Effects of Salinity on Distribution of Alien Invasive and Indigenous Byssally-attached Bivalves in the Songkhla Lake Basin

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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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ชื่อวิทยานิพนธ์ ผลของความเค็มต่อการกระจายของหอยสองฝาต่างถิ่นและพื้นเมืองในพื้นที่ลุ่มน้ำทะเลสาบสงขลา
ผู้เขียน นางสาวณัฐชา ขันทะสีมาเฉลิม
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บทคัดย่อ

Mytilopsis sallei (Récluz, 1849) และ *Mytella strigata* (Hanley, 1843) เป็นหอยสองฝาต่างถิ่นที่รุกรานในทะเลสาบสงขลา ซึ่งเป็นส่วนหนึ่งของระบบลากูนที่ใหญ่ที่สุดในประเทศไทย หอยสองฝาอีกชนิด *Perna viridis* (Linnaeus, 1758) เป็นหอยสองฝาพื้นเมือง มักพบการกระจายอย่างชุกชุมในทะเลสาบสงขลา และชายฝั่งทะเลอ่าวไทยใกล้ปากทะเลสาบ แต่ในช่วงไม่กี่ปีที่ผ่านมา พบหอยชนิดนี้ได้น้อยมากในทะเลสาบสงขลา และพบการกระจายเฉพาะบริเวณชายฝั่งทะเลอ่าวไทยเท่านั้น งานวิจัยวิทยานิพนธ์นี้ศึกษารูปแบบการกระจาย, ความชุกชุม และความแปรผันการเข้าสู่พื้นที่ในเชิงพื้นที่และเวลาของหอยสองฝาเหล่านี้ในทะเลสาบสงขลา โดยเก็บข้อมูลการกระจาย และประเมินความชุกชมของหอยสองฝาตัวเต็มวัย อีกทั้งนับการลงเกาะของตัวอ่อนหอยสองฝาเพื่อศึกษาความแปรผันการเข้าสู่พื้นที่ โดยเน้นที่อิทธิผลของความเค็มซึ่งมีความแปรผันอย่างมากในระบบลากูนนี้ รวมถึงทดลองเลี้ยงลูกหอยวัยรุ่น (juveniles) ในความเค็มที่ต่างกัน (0, 20, 35) เพื่อศึกษาอัตราการเจริญเติบโต ดัชนีความสมบูรณ์ และการอยู่รอดของหอยสองฝาในสภาวะความเค็มที่ต่างกัน ผลการศึกษา พบว่ารูปแบบการกระจายของตัวอ่อนที่เข้าสู่พื้นที่ (recruits) และการกระจายของตัวเต็มวัยสอดคล้องกัน การเข้าสู่พื้นที่ของ *M. sallei* และการกระจายของตัวเต็มวัย พบที่ความเค็มตั้งแต่บริเวณน้ำจืดไปจนถึงความเค็มระดับกลาง การเข้าสู่พื้นที่และการกระจายของตัวเต็มวัย *M. strigata* พบบริเวณความเค็มจากน้ำกร่อยถึงน้ำเค็ม และพบการเข้าสู่พื้นที่และการกระจายของตัวเต็มวัย *P. viridis* บริเวณปากทะเลสาบสงขลาและชายฝั่งทะเลอ่าวไทยที่มีความเค็มสูง อย่างไรก็ตามจำนวนของตัวอ่อนและตัวเต็มวัยของ *P. viridis* ในทะเลสาบมีน้อยมาก การสำรวจการกระจายรอบทะเลสาบยังแสดงให้เห็นว่าการเปลี่ยนแปลงตามฤดูกาลของความเค็มในทะเลสาบทำให้การกระจายตัวของหอยสองฝาที่โตเต็มวัยเปลี่ยนไป *M. strigata* และ *P. viridis* ดูเหมือนจะอพยพไปยังส่วนในของทะเลสาบเมื่อความเค็มสูงขึ้น นอกจากนี้ การทดลองเลี้ยงลูกหอยวัยรุ่นในสภาวะความเค็มต่างๆ พบว่า *M. sallei* รอดชีวิตในทุกความเค็ม และมีดัชนีความสมบูรณ์สูงสุดที่ความเค็ม 35 ส่วน *M. strigata* และ *P. viridis* รอดชีวิตที่ความเค็ม 20 และ 35 เท่านั้น การศึกษานี้แสดงให้เห็นว่าความเค็มเป็นปัจจัยสำคัญที่มีอิทธิพลต่อการเข้าสู่พื้นที่ ซึ่งควบคุมการกระจายของหอยสองฝาในทะเลสาบสงขลา

