA
So01	Khlong Kae	41.64	49.99	39.44	42.21	25.94	NA	NA	NA	NA	NA	NA	NA
So01	Khlong Kae	32.64	50.32	43.83	32.39	18.88	NA	NA	NA	NA	NA	NA	NA
So04	Khlong Kae	29.80	42.43	29.06	19.40	15.71	NA	NA	NA	NA	NA	NA	NA
So04	Khlong Kae	39.11	22.78	19.84	23.80	6.55	NA	NA	NA	NA	NA	NA	NA
So10	Khlong Kae	59.83	65.63	15.98	9.56	4.08	2.82	1.88	2.03	3.47	1.81	1.28	1.77
So10	Khlong Kae	64.18	61.72	51.04	10.86	3.09	5.52	2.10	NA	NA	NA	NA	NA
So10	Khlong Kae	51.59	45.23	32.71	16.70	6.26	NA	NA	NA	NA	NA	NA	NA
So14	Khlong Kae	53.77	64.78	22.14	5.01	0.76	0.65	0.16	NA	NA	NA	NA	NA
SoHT01	Hua Tang	48.70	28.17	32.62	22.42	10.45	NA	NA	NA	NA	NA	NA	NA
SoHT01	Hua Tang	73.94	61.86	22.29	3.69	1.24	NA	NA	NA	NA	NA	NA	NA
SoHT01	Hua Tang	71.13	54.28	25.94	8.17	1.39	NA	NA	NA	NA	NA	NA	NA
SoHT02	Hua Tang	79.25	68.75	47.14	7.68	0.53	NA	NA	NA	NA	NA	NA	NA
SoHT02	Hua Tang	68.44	46.55	28.15	2.40	0.63	NA	NA	NA	NA	NA	NA	NA
SoHT02	Hua Tang	51.44	65.51	47.38	7.51	0.00	NA	NA	NA	NA	NA	NA	NA
SoHT03	Hua Tang	11.24	47.62	44.36	6.60	0.15	NA	NA	NA	NA	NA	NA	NA

Tree ID	Location	0 h	12 h	24 h	36 h	48 h	60 h	72 h	84 h	96 h	108 h	120 h	132 h
SoHT05	Hua Tang	32.81	29.24	33.73	24.73	16.71	NA	NA	NA	NA	NA	NA	NA
SoHT05	Hua Tang	50.32	74.09	64.31	27.74	13.80	NA	NA	NA	NA	NA	NA	NA
SoHT08	Hua Tang	63.08	50.70	38.54	26.84	10.61	NA	NA	NA	NA	NA	NA	NA
SoHT08	Hua Tang	50.93	76.28	68.57	34.77	7.59	NA	NA	NA	NA	NA	NA	NA
SoHT08	Hua Tang	56.55	68.79	14.48	3.90	0.15	NA	NA	NA	NA	NA	NA	NA

Appendix 9: The average percentage of *Sonneratia griffithii* pollen that germinated in 15% sucrose solution when collected every 12 hours (h) after anther dehiscence.

Tree ID	Location	0 h	12 h	24 h	36 h	48 h	60 h	72 h	84 h	96 h	108 h	120 h	132 h
SgHT01	Hua Tang	37.84	56.35	48.00	52.38	43.54	39.43	20.10	14.78	NA	NA	NA	NA
SgHT01	Hua Tang	47.94	57.42	54.19	52.54	42.69	17.82	19.93	15.95	NA	NA	NA	NA
SgHT01	Hua Tang	50.92	38.53	40.83	28.99	17.42	12.62	6.89	5.94	NA	NA	NA	NA
SgHT02	Hua Tang	37.24	28.96	31.33	37.98	26.10	31.58	25.65	24.24	23.78	21.00	6.74	3.24
SgHT02	Hua Tang	31.91	46.13	39.88	48.05	41.96	43.74	45.47	51.36	40.12	36.23	18.23	21.16
SgHT02	Hua Tang	39.18	49.64	48.76	38.00	32.88	16.36	13.50	4.14	NA	NA	NA	NA
Sg495B	Koh Nok	54.10	58.30	54.29	62.45	47.85	39.72	37.76	17.21	13.36	4.07	1.81	1.12

Appendix 10: Amount of nectar (µL) per *Sonneratia ovata* flower collected at 19h00, 21h00, 23h00, 01h00, 04h00, and 07h00 during anthesis.

Tree	Season	Location	19h00	21h00	23h00	01h00	04h00	07h00
So01	Dry	Khlong Kae	0.00	39.01	60.15	21.06	2.53	0.00
So01	Dry	Khlong Kae	57.30	15.52	7.86	3.30	1.90	0.00
So01	Dry	Khlong Kae	61.36	25.27	39.71	2.94	0.00	0.00
So01	Dry	Khlong Kae	43.56	45.20	66.76	5.55	1.76	0.00
So01	Dry	Khlong Kae	32.84	50.55	19.77	34.25	29.22	0.00
So11	Dry	Khlong Kae	32.65	39.87	23.55	219.95	102.49	0.00
SoHT01	Wet	Hua Tang	98.52	217.84	145.92	138.84	71.50	NA
SoHT01	Wet	Hua Tang	87.92	202.44	126.62	101.22	112.44	NA
SoHT01	Wet	Hua Tang	82.70	56.60	67.04	10.38	0.00	NA
SoHT01	Wet	Hua Tang	119.98	181.70	336.68	271.00	97.16	NA
SoHT01	Wet	Hua Tang	62.82	167.64	161.96	41.28	0.00	0.00
SoHT02	Wet	Hua Tang	87.34	197.14	185.60	146.78	71.58	NA
SoHT02	Wet	Hua Tang	100.7	231.84	178.44	122.90	68.96	NA
SoHT02	Wet	Hua Tang	162.02	225.36	225.04	142.56	29.66	NA
SoHT04	Wet	Hua Tang	173.02	273.50	135.62	147.34	49.08	NA
SoHT04	Wet	Hua Tang	169.42	289.48	213.74	73.00	70.04	NA