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ABSTRACT

Mytilopsis sallei (Récluz, 1849) and *Mytella strigata* (Hanley, 1843) are alien invasive byssally-attached bivalves that invaded Songkhla Lake which is a part of the largest lagoon system in Thailand. Another bivalve, a native *Perna viridis* (Linnaeus, 1758) was commonly found in the lake before, but it has been very rare in recent years, instead it occurs seasonally on the coast near the mouth of the lake. This work investigated distribution pattern and recruitment variability of these dominant bivalves in the Songkhla Lake with a focus on the effects of salinity which fluctuates greatly in this estuarine system. Growth rate, condition index and survival of juvenile bivalves rearing at different salinities (0, 20, 35) were also monitored to examine mechanisms influencing distribution of these bivalves. Spatial pattern of bivalve recruitment and adult distributions were congruent. Recruits of *M. sallei* and adults were found at salinities ranging from freshwater to mid-range salinity. *M. strigata* recruited at salinities from brackish to saline condition, where the adults were present. Recruits and adults of *P. viridis* were found at the mouth of the lake and on the shore of the Gulf of Thailand where salinity was high. Observations around the lake also showed that the distribution of the adult bivalves changed due to the seasonal variations of salinity in the lake. *M. strigata* and *P. viridis* seemed to migrate to the inner part of the lake when salinity became higher. From the experiment, *M. sallei* survived at all salinities and having the highest condition index at salinity 35. *M. strigata* and *P. viridis* survived only at salinity 20 and 35. The study suggests that salinity is the key factor influencing recruitment process that regulates distribution of the dominant byssally-attached bivalves in this estuarine system.

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CHAPTER 1

General introduction

Background

Invasion by alien invasive species is one of the main threats to natural ecosystems because it can cause biodiversity loss and alteration of ecosystem functioning (Branch & Steffani, 2004; Morales et al., 2007; Miehls et al., 2009). Alien invasive species may be accidentally, or intentionally introduced to the marine and brackish water environments through human activities (Cohen & Carlton, 1998; Wonham & Carlton, 2005). The four main causes of anthropogenic introduction are via aquaculture, transportation in ballast water, attachment to ships, and dispersal through new connections between canals and oceans (Branch & Steffani, 2004). These species can adapt and establish their populations to the new environments (Caro et al., 2011). They have significant effects on ecosystems by altering habitats, nutrient cycles and energy budgets. Moreover, they can compete with or predate on indigenous species (Mack et al., 2000; Baxter et al., 2004; Miehls et al., 2009).

Both dreissenids and mytilids bivalves are frequently reported as alien invasive species. For example, zebra mussel *Dreissena polymorpha* (Pallas, 1771), is native to Ponto-Caspian region (Enders et al., 2019). It had been introduced and become a well-known invasive species in many countries worldwide (Aldridge et al., 2004; Lori & Cianfanelli, 2006; Carlton, 2008). The Caribbean false mussel *Mytilopsis sallei* (Recluz, 1849) is a brackish water dreissenid, native to the Caribbean islands and the Bay of Mexico (Marelli & Gray, 1983). It has invaded estuaries, lagoons and marinas of many countries in the Indo-Pacific region since the nineteenth century (Tan & Tay, 2018; Sa-nguansil & Wangkulangkul, 2020). A mytilid, *Perna viridis* (Linnaeus, 1758) is native to the Indo-Pacific region (Rajagopal et al., 2006). It has been introduced to the Caribbean Sea, Atlantic Ocean and the Gulf Coast of Florida, North America (Benson et al., 2001; Buddo et al., 2003; de Messano et al., 2019). American brackish water mussel *Mytella strigata* (Hanley, 1843), is native to Central and South America (Lim et al., 2018). It had been invaded Philippines, Singapore, Thailand and India (Lim et al., 2018; Biju Kumar et al., 2019; Sanpanich & Wells, 2019; Fuertes et al., 2021).

Songkhla Lagoon System is a shallow coastal lagoon, located in southern region of Thailand with a total area of 1,042 km^2. The lagoon can be divided into four parts from north to south; Thale Noi, Inner Lake, Middle Lake and Outer Lake or Songkhla Lake (Kumblad et al., 2001; Pradit et al., 2013). There is a salinity gradient, fresh to saline from the northern to the southern part of the lagoon system as the Songkhla Lake is connected to the Gulf of Thailand by a narrow channel (Wangkulangkul & Lheknim, 2008). Generally, coastal lagoon ecosystem has high biodiversity because it can provide habitat that serve as nursery, feeding area and also shelter for many species (Kennish & Paerl, 2010). Songkhla Lake is known as one of a productive estuarine ecosystems in Thailand. It has high productivity of economically important aquatic fauna such as fish, and shrimps. Local people have intensively utilized this ecosystem for fisheries and aquaculture (Lateh et al., 2006; Chesoh & Lim, 2008; Pornpinatepong et al., 2016; Hue et al., 2018; Yolanda & Lheknim, 2020). In addition, there is a deep seaport in adjacent to the Songkhla Lake, which makes the lake susceptible to invasion by marine or brackish water alien invasive species.

Nowadays, *Mytilopsis sallei* and *Mytella strigata* had been reported in many countries in Asia including in Songkhla Lake in southern Thailand. Lutaenko et al. (2019) recorded the occurrence of *M. sallei* in brackish water of Ba Tai Lake, Vietnam. They were attached to stones on the muddy bottom at salinities ranging from 15-20. Tan & Morton (2006) reported the establishment of *M. sallei* in Singapore and Malaysia. Colonies of this species were found on vertical and sloping walls as well as on the floor of monsoon drains in a range of salinities from 2 to 22. In Thailand, Wangkulangkul & Lheknim (2008) reported the establishment of *M. sallei* population (reported as *Mytilopsis adamsi* Morrison, 1946) in Haad-kaew Lagoon, Songkhla Lagoon System, and the Pak Phanang Estuary. This work showed that *M. sallei* can survive in brackish water at salinity of 6 to 31. In Haad-kaew Lagoon, this species was found on the substratum, on boat piers, and other hard submerged substrate. In Pak Phanang Estuary, they were observed on the walls of the water gate. Recently, Wangkulangkul (2018) showed that *M. sallei* were found in the rivers or canals that running into the lower part of Songkhla Lake (Fig. 1) in a wide range of salinities from 0 to 35. However, they were not present in the lake. Impacts of *M. sallei* invasion have been reported. Cai et al. (2014) reported that *M. sallei* has changed the species composition and reduced the species diversity index of macrofauna on fouling panels in Xiamen, China. Occurrence of *M. strigata* was reported in Taiwan by Huang et al. (2021). They were attached to the concrete walls and drainage system of the clam ponds as well as on the hulls of boats, bottom sediment, and riverbanks of the estuary at salinities ranging from 15-42. In the Philippines, *M. strigata* were observed in downstream part of rivers and estuaries and in semi-enclose marine waters at salinity of 2-35 (Fuertes et al., 2021). In Thailand, Sanpanich & Wells (2019) reported that *M. strigata* has invaded the inner Gulf of Thailand in 2017. They were found on the bamboo poles at aquaculture sites in a range of salinities from 7 to 35. From preliminary observation in 2022, *M. strigata* has been observed in the southernmost part of Songkhla Lake (Fig. 1). It was found on rocks, submerged wood, and soft bottom at salinity of 6-26 (Personal observation). Impacts of *M. strigata* invasion have been reported. Sanpanich & Wells (2019) reported that *M. strigata* has replaced the Asian green mussels *Perna viridis*, that economically important mussel species in the Gulf of Thailand and Singapore. In addition, Wangkulangkul et al. (2022) who compared the data of benthic macro-invertebrate species between before and after the invasion of *M. sallei* and *M. strigata* in Songkhla Lake. The results indicated that benthic macro-invertebrate assemblages have changed after the invasion of both invasive species.

The green mussel *Perna viridis* is a native mytilid bivalve in the Indo-Pacific region (Rajagopal et al., 2006). This species is economically important to local fisheries and widely used for food resources in Southeast Asia (Wells, 2017). Generally, *P. viridis* inhabits intertidal and subtidal zone of marine environments and estuary in areas with relative high salinity (Rajagopal et al., 2006). Vakily (1989) reported that *P. viridis* has been documented in a range of salinities between 10 and 44. It has been also found in the intertidal rocky shores on the coast of the Gulf of Thailand near the mouth of Songkhla Lake (Personal observation) (Fig. 1). *P. viridis* were found and utilized in fishery and aquaculture in Songkhla Lake (Mardnui & Plathong, 2009). However, it has rarely been observed in the lake in the recent years (Personal observation).

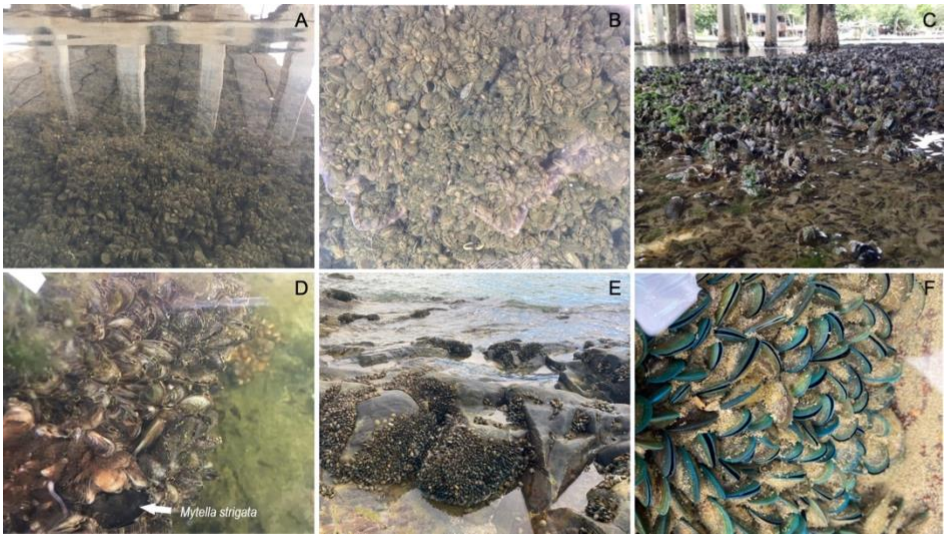

Fig. 1. (A-B) *Mytilopsis sallei* patches cover the soft bottom of Pawong canal; (C) *Mytella strigata* in Pawong canal; (D) *M. strigata* were found among patches of *M. sallei*. (E-F) *Perna viridis* patches attaching on rocky substrate in the intertidal zone of Samila beach, Songkhla.

The relative importance of recruitment variability and post-recruitment processes in regulating the structure and dynamics of adult population have been extensively discussed. Previous works suggested that larval recruitment can have significant effects on adult distribution and abundance (Gaines & Roughgarden, 1985; Minchinton & Scheibling, 1991; Arribas et al., 2015). In addition, variation in recruitment can also influence post-recruitment processes (competition and predation), which may have an impact on adult population size, species distribution, and community structure (Menge, 2000).