Tree	Season	Location	19h00	21h00	23h00	01h00	04h00	07h00
SoHT04	Wet	Hua Tang	176.9	128.90	200.86	78.32	68.32	0.70
SoHT05	Wet	Hua Tang	193.76	254.02	208.02	67.56	68.60	NA
SoHT05	Wet	Hua Tang	137.58	148.70	165.30	108.76	56.80	NA
SoHT05	Wet	Hua Tang	50.00	108.84	88.74	152.80	21.00	0.00
SoHT08	Wet	Hua Tang	93.16	306.00	141.96	134.12	69.66	NA
So10	Wet	Khlong Kae	101.80	147.23	173.18	191.40	115.95	NA
So10	Wet	Khlong Kae	76.20	276.23	272.22	128.10	42.00	NA
So10	Wet	Khlong Kae	71.50	276.23	214.68	245.10	165.25	NA
So14	Wet	Khlong Kae	83.25	157.46	124.34	106.90	57.45	NA
So14	Wet	Khlong Kae	73.80	230.78	140.88	117.55	1.40	NA
So14	Wet	Khlong Kae	116.05	185.25	112.85	121.00	54.05	NA
KY01	Wet	Koh Yor	199.74	168.30	158.16	80.58	67.46	NA
KY01	Wet	Koh Yor	124.72	110.74	88.46	82.54	8.76	NA
KY01	Wet	Koh Yor	127.58	184.78	117.76	76.10	11.88	NA
KY01	Wet	Koh Yor	209.00	187.56	135.36	68.34	11.80	NA
KY01	Wet	Koh Yor	79.90	137.08	100	52.86	17.20	NA
KY02	Wet	Koh Yor	61.66	136.70	120.99	111.68	80.28	NA
KY02	Wet	Koh Yor	135.08	120.96	130.12	151.42	150.26	NA
KY02	Wet	Koh Yor	27.02	152.20	142.48	124.71	81.68	NA

Tree	**Season**	**Location**	**19h00**	**21h00**	**23h00**	**01h00**	**04h00**	**07h00**
KY02	Wet	Koh Yor	137.18	136.66	128.24	129.28	4.8	NA
KY02	Wet	Koh Yor	38.83	191.22	145.74	124.42	66.50	NA
KY03	Wet	Koh Yor	67.78	221.48	166.68	60.40	143.6	1.74
KY03	Wet	Koh Yor	60.44	212.56	154.66	9.920	55.56	0.00
KY03	Wet	Koh Yor	28.20	93.62	259.32	109.36	48.46	1.64
KY03	Wet	Koh Yor	67.20	197.04	191.12	125.56	48.64	NA
KY03	Wet	Koh Yor	131.96	229.18	57.92	162.6	25.90	NA
KY04	Wet	Koh Yor	117.62	71.56	113.36	93.34	86.32	NA
KY04	Wet	Koh Yor	35.98	148.18	157.32	104.98	17.08	NA
KY04	Wet	Koh Yor	63.54	117.80	117.16	50.78	54.26	NA
KY05	Wet	Koh Yor	114.86	35.18	113.34	78.28	52.48	NA

Appendix 11: Amount of nectar (µL) per *Sonneratia griffithii* flower collected at 19h00, 21h00, 23h00, 01h00, 04h00, and 07h00 during anthesis.

Tree ID	Location	19h00	21h00	23h00	01h00	04h00	07h00
SgHT01	Hua Tang	70.06	213.04	347.96	157.78	88.58	0.00
SgHT01	Hua Tang	126.44	225.36	281.32	78.42	50.60	1.98
SgHT01	Hua Tang	93.52	389.52	239.46	43.94	27.88	0.00
Sg441	Koh Nok	110.66	219.96	196.10	122.98	1.98	NA
Sg441	Koh Nok	183.34	254.94	209.54	97.82	24.32	NA

Appendix 12: Nectar sugar concentration (% Brix) of *Sonneratia ovata* flowers from nectar collected at 19h00, 21h00, 23h00, 01h00, and 04h00 during anthesis.

Tree ID	Season	Location	19h00	21h00	23h00	01h00	04h00
So01	Dry	Khlong Kae	18.3	19.6	19.2	18.0	15.8
So01	Dry	Khlong Kae	18.4	19.0	17.8	16.2	14.2
So01	Dry	Khlong Kae	18.6	18.8	18.0	17.2	16.6
So11	Dry	Khlong Kae	18.0	19.2	19.6	19.0	15.4
KY01	Wet	Koh Yor	21.2	21.2	17.8	14.8	13.4
KY01	Wet	Koh Yor	22.6	21.4	17.8	15.0	13.8
KY01	Wet	Koh Yor	21.8	21.2	18.4	15.6	13.2
KY01	Wet	Koh Yor	21.2	20.8	18.2	15.6	14.0
KY02	Wet	Koh Yor	19.4	20.6	19.2	17.4	14.4
KY02	Wet	Koh Yor	19.0	20.4	19.2	17.4	15.6
KY02	Wet	Koh Yor	19.0	19.6	18.6	17.0	15.0
KY02	Wet	Koh Yor	21.0	21.0	19.2	16.8	14.2
KY02	Wet	Koh Yor	21.4	21.6	19.0	16.0	13.8
KY03	Wet	Koh Yor	21.0	21.6	19.8	16.2	15.2
KY03	Wet	Koh Yor	21.2	21.2	18.8	16.0	15.0
KY03	Wet	Koh Yor	21.0	21.2	19.6	15.2	13.4