Salinity is considered the main environmental factor determining the distribution of aquatic organisms due to the potential to have an impact on organisms to carry out biological activities, and their ability to grow and survive (Smyth & Elliott, 2016; Perez-Miguel et al., 2019). Previous works proposed that variation in salinity plays an important role in marine species via reproduction, larval dispersal and recruitment, distribution, and behavior (van der Gaag et al., 2016; Galimany et al., 2018; Pourmozaffar et al., 2020). Consequently, changes in salinity will influence community structure and species distribution (Smyth & Elliott, 2016).

M. sallei, *M. strigata* and *P. viridis* are dominant bivalve species in Songkhla Lake and the surrounding area. All three species are filter feeding, byssally attached bivalves that are often recognized as ecosystem engineers, of which their presence can determine the composition and structure of both pelagic and benthic communities (e.g., phytoplankton, polychaetes, crustaceans and molluscs) (Qing-liang, 2006; Cai et al., 2014; Wangkulangkul et al., 2022). *M. sallei* has been established in the system for more than two decades while *M. strigata* was recently observed. Interactions between the two alien species as well as the indigenous *P. viridis* in the Songkhla Lagoon System is largely unknown. Recent observations suggested that the three bivalves seem to occupy areas with different salinity ranges. *M. sallei* were observed in the rivers or canals that running into the lower part of the lake where salinity is relatively low. *M. strigata* were found in the southernmost part of the lake where it is open to the sea at mid to high salinity. There were some areas in the lake that *M. sallei* and *M. strigata* co-occur. *P. viridis* were reported from the lake before but they have been found only on the coast around mouth of the lake at fully marine condition in the recent years. This thesis research aims to investigate the mechanisms that shape the pattern of distribution of these bivalves by focusing on role of salinity as a main driver that regulate the distribution. Variation in recruitment of the bivalves was quantified monthly throughout a year at different sampling locations along a salinity gradient, from the mouth of Songkhla Lake to the inner part of a canal that runs into the lake. Environmental parameters, such as dissolved oxygen, pH, salinity, water temperature and chlorophyll-*a* concentration were also monitored to evaluate their relationships with bivalve recruitment. Moreover, an experiment on effects of salinity on survival, growth, and condition of juvenile bivalves was performed for *M. sallei*, *M. strigata* and *P. viridis* to examine role of the bivalves' salinity tolerance as part of post-recruitment process that might determine their distribution. Information obtained will help shed some light on the life history traits of these bivalves which is important for management of alien invasive species and conservation of this ecosystem.

Literature review

1. *Mytilopsis sallei* (Récluz, 1849)

Classification

Phylum: Mollusca
  Class: Bivalvia
    Subclass: Autobranchia
      Order: Myida
        Superfamily: Dreissenoidea
          Family: Dreissenidae
            Genus: *Mytilopsis*
              Species: *Mytilopsis sallei* (Récluz, 1849)

Morphology

Mytilopsis sallei is a small, fingernail sized mussel. The shell coloration is varying from black through to a light colour. A light and dark zig-zag pattern is present in some small individuals (NIMPIS, 2021).

The shell and anatomy of adult *M. sallei* have been described in Morton (1981). The shell is widest at the posterior and narrowest at the anterior, which is called heteromyarian or mytiliform valves. The left valve is slightly smaller than the right valve. The ambones usually located in ventral terminally. A byssal notch is located on the anterior ventral margin of the valves. The external shell surface is usually covered with a thin periostracum that has a pale brown color. Under the periostracum, the shell of this species is mostly dirty white, but in some individuals may have light to dark grey concentric markings. The internal shell surface is white or bluish white. The ligament, septum (hinge plate), and apophysis (hinge lobe) are located on the anterior end of each valve, in which the septum receives the anterior adductor muscles, while the apophysis anchors the anterior byssal retractor muscles.

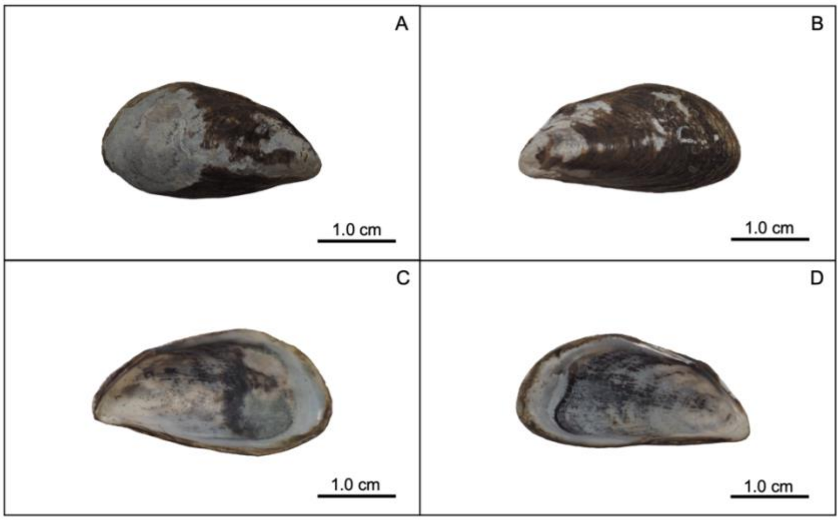

Fig. 2. *Mytilopsis sallei* (Récluz, 1849) (A-B) Right and left valves of *M. sallei* (C-D) Interior view of right and left valves of *M. sallei*.

Growth and reproduction

Mytilopsis sallei has high fecundity, rapid growth and a fast maturity rate (Morton, 1981). Karande & Menon (1975) reported that *M. sallei* can be functioning as a male, female or hermaphrodite. During their lifespan, individuals can reverse sex from male to female that usually occurred after the resting phase (Nagabhushanam & Sarojini, 1997). It is reproductively mature by about 8 mm shell length and reaches sexual maturity within one year of settlement (Morton, 1989). Eggs and sperm can be released into the water column where external fertilization takes place (NIMPIS, 2021). Kalynasundaram (1975) reported that the larvae settlement occurs within four days after fertilization. Morton (1981) suggested that in one month of settlement, individuals can attain shell lengths between 6 and 10 mm, and required another three months to reach 20 mm. A maximum life cycle is about 22 months, with an average of fewer than 18 months was observed in Hong Kong (Morton, 1989).

Feeding

Mytilopsis sallei is a suspension feeder. Water flow through its gills, then zooplankton, phytoplankton and other organic matter particle that suspended from the water column are filtered and send to its mouth (NIMPIS, 2021).

Habitat

Mytilopsis sallei can survive in wide range of temperature (10-35°C), salinity (0-27 ppt) and low oxygen concentration (Morton, 1981). In invaded areas, *M. sallei* is usually forming colonies on submerged substrates in shallow coastal areas (Morton, 1981), and the species likely prefer man-made structures in confined water of harbours (Udhayakumar & Karande, 1989; Bax et al., 2002).

Distribution

Mytilopsis sallei is a native species in centers of the Caribbean islands and the Bay of Mexico (Marelli & Gray, 1983). Morton (1980) and Nuttall (1990a, b) reported that it is generally believed that the opening of Panama Canal in 1915 leads to the introduction of the species to the Eastern Pacific, consist of Fiji report as *M. allyneana* in Hertlein & Hanna (1949), India (Karande & Menon, 1975), Japan (Ishibashi & Kasaka, 1980), Hong Kong (Morton, 1980), Taiwan (Chang, 1985), China (Wang et al., 1999), Singapore, Malaysia (Tan & Morton, 2006), Thailand report as *M. adamsi* in Wangkulangkul & Lheknim (2008), Philippines (Vallejo, 2010).

Impacts

Impacts of *M. sallei* invasion on biodiversity, ecosystem functioning, and economy have been reported. It grows, reproduces rapidly and spreads aggressively. It forms dense colonies, mostly without other species that cause biodiversity reduction in invaded areas (NIMPIS, 2021). Cai et al. (2014) reported that *M. sallei* changed the species composition and reduced the species diversity index of macrofauna on fouling panels in Xiamen, China. Moreover, it threatens marine industries by fouling on vessels, ports, seawater systems (e.g., pumping stations, vessel ballast and cooling systems) and marine farms (NIMPIS, 2021).

2. *Mytella strigata* (Hanley, 1843)

Classification

Phylum: Mollusca
Class: Bivalvia
Subclass: Autobranchia
Order: Mytilida
Superfamily: Mytiloidea
Family: Mytilidae
Genus: *Mytella*
Species: *Mytella strigata* (Hanley, 1843)

Morphology

Mytella strigata is a moderately large and symmetrical shelled bivalve. Introíni et al. (2010) and Lim et al. (2018) described that the shell of this species is elongate, mytiliform to modioliform and have a subterminal umbo. The external shell is smooth and shiny that has a variety of colors from black, dark bluish, brown, grey, orange, and (rarely) green. The external shell patterns have a pattern range from zig-zags, spots, or concentric bands. The internal shell has a hinge, which a pitted ridge along the base of the ligament on each valve. While generally 3–4 teeth located in the anterior ventral regions of valves. The beak is generally short and rounded with a prominent dorsal. The anterior adductor muscle scar is an oval shape, which depression ventrally against the umbonal region. Although, the anterior retractor muscle scar is smaller, circular in shape, and slightly in the back of the anterior adductor muscle. Another scar is present between the two scars, which are small, circular and connect to the anterior ends of the labial palps. The posterior adductor muscle scar is confluent with the posterior retractor muscle scars.

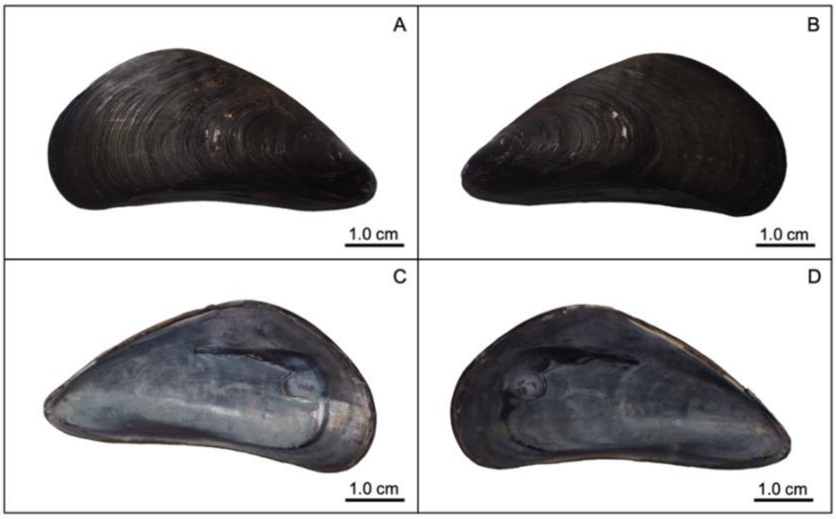

Fig. 3. *Mytella strigata* (Hanley, 1843) (A-B) Right and left valves of *M. strigata* (C-D) Interior view of right and left valves of *M. strigata*.