Tree ID	Season	Location	19h00	21h00	23h00	01h00	04h00
KY03	Wet	Koh Yor	21.0	21.6	20.4	16.4	14.0
KY03	Wet	Koh Yor	21.8	22.0	19.2	16.2	13.2
KY04	Wet	Koh Yor	18.8	20.0	19.8	18.4	15.8
KY04	Wet	Koh Yor	21.2	21.0	20.2	17.2	17.4
KY05	Wet	Koh Yor	20.0	20.0	18.2	15.8	13.2
So10	Wet	Khlong Kae	16.0	19.4	19.2	18.0	15.4
So10	Wet	Khlong Kae	17.4	20.0	19.2	17.4	15.4
So10	Wet	Khlong Kae	17.2	19.4	18.8	17.0	13.6
So14	Wet	Khlong Kae	17.6	19.0	17.4	14.6	12.6
So14	Wet	Khlong Kae	17.8	18.6	17.6	16.0	14.2
SoHT01	Wet	Hua Tang	19.0	20.2	19.8	16.4	14.8
SoHT01	Wet	Hua Tang	19.0	19.4	18.0	15.4	15.0
SoHT01	Wet	Hua Tang	18.4	20.4	18.8	15.6	12.8
SoHT02	Wet	Hua Tang	17.6	19.2	17.0	13.6	11.2
SoHT02	Wet	Hua Tang	18.8	19.6	17.6	14.4	12.0
SoHT02	Wet	Hua Tang	19.0	19.8	17.0	18.8	12.0
SoHT04	Wet	Hua Tang	18.0	19.6	17.4	14.6	13.0
SoHT04	Wet	Hua Tang	18.8	19.2	16.8	19.2	12.8
SoHT04	Wet	Hua Tang	17.8	18.0	15.6	18.0	11.4
SoHT05	Wet	Hua Tang	18.8	19.0	16.4	18.6	12.4
SoHT05	Wet	Hua Tang	18.8	19.0	16.8	13.6	11.0
SoHT05	Wet	Hua Tang	17.2	19.6	18.0	16.0	12.4

Appendix 13: Nectar sugar concentration (% Brix) of *Sonneratia griffithii* flowers from nectar collected at 19h00, 21h00, 23h00, 01h00, and 04h00 during anthesis.

Tree ID	Location	19h00	21h00	23h00	01h00	04h00
SgHT01	Hua Tang	17.6	19.4	17.4	14.8	13.2
SgHT01	Hua Tang	16.6	19.2	16.8	13.4	12.0
SgHT01	Hua Tang	18.4	19.0	17.0	13.8	12.2
Sg441	Koh Nok	15.6	18.2	16.6	14.0	11.0

Appendix 14: Results of Pollination experiment on *Sonneratia ovata,* where A = aborted, L = lost, and M = mature. The treatments were OP = open pollination, IP = insect pollination, HC = hand-cross pollination, HS = hand-self pollination, and SA = spontaneous autogamy.

Tree ID	Location	Season	Treatment	Result	Weeks after Anthesis
KY01	Koh Yor	Wet	HS	M	8th
KY01	Koh Yor	Wet	HS	M	8th
KY01	Koh Yor	Wet	HS	M	8th
KY01	Koh Yor	Wet	HS	M	8th
KY01	Koh Yor	Wet	HC	M	8th
KY01	Koh Yor	Wet	HC	M	8th
KY01	Koh Yor	Wet	HC	M	8th
KY01	Koh Yor	Wet	IP	M	8th
KY01	Koh Yor	Wet	IP	M	8th
KY01	Koh Yor	Wet	IP	A	1st
KY01	Koh Yor	Wet	OP	A	3rd
KY01	Koh Yor	Wet	SA	M	8th
KY02	Koh Yor	Wet	HS	M	8th
KY02	Koh Yor	Wet	HS	L	4th
KY02	Koh Yor	Wet	HS	L	4th

Tree ID	Location	Season	Treatment	Result	Weeks after Anthesis
KY02	Koh Yor	Wet	HS	M	8th
KY02	Koh Yor	Wet	HS	M	8th
KY02	Koh Yor	Wet	HC	M	8th
KY02	Koh Yor	Wet	HC	M	8th
KY02	Koh Yor	Wet	HC	L	4th
KY02	Koh Yor	Wet	HC	M	8th
KY02	Koh Yor	Wet	IP	M	8th
KY02	Koh Yor	Wet	IP	M	8th
KY02	Koh Yor	Wet	IP	M	8th
KY02	Koh Yor	Wet	IP	A	3rd
KY02	Koh Yor	Wet	IP	M	8th
KY02	Koh Yor	Wet	IP	A	4th
KY02	Koh Yor	Wet	OP	M	8th
KY02	Koh Yor	Wet	OP	A	1st
KY02	Koh Yor	Wet	OP	M	8th
KY02	Koh Yor	Wet	OP	L	5th
KY02	Koh Yor	Wet	OP	L	5th
KY02	Koh Yor	Wet	OP	M	8th
KY02	Koh Yor	Wet	SA	M	8th
KY02	Koh Yor	Wet	SA	A	3rd
KY02	Koh Yor	Wet	SA	M	8th
KY02	Koh Yor	Wet	SA	A	3rd
KY02	Koh Yor	Wet	SA	A	3rd
KY02	Koh Yor	Wet	SA	A	5th
KY03	Koh Yor	Wet	HS	M	8th
KY03	Koh Yor	Wet	HS	A	2nd
KY03	Koh Yor	Wet	HS	L	1st
KY03	Koh Yor	Wet	HC	M	8th
KY03	Koh Yor	Wet	HC	M	8th
KY03	Koh Yor	Wet	HC	A	3rd