Growth and reproduction

Mytella strigata is highly fecund, grows rapidly, and reaches sexual maturity at a minimum size of 12.5 mm (Stenyakina et al., 2010). Stenyakina et al. (2010) reported that *M. strigata* is a sequential hermaphrodite because in the experiment, it is likely to maintains separate sexes when food is not limited, but under starvation conditions, many individuals change the sex from female to male. (Raúl et al., 2000) reported that eggs and sperms can be released into the water column during the warmer months, however production of eggs and sperm can take place year-round. Larvae remain in the water column for 10-15 days before producing thin byssus threads in order to attach to the substrate (Oliveira et al., 2005).

Feeding

Mytella strigata is a filter feeder. It actively pumping water through its gills in order to remove particles from the water column. It feeds on bacteria, phytoplankton and detritus (Walters et al., 2010; Galimany et al., 2017, 2018).

Habitat

Mytella strigata is generally distributed in the middle intertidal and the shallow subtidal waters of estuaries and near coastal environments (Vallejo et al., 2017). It can live in a wide variety of habitats by using byssus treads to attach on hard substrates such as rocky substrates, submerged woods, vessels, other sessile organisms, and it also lives on soft substrate such as sand and clay (Carranza et al., 2009; Vallejo et al., 2017). Yuan et al. (2016) reported that *M. strigata* can survive in a large salinity tolerance range (2-40 ppt) at temperatures around 20 °C. However, it is observed more frequently in brackish waters more than areas with higher salinities (Huang et al., 2021).

Distribution

Mytella strigata is a native species in Central and South America (Lim et al., 2018). It is present on both the Atlantic and Pacific coasts of tropical South and Central America. It had been reported in Taiwan (Huang et al., 2021), Philippines (Fabiosa et al., 2021), Singapore (Lim et al., 2018), the Gulf of Thailand (Sanpanich & Wells, 2019), the west coast of India (Jayachandran et al., 2019), and the south-eastern United States (Boudreaux & Walters, 2006).

Impacts

Mytella strigata had recently been reported as an invasive species in many countries of Southeast Asia. Some publication reported it can compete with native sessile invertebrates in all countries that it has invaded (Galimany et al., 2017; Mediodia et al., 2017; Lim et al., 2018; Sanpanich & Wells, 2019; Huang et al., 2021). It has replaced the Asian green mussels *Perna viridis*, that economically important mussel species in the Gulf of Thailand and Singapore (Sanpanich & Wells, 2019). In India, Jayachandran et al. (2019) reported the concern that this species will affect the Asian green mussel industry in Kerala. In Taiwan, *M. strigata* cause fouling problem on aquaculture equipment that impacted clam aquaculture. Moreover, it impedes the growth and survival of clam through competition or translocation of pathogens (Huang et al., 2021).

3. *Perna viridis* (Linnaeus, 1758)

Classification

Phylum: Mollusca
Class: Bivalvia
Subclass: Autobranchia
Order: Mytilida
Superfamily: Mytiloidea
Family: Mytilidae
Genus: *Perna*
Species: *Perna viridis* (Linnaeus, 1758)

Morphology

Perna viridis is a large-sized bivalve, with 80-100 mm in shell length. The external shell surface is smooth, characterized by concentric growth lines and slightly concave ventral margin. The shell is covered with periostracum that has bright green color in juveniles and fading to brown with green edges in older individuals. The internal shell surface is smooth, which is bluish-green hue. The ridge is finely pitted that helps the ligament connecting the two shell valves. The beak has three interlocking teeth that located one in the right valve and two in the left valve. Characteristic features of this species are wavy posterior end of the pallial line, the large kidney-shaped retractor muscle scar, and absent of anterior adductor muscle. The foot is small, tongue-like in shape, and the ventral surface has groove that connecting to byssal pit (Rajagopal et al., 2006; Soon & Ransangan, 2014).

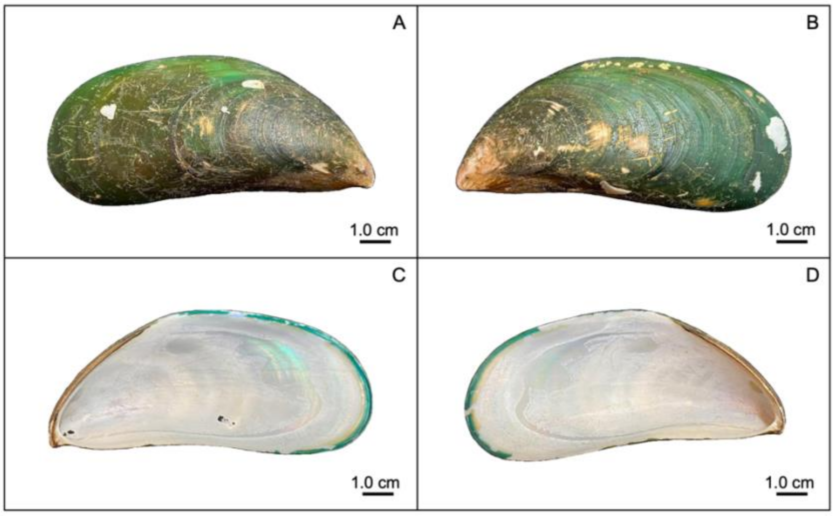

Fig. 4. *Perna viridis* (Linnaeus, 1758) (A-B) Right and left valves of *P. viridis* (C-D) Interior view of right and left valves of *P. viridis*.

Growth and reproduction

Perna viridis has a high growth rate and reaches sexual maturity at 15-30 mm shell length (about 2-3 months) (Siddall, 1980). Sex is separated (gonochoristic) and the difference between males and females by using external morphology is not distinguishable (Soon & Ransangan, 2014). Rajagopal (1991) reported that males show milky white gonads, while females show bright orange or brick red colored gonads. Adults sexually reproduce by releasing gametes into the water column where external fertilization takes place (Soon & Ransangan, 2014). Spawning generally occurs twice a year between early spring and late autumn, however in the tropical countries spawning occurs year-round (Sivalingam, 1977). Hayes et al. (2005) reported that free swimming larvae (trochophore stage) develop after 7 or 8 hours of fertilization. Larvae remain in the water column for 2-3 weeks to producing byssus threads before they settle and attach on substrate (Yap et al., 2002). Rajagopal et al. (1998) reported that the maximum life cycle of *P. viridis* is typically 2-3 years, and environmental factors (temperature, food availability and water movement) can affect growth rate.

Feeding

Perna viridis is a suspension feeder. It feeds on zooplankton, phytoplankton, detritus and other suspended organic particle from the water column by pumping water through its gills (Soon & Ransangan, 2014).

Habitat

Perna viridis can be found in intertidal, subtidal and estuarine areas (Iqbal et al., 2017). It can live on a wide variety of submerged structures including wood, rope, vessels, wharves, mariculture equipment, buoys and other hard substrates by using byssus threads (Vakily, 1989). It survives in a broad range of salinity and temperature tolerance with salinities ranging from 0-80 ppt and temperatures from 7-37.5 °C (Sivalingam, 1977; Segnini De Bravo et al., 1998).

Distribution

Perna viridis is a native species found on the Indo-Pacific coasts. This species widely distributes in the Persian Gulf, India, Malaysia, New Guinea and the South Pacific Island (Sivalingam, 1977; Siddall, 1980; Vakily, 1989; Cheung, 1993; Rajagopal et al., 2006). In the mid-1990s, Agard et al. (1992) reported the first record of this species in Trinidad then it was introduced to the Gulf of Paria via prevailing currents. Afterward, some publications believed that the population from Trinidad have been dispersed into Venezuela by currents and human activities in 1993 (Agard et al., 1992; Rylander et al., 1996). Benson et al. (2001), who recently reported that the establishment of this species in Tampa Bay, the United States, where scientists believe that larvae in ballast ships are the vector of transportation into this area.

Commercial and ecological significance

Perna viridis is a global commercially mussel species, especially in Indo-Pacific region (Asaduzzaman et al., 2019). This species is the main food resource for human consumption and most suitable use in aquaculture due to its dense and fast growth (Soon & Ransangan, 2014). This species has been important not only as a commercial species but also as a bioindicator of pollution (Rajagopal et al., 2006) by using to study contamination of heavy metal in coastal waters (Phillips, 1985; Putri et al., 2012; Vasanthi et al., 2012).

Hydrology of Songkhla Lagoon System

Songkhla Lagoon system is the largest lagoon system in Thailand, located in southern region of Thailand (7°08′N–7°48′N and 100°07′E–100°35′E) (Kumblad et al., 2001; Lheknim & Yolanda, 2020). The system consists of four connected lakes: Thale Noi, Inner Lake, Middle Lake and Outer Lake or Songkhla Lake, with the mouth of Songkhla Lake is connected by a narrow channel to the Gulf of Thailand (Lheknim & Yolanda, 2020). The system is a water body with three distinct water regimes (fresh, brackish, and saline water) with salinities gradient from practically zero to 34, along the northern in Thale Noi to the southern in Songkhla Lake (Lheknim & Yolanda, 2020). Previous works in the Songkhla Lagoon system reported that rainfall, freshwater runoff and seawater surges cause seasonal fluctuation in salinity of the system (Angsupanich & Rakkheaw, 1997; Bunsom & Prathep, 2012). Evenson (1983) reported that this region has two monsoons per year: the northeast monsoon (October to January) and the southwest monsoon (May to September), and it also has an intermediate period between February and March. At this time of the year, freshwater runoff due to the heavy rainfall period is dominant (Phasook and Sojisuporn, 2005). The water current flows toward the mouth of Songkhla Lake to the Gulf of Thailand, and so salinity in the system is generally low (10-20 in Angsupanich & Kuwabara, 1995). While in the dry season, the hydrology of the system is influenced by seawater surge (Phasook and Sojisuporn, 2005). The water current from the sea flows into the Songkhla Lake; therefore, the salinity in the system is generally higher (the average salinity was 28 in Angsupanich, 1997) compared to the monsoon season.