Tree ID	Location	Season	Treatment	Result	Weeks after Anthesis
KY03	Koh Yor	Wet	OP	A	4th
KY04	Koh Yor	Wet	IP	A	3rd
KY04	Koh Yor	Wet	OP	A	2nd
KY04	Koh Yor	Wet	OP	M	8th
KY05	Koh Yor	Wet	SA	A	1st
KY05	Koh Yor	Wet	SA	M	8th
So01	Khlong Kae	Dry	OP	A	3rd
So01	Khlong Kae	Dry	OP	A	2nd
So01	Khlong Kae	Dry	OP	A	1st
So01	Khlong Kae	Dry	OP	A	1st
So01	Khlong Kae	Dry	HS	A	2nd
So01	Khlong Kae	Wet	HS	M	8th
So01	Khlong Kae	Dry	HS	A	1st
So01	Khlong Kae	Wet	HS	A	4th
So01	Khlong Kae	Dry	HS	A	1st
So01	Khlong Kae	Wet	HS	A	2nd
So01	Khlong Kae	Dry	HC	A	4th
So01	Khlong Kae	Wet	HC	A	1st
So01	Khlong Kae	Dry	HC	A	3rd
So01	Khlong Kae	Dry	HC	A	2nd
So01	Khlong Kae	Wet	HC	M	8th
So01	Khlong Kae	Dry	IP	A	1st
So01	Khlong Kae	Wet	IP	M	8th
So01	Khlong Kae	Dry	IP	A	2nd
So01	Khlong Kae	Wet	IP	A	2nd
So01	Khlong Kae	Wet	IP	A	1st
So01	Khlong Kae	Wet	OP	M	8th
So01	Khlong Kae	Wet	OP	A	1st
So01	Khlong Kae	Dry	SA	A	1st
So01	Khlong Kae	Wet	SA	A	1st

Tree ID	Location	Season	Treatment	Result	Weeks after Anthesis
So01	Khlong Kae	Wet	SA	A	2nd
So01	Khlong Kae	Wet	SA	A	2nd
So02	Khlong Kae	Dry	OP	A	2nd
So02	Khlong Kae	Dry	OP	A	2nd
So02	Khlong Kae	Dry	OP	A	1st
So02	Khlong Kae	Dry	HS	L	2nd
So02	Khlong Kae	Dry	HC	A	1st
So02	Khlong Kae	Dry	IP	A	2nd
So02	Khlong Kae	Dry	IP	A	1st
So02	Khlong Kae	Dry	SA	A	2nd
So02	Khlong Kae	Dry	SA	A	1st
So02	Khlong Kae	Dry	SA	A	1st
So03	Khlong Kae	Dry	OP	L	2nd
So03	Khlong Kae	Dry	HS	A	3rd
So03	Khlong Kae	Dry	HC	A	2nd
So03	Khlong Kae	Dry	HC	A	1st
So03	Khlong Kae	Dry	IP	A	2nd
So03	Khlong Kae	Dry	IP	A	1st
So03	Khlong Kae	Dry	SA	A	2nd
So03	Khlong Kae	Dry	SA	A	1st
So04	Khlong Kae	Dry	SA	A	3rd
So04	Khlong Kae	Dry	SA	A	4th
So04	Khlong Kae	Dry	HS	A	3rd
So04	Khlong Kae	Wet	HS	M	8th
So04	Khlong Kae	Wet	HC	A	7th
So04	Khlong Kae	Wet	HC	M	8th
So04	Khlong Kae	Dry	IP	A	2nd
So04	Khlong Kae	Wet	IP	M	8th
So04	Khlong Kae	Wet	OP	M	8th
So04	Khlong Kae	Wet	OP	M	8th

Tree ID	Location	Season	Treatment	Result	Weeks after Anthesis
So05	Khlong Kae	Dry	OP	A	1st
So05	Khlong Kae	Dry	HC	A	2nd
So06	Khlong Kae	Dry	OP	A	1st
So06	Khlong Kae	Dry	HS	A	3rd
So06	Khlong Kae	Dry	HS	A	2nd
So06	Khlong Kae	Dry	HC	A	1st
So06	Khlong Kae	Dry	HC	A	2nd
So06	Khlong Kae	Dry	IP	A	1st
So06	Khlong Kae	Dry	IP	A	2nd
So06	Khlong Kae	Dry	SA	A	3rd
So07	Khlong Kae	Dry	OP	A	3rd
So07	Khlong Kae	Dry	HC	A	3rd
So07	Khlong Kae	Dry	SA	A	1st
So08	Khlong Kae	Dry	HS	A	3rd
So08	Khlong Kae	Dry	HC	A	3rd
So08	Khlong Kae	Dry	IP	A	1st
So08	Khlong Kae	Dry	SA	A	1st
So09	Khlong Kae	Dry	OP	A	1st
So09	Khlong Kae	Dry	IP	A	2nd
So09	Khlong Kae	Dry	SA	A	4th
So10	Khlong Kae	Dry	OP	A	3rd
So10	Khlong Kae	Dry	SA	A	1st
So10	Khlong Kae	Dry	HS	A	2nd
So10	Khlong Kae	Dry	HS	A	1st
So10	Khlong Kae	Wet	HS	M	8th
So10	Khlong Kae	Dry	HS	A	2nd
So10	Khlong Kae	Wet	HS	M	8th
So10	Khlong Kae	Dry	HC	A	2nd
So10	Khlong Kae	Wet	HC	L	5th
So10	Khlong Kae	Dry	HC	A	3rd

Tree ID	Location	Season	Treatment	Result	Weeks after Anthesis
So10	Khlong Kae	Dry	HC	A	2nd
So10	Khlong Kae	Dry	IP	A	2nd
So10	Khlong Kae	Wet	IP	A	1st
So10	Khlong Kae	Wet	IP	A	3rd
So10	Khlong Kae	Wet	IP	M	9th
So10	Khlong Kae	Wet	IP	A	1st
So10	Khlong Kae	Dry	OP	A	1st
So10	Khlong Kae	Wet	OP	A	1st
So10	Khlong Kae	Dry	OP	A	2nd
So10	Khlong Kae	Wet	OP	A	1st
So10	Khlong Kae	Dry	OP	A	4th
So10	Khlong Kae	Wet	OP	A	4th
So10	Khlong Kae	Dry	OP	A	4th
So10	Khlong Kae	Dry	SA	A	1st
So10	Khlong Kae	Wet	SA	A	1st
So10	Khlong Kae	Wet	SA	A	2nd
So10	Khlong Kae	Dry	SA	A	1st
So10	Khlong Kae	Wet	SA	M	9th
So11	Khlong Kae	Dry	HS	A	4th
So11	Khlong Kae	Dry	HS	A	4th
So11	Khlong Kae	Dry	HS	A	4th
So11	Khlong Kae	Dry	HS	A	2nd
So11	Khlong Kae	Dry	HC	A	4th
So11	Khlong Kae	Dry	HC	A	2nd
So11	Khlong Kae	Dry	HC	A	1st
So11	Khlong Kae	Dry	IP	A	2nd
So11	Khlong Kae	Dry	IP	A	2nd
So11	Khlong Kae	Dry	OP	A	1st
So11	Khlong Kae	Dry	OP	A	5th
So11	Khlong Kae	Dry	OP	A	1st

Tree ID	Location	Season	Treatment	Result	Weeks after Anthesis
So11	Khlong Kae	Dry	OP	A	1st
So11	Khlong Kae	Dry	SA	A	3rd
So11	Khlong Kae	Dry	SA	A	1st
So11	Khlong Kae	Dry	SA	A	3rd
So11	Khlong Kae	Dry	SA	L	2nd
So12	Khlong Kae	Dry	HS	A	4th
So12	Khlong Kae	Dry	HS	A	1st
So12	Khlong Kae	Dry	HS	A	2nd
So12	Khlong Kae	Dry	HC	A	3rd
So12	Khlong Kae	Dry	HC	A	3rd
So12	Khlong Kae	Dry	HC	A	1st
So12	Khlong Kae	Dry	HC	A	2nd
So12	Khlong Kae	Dry	IP	A	1st
So12	Khlong Kae	Dry	IP	A	1st
So12	Khlong Kae	Dry	IP	A	1st
So12	Khlong Kae	Dry	IP	A	4th
So12	Khlong Kae	Dry	OP	A	1st
So12	Khlong Kae	Dry	OP	A	1st
So12	Khlong Kae	Dry	OP	A	2nd
So12	Khlong Kae	Dry	OP	A	2nd
So12	Khlong Kae	Dry	SA	A	1st
So12	Khlong Kae	Dry	SA	A	1st
So12	Khlong Kae	Dry	SA	A	1st
So14	Khlong Kae	Wet	HC	A	1st
So14	Khlong Kae	Wet	IP	A	1st
So14	Khlong Kae	Wet	OP	A	1st
So14	Khlong Kae	Wet	OP	M	8th
So14	Khlong Kae	Wet	OP	M	8th
So14	Khlong Kae	Wet	SA	A	1st
So14	Khlong Kae	Wet	SA	A	1st

Tree ID	Location	Season	Treatment	Result	Weeks after Anthesis
So14	Khlong Kae	Wet	SA	A	1st
SoHT01	Hua Tang	Wet	HS	M	8th
SoHT01	Hua Tang	Wet	HS	A	6th
SoHT01	Hua Tang	Wet	HC	M	8th
SoHT01	Hua Tang	Wet	HC	M	8th
SoHT01	Hua Tang	Wet	HC	M	8th
SoHT01	Hua Tang	Wet	IP	A	1st
SoHT01	Hua Tang	Wet	IP	L	1st
SoHT01	Hua Tang	Wet	IP	M	8th
SoHT01	Hua Tang	Wet	OP	M	9th
SoHT01	Hua Tang	Wet	OP	M	8th
SoHT01	Hua Tang	Wet	OP	M	8th
SoHT01	Hua Tang	Wet	SA	A	1st
SoHT01	Hua Tang	Wet	SA	A	1st
SoHT01	Hua Tang	Wet	SA	M	8th
SoHT02	Hua Tang	Wet	HS	M	8th
SoHT02	Hua Tang	Wet	HS	M	8th
SoHT02	Hua Tang	Wet	HS	A	7th
SoHT02	Hua Tang	Wet	HC	A	1st
SoHT02	Hua Tang	Wet	HC	A	1st
SoHT02	Hua Tang	Wet	HC	M	8th
SoHT02	Hua Tang	Wet	HC	M	8th
SoHT02	Hua Tang	Wet	IP	A	1st
SoHT02	Hua Tang	Wet	IP	A	1st
SoHT02	Hua Tang	Wet	IP	A	1st
SoHT02	Hua Tang	Wet	IP	M	8th
SoHT02	Hua Tang	Wet	OP	L	2nd
SoHT02	Hua Tang	Wet	OP	M	8th
SoHT02	Hua Tang	Wet	OP	L	1st
SoHT02	Hua Tang	Wet	SA	A	1st

Tree ID	Location	Season	Treatment	Result	Weeks after Anthesis
SoHT02	Hua Tang	Wet	SA	A	1st
SoHT02	Hua Tang	Wet	SA	A	1st
SoHT03	Hua Tang	Wet	HS	M	12th
SoHT03	Hua Tang	Wet	HS	L	2nd
SoHT03	Hua Tang	Wet	HS	M	8th
SoHT03	Hua Tang	Wet	HC	A	2nd
SoHT03	Hua Tang	Wet	HC	M	8th
SoHT03	Hua Tang	Wet	HC	A	1st
SoHT03	Hua Tang	Wet	IP	M	9th
SoHT03	Hua Tang	Wet	IP	A	1st
SoHT03	Hua Tang	Wet	IP	M	8th
SoHT03	Hua Tang	Wet	OP	M	9th
SoHT03	Hua Tang	Wet	OP	M	8th
SoHT03	Hua Tang	Wet	OP	M	8th
SoHT03	Hua Tang	Wet	SA	L	1st
SoHT03	Hua Tang	Wet	SA	L	2nd
SoHT03	Hua Tang	Wet	SA	A	1st
SoHT03	Hua Tang	Wet	SA	A	1st
SoHT04	Hua Tang	Wet	HS	A	3rd
SoHT04	Hua Tang	Wet	HS	L	2nd
SoHT04	Hua Tang	Wet	HS	M	8th
SoHT04	Hua Tang	Wet	HC	A	1st
SoHT04	Hua Tang	Wet	HC	M	8th
SoHT04	Hua Tang	Wet	HC	M	8th
SoHT04	Hua Tang	Wet	IP	A	1st
SoHT04	Hua Tang	Wet	IP	A	1st
SoHT04	Hua Tang	Wet	IP	A	1st
SoHT04	Hua Tang	Wet	IP	A	1st
SoHT04	Hua Tang	Wet	OP	A	1st
SoHT04	Hua Tang	Wet	OP	M	8th

Tree ID	Location	Season	Treatment	Result	Weeks after Anthesis
SoHT04	Hua Tang	Wet	OP	M	8th
SoHT04	Hua Tang	Wet	SA	A	1st
SoHT04	Hua Tang	Wet	SA	M	8th
SoHT04	Hua Tang	Wet	SA	A	1st
SoHT05	Hua Tang	Wet	HS	M	8th
SoHT05	Hua Tang	Wet	HS	M	8th
SoHT05	Hua Tang	Wet	HS	M	8th
SoHT05	Hua Tang	Wet	HC	M	8th
SoHT05	Hua Tang	Wet	HC	M	9th
SoHT05	Hua Tang	Wet	HC	M	8th
SoHT05	Hua Tang	Wet	IP	M	9th
SoHT05	Hua Tang	Wet	IP	A	1st
SoHT05	Hua Tang	Wet	IP	A	1st
SoHT05	Hua Tang	Wet	OP	M	9th
SoHT05	Hua Tang	Wet	SA	M	9th
SoHT05	Hua Tang	Wet	SA	M	9th
SoHT07	Hua Tang	Wet	HS	M	8th
SoHT07	Hua Tang	Wet	HS	M	8th
SoHT07	Hua Tang	Wet	HS	A	2nd
SoHT07	Hua Tang	Wet	HC	A	1st
SoHT07	Hua Tang	Wet	HC	M	8th
SoHT07	Hua Tang	Wet	HC	M	8th
SoHT07	Hua Tang	Wet	IP	M	8th
SoHT07	Hua Tang	Wet	IP	M	8th
SoHT07	Hua Tang	Wet	IP	M	8th
SoHT07	Hua Tang	Wet	IP	M	9th
SoHT07	Hua Tang	Wet	OP	M	10th
SoHT07	Hua Tang	Wet	OP	M	9th
SoHT07	Hua Tang	Wet	OP	A	2nd
SoHT07	Hua Tang	Wet	SA	M	10th

Tree ID	Location	Season	Treatment	Result	Weeks after Anthesis
SoHT07	Hua Tang	Wet	SA	M	9th
SoHT07	Hua Tang	Wet	SA	A	1st
SoHT07	Hua Tang	Wet	SA	A	1st
SoHT08	Hua Tang	Wet	HS	M	9th
SoHT08	Hua Tang	Wet	HS	A	1st
SoHT08	Hua Tang	Wet	HS	M	8th
SoHT08	Hua Tang	Wet	HC	M	8th
SoHT08	Hua Tang	Wet	HC	A	1st
SoHT08	Hua Tang	Wet	HC	M	8th
SoHT08	Hua Tang	Wet	IP	A	2nd
SoHT08	Hua Tang	Wet	OP	M	8th
SoHT08	Hua Tang	Wet	OP	M	8th
SoHT08	Hua Tang	Wet	SA	M	8th

Appendix 15: Results of Pollination experiment on *Sonneratia griffithii,* where A = aborted, L = lost, and M = mature. The treatments were OP = open pollination, IP = insect pollination, HC = hand-cross pollination, HS = hand-self pollination, and SA = spontaneous autogamy.

Tree ID	Location	Treatment	Result	Weeks after Anthesis
Sg435	Koh Nok	IP	L	2nd
Sg440	Koh Nok	IP	A	4th
Sg440	Koh Nok	OP	A	1st
Sg440	Koh Nok	SA	A	1st
Sg441	Koh Nok	HS	A	5th
Sg441	Koh Nok	IP	A	1st
Sg441	Koh Nok	IP	A	1st
Sg441	Koh Nok	OP	A	2nd
Sg441	Koh Nok	OP	A	3rd
Sg441	Koh Nok	OP	A	3rd
Sg441	Koh Nok	SA	A	3rd
Sg441	Koh Nok	SA	L	1st
Sg446A	Koh Nok	HS	L	1st
Sg446A	Koh Nok	HS	A	2nd
Sg446A	Koh Nok	IP	A	2nd
Sg446A	Koh Nok	IP	L	1st
Sg446A	Koh Nok	OP	A	4th
Sg446A	Koh Nok	OP	A	2nd
Sg446A	Koh Nok	SA	M	9th
Sg446A	Koh Nok	SA	A	1st
Sg446A	Koh Nok	SA	A	6th
Sg448A	Koh Nok	HS	A	2nd
Sg448A	Koh Nok	HS	A	2nd
Sg448A	Koh Nok	HS	A	3rd
Sg448A	Koh Nok	HC	A	5th

Tree ID	**Location**	**Treatment**	**Result**	**Weeks after Anthesis**
Sg448A	Koh Nok	HC	A	3rd
Sg448A	Koh Nok	HC	A	4th
Sg448A	Koh Nok	IP	A	1st
Sg448A	Koh Nok	IP	A	2nd
Sg448A	Koh Nok	IP	A	1st
Sg448A	Koh Nok	OP	A	2nd
Sg448A	Koh Nok	OP	A	1st
Sg448A	Koh Nok	OP	A	3rd
Sg448A	Koh Nok	SA	A	1st
Sg448A	Koh Nok	SA	A	3rd
Sg448A	Koh Nok	SA	A	1st
Sg495A	Koh Nok	HS	M	9th
Sg495A	Koh Nok	HS	A	1st
Sg495A	Koh Nok	HC	M	9th
Sg495A	Koh Nok	HC	A	3rd
Sg495A	Koh Nok	IP	A	1st
Sg495A	Koh Nok	OP	A	4th
Sg495A	Koh Nok	OP	A	1st
Sg495A	Koh Nok	OP	M	9th
Sg495A	Koh Nok	SA	A	1st
Sg495A	Koh Nok	SA	A	1st
Sg495A	Koh Nok	SA	A	1st
SgHT01	Hua Tang	HS	A	1st
SgHT01	Hua Tang	HS	A	1st
SgHT01	Hua Tang	HS	L	1st
SgHT01	Hua Tang	HC	L	3rd
SgHT01	Hua Tang	HC	M	10th
SgHT01	Hua Tang	HC	M	8th
SgHT01	Hua Tang	IP	M	9th
SgHT01	Hua Tang	IP	M	8th

Tree ID	**Location**	**Treatment**	**Result**	**Weeks after Anthesis**
SgHT01	Hua Tang	IP	A	1st
SgHT01	Hua Tang	OP	A	3rd
SgHT01	Hua Tang	OP	A	1st
SgHT01	Hua Tang	OP	M	8th
SgHT01	Hua Tang	OP	A	1st
SgHT01	Hua Tang	SA	A	3rd
SgHT01	Hua Tang	SA	L	3rd
SgHT01	Hua Tang	SA	A	3rd
SgHT02	Hua Tang	HS	A	2nd
SgHT02	Hua Tang	HS	A	2nd
SgHT02	Hua Tang	HC	A	1st
SgHT02	Hua Tang	IP	A	2nd
SgHT02	Hua Tang	OP	M	9th
SgHT02	Hua Tang	OP	M	8th
SgHT02	Hua Tang	SA	A	2nd

Appendix 16: Seed sets of undamaged mature fruits resulting from the pollination experiment on *Sonneratia ovata.* The treatments were OP = open pollination, IP = insect pollination, HC = hand-cross pollination, HS = hand-self pollination, and SA = spontaneous autogamy.

Tree	Location	Treatment	Seed Count
KY01	Koh Yor	SA	181
KY01	Koh Yor	IP	229
KY01	Koh Yor	HS	473
KY01	Koh Yor	HS	106
KY01	Koh Yor	HC	76
KY01	Koh Yor	HC	129
KY01	Koh Yor	HC	357
KY02	Koh Yor	OP	111
KY02	Koh Yor	OP	136
KY02	Koh Yor	HS	176
KY02	Koh Yor	HS	133
KY02	Koh Yor	HC	262
KY02	Koh Yor	HC	129
KY02	Koh Yor	HC	128
KY02	Koh Yor	IP	67
KY02	Koh Yor	IP	92
KY02	Koh Yor	IP	163
KY03	Koh Yor	HS	313
So01	Khlong Kae	HS	615
So01	Khlong Kae	IP	401
So01	Khlong Kae	OP	419
So04	Khlong Kae	HS	506
So04	Khlong Kae	HC	634
So04	Khlong Kae	HC	387
So04	Khlong Kae	IP	363
So04	Khlong Kae	OP	327

Tree	**Location**	**Treatment**	**Seed Count**
So04	Khlong Kae	OP	671
So10	Khlong Kae	IP	311
So10	Khlong Kae	SA	50
SoHT01	Hua Tang	HS	118
SoHT01	Hua Tang	HC	888
SoHT01	Hua Tang	HC	179
SoHT01	Hua Tang	HC	524
SoHT01	Hua Tang	OP	562
SoHT01	Hua Tang	OP	324
SoHT01	Hua Tang	SA	157
SoHT02	Hua Tang	HS	343
SoHT02	Hua Tang	HS	345
SoHT02	Hua Tang	HC	616
SoHT02	Hua Tang	OP	219
SoHT03	Hua Tang	HS	515
SoHT03	Hua Tang	HS	731
SoHT03	Hua Tang	IP	559
SoHT03	Hua Tang	OP	557
SoHT03	Hua Tang	OP	642
SoHT03	Hua Tang	OP	514
SoHT04	Hua Tang	HC	593
SoHT04	Hua Tang	OP	416
SoHT05	Hua Tang	HS	406
SoHT05	Hua Tang	HS	1020
SoHT05	Hua Tang	HC	405
SoHT05	Hua Tang	HC	540
SoHT05	Hua Tang	OP	572
SoHT05	Hua Tang	SA	283
SoHT05	Hua Tang	SA	48
SoHT07	Hua Tang	HC	262

Tree	Location	Treatment	Seed Count
SoHT07	Hua Tang	IP	408
SoHT07	Hua Tang	IP	271
SoHT07	Hua Tang	IP	299
SoHT07	Hua Tang	IP	581
SoHT07	Hua Tang	OP	402
SoHT07	Hua Tang	OP	193
SoHT08	Hua Tang	HS	93
SoHT08	Hua Tang	OP	215

Appendix 17: Seed sets of undamaged mature fruits resulting from the pollination experiment on *Sonneratia griffithii.* The treatments were OP = open pollination, IP = insect pollination, HC = hand-cross pollination, HS = hand-self pollination, and SA = spontaneous autogamy.

TreeID	Location	Treatment	Seed Count
SgHT01	Hua Tang	HC	280
SgHT01	Hua Tang	HC	233
SgHT01	Hua Tang	IP	283
SgHT01	Hua Tang	IP	129
SgHT01	Hua Tang	OP	168
SgHT02	Hua Tang	OP	103
SgHT02	Hua Tang	OP	107

Appendix 18: ***Macroglossus minimus*** **(Mm) and** ***Eonycteris spelaea*** **(Es) captured by mistnests near** ***Sonneratia ovata*** **(So) and** ***S. griffithii*** **(Sg) flowers. Nets were closed at 00h00 on 2-3 April 2016 and at 23h00 on 15 Dec 2016. Gender: male (M), female (F). Age: juvenile (J), sub-adult (SA), adult (A).**

Date	Location	Time	Species	Gender	Age	Forearm (mm)	Bat weight (gm)	Notes
2-Apr-2016	So01	Before 20h00	Mm	M	A	42	20	-
2-Apr-2016	So01	Before 20h00	Mm	M	A	42	16	-
2-Apr-2016	So01	20h00	Mm	M	A	29	11	-
2-Apr-2016	So01	20h30	Mm	F	J	40	16	-
2-Apr-2016	So01	20h30	Mm	F	A	40	17	-
2-Apr-2016	So01	21h00	Mm	F	A	41	13	*
2-Apr-2016	So01	21h40	Mm	M	A	36	11	-
2-Apr-2016	So01	21h55	Mm	F	A	42	19	possibly pregnant
2-Apr-2016	So01	22h15	Mm	F	A	41	13	possible recapture (see *)
3-Apr-2016	So04 & 05	20h00	Mm	F	A	38	17	-
3-Apr-2016	So04 & 05	20h00	Mm	F	A	42	18	pregnant
3-Apr-2016	So04 & 05	20h00	Mm	F	A	41	14	lactating
3-Apr-2016	So04 & 05	20h20	Mm	M	A	41	14	-
3-Apr-2016	So04 & 05	20h50	Mm	F	A	40	16	pregnant

Date	**Location**	**Time**	**Species**	**Gender**	**Age**	**Forearm (mm)**	**Bat weight (gm)**	**Notes**
3-Apr-2016	So04 & 05	20h05	Mm	F	SA	41	18	pregnant
3-Apr-2016	So04 & 05	20h05	Mm	F	SA	42	14	-
3-Apr-2016	So04 & 05	20h05	Mm	F	A	40	16	possibly pregnant
3-Apr-2016	So04 & 05	20h45	Mm	F	A	39	16	-
15-Dec-2016	Sg495A	Before 20h00	Mm	M	A	42	14	-
15-Dec-2016	Sg495A	Before 20h00	Mm	F	A	40	13	post lactating; very thin
15-Dec-2016	Sg495A	Before 20h00	Es	F	A	66	47	lactating
15-Dec-2016	Sg495A	Before 20h00	Es	F	A	64	45	nulliparous
15-Dec-2016	Sg495A	Before 20h00	Mm	M	A	39	14	-
15-Dec-2016	Sg495A	Before 20h00	Es	F	A	68	48	-
15-Dec-2016	Sg495A	~21h00	Es	M	J	57	29	-
15-Dec-2016	Sg495A	~21h00	Mm	F	A	39	15	Non-lactating

Appendix 19: Visual observations of bats visiting *Sonneratia ovata* (So) and *S. griffithii* (Sg) flowers. All observations were from ~19h30. Observations for 28 December 2016 for *S. ovata* were until 21h00 and until 22h00 for *S. griffithii*. Observations for 17 January 2016 were only until 20h45.

Date	Tree	Directly Illuminated?	Time	Possible visitor
28-Dec-15	SoHT07	No; ~3m away from bright inflorescent light	20h30	*M. minimus*
28-Dec-15	SgHT01	No; ~6m away from bright yellow streetlight	20h35	*M. minimus*
28-Dec-15	SgHT01	No; ~6m away from bright yellow streetlight	21h35	*M. minimus*
28-Dec-15	SgHT02	Yes; ~6 m away from bright yellow streetlight	none	none
17-Jan-16	Sg440	No; streetlight > 10 m away	19h00	several *E. spelaea*
17-Jan-16	Sg440	No; streetlight > 10 m away	20h45	several *E. spelaea*

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List of Publications

NUEVO-DIEGO, C. E., STEWART, A. B. & BUMRUNGSRI, S. Pollinators increase reproductive success of a self-compatible mangrove, *Sonneratia ovata*, in southern Thailand. *Journal of Tropical Ecology* **(Submitted)**.

NUEVO-DIEGO, C. E., STEWART, A. B. & BUMRUNGSRI, S. Pollinators required for reproductive success of largely self-incompatible critically endangered mangrove, *Sonneratia griffithii*, in Satun, Thailand **(To be submitted)**.