Recruitment and post-recruitment processes

The term “recruitment processes” was defined by Keough & Downes (1982) as the processes by which a number of individuals have settled and survived for a certain length of time, including a period of post-settlement mortality. Afterward, recruits are exposed to environmental stress, physical disturbance, competition, and predation, which refer to “post-recruitment processes”. The importance of recruitment and post-recruitment processes in regulating the structure and dynamics of adult population have been extensively discussed. According to the recruitment limitation hypothesis, when the larval supply is insufficient to support the total population size. As a result, an increase in recruitment rate will directly result in an increase in the size of adult population. This means that the recruitment process that enters new individuals into the population can greatly determine the size of adult population (Jenkins et. al., 2009). However, Menge (2000) proposed “the recruit-adult hypothesis” which suggested that recruitment intensity may potentially influence to the relative importance of recruitment and post-recruitment processes. When the recruitment is insufficient, recruitment processes is a significant factor in determining abundance of adult population. While in the high recruitment period, post-recruitment processes become more important to determining adult population via density-dependent factors (Gaines & Roughgarden, 1985). Because the mortality after recruits exposed to several physical and biological stress may increase the availability of space or food supply.

Research questions

1. What are distribution patterns of *M. sallei*, *M. strigata* and *P. viridis* in Songkhla Lake and its tributaries?
2. Is there spatial and temporal variation in recruitment of *M. sallei*, *M. strigata* and *P. viridis* in Songkhla Lake; and do recruitment variability differ between species?
3. Do survival rate, growth rate, and condition indices of *M. sallei*, *M. strigata* and *P. viridis* differ under different salinities?

CHAPTER 2

Distribution patterns of *Mytilopsis sallei*, *Mytella strigata* and *Perna viridis* in Songkhla Lake and its tributaries

Introduction

This chapter aims to assess the distribution pattern and abundance of *M. sallei*, *M. strigata* and *P. viridis* which are abundant bivalves in Songkhla Lake and its tributaries, by estimating abundance of these bivalves at the bank around the lake, as well as canals and rivers running into the lake, and the coast around the mouth of the lake. In addition, the salinity was examined, as the gradient of salinity may affect the distribution of the bivalves.

Research methodology

Study site

Songkhla Lagoon System (7°08′N–7°48′N and 100°07′E–100°35′E, Fig. 5A) is a shallow coastal lagoon, located in southern region of Thailand with a total area of 1,042 km^2. The lagoon can be divided into four parts from north to south; Thale Noi, Inner Lake, Middle Lake and Outer Lake or Songkhla Lake (Kumblad et al., 2001; Pradit et al., 2013). There is a salinity gradient, fresh to saline from the northern to the southern part of the lagoon system as the Songkhla Lake is connected to the Gulf of Thailand by a narrow channel (Wangkulangkul & Lheknim, 2008). Songkhla Lake (7°10′34.40″N, 100°34′00″E, Fig. 5B) has several rivers and canals running into the lake that bring in the freshwater. The surveys were carried out at 38 stations at the bank around the Songkhla Lake, as well as canals and rivers running into the lake, and the coast around mouth of the lake where the bivalves have been found in preliminary observation and previous study in Wangkulangkul (2018) (Fig. 5, Table 1). The stations were easy to access and not placed at areas with depth of water exceeding 1 meter as most bivalves were found at shallow level.

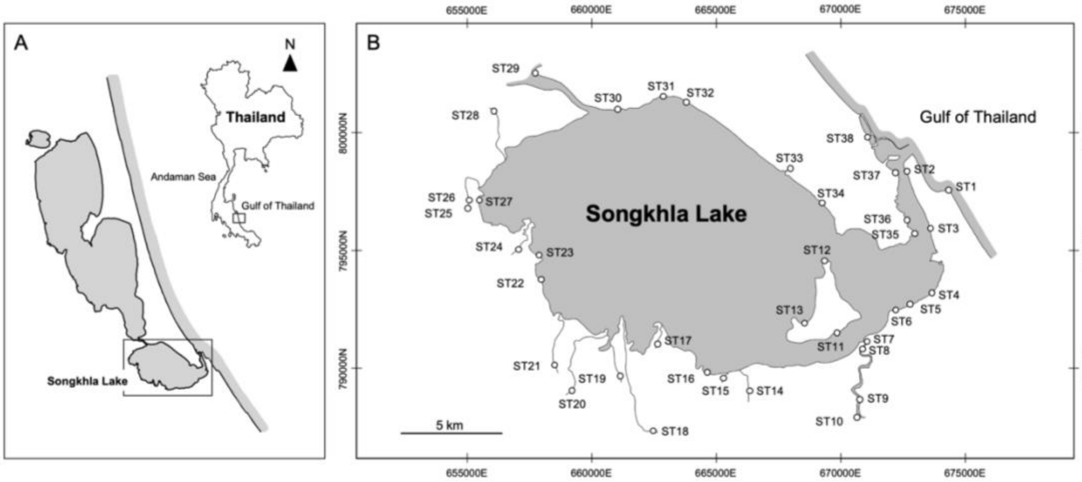

Fig. 5. (A) The Songkhla Lagoon System. (B) Location of sampling stations (ST1-ST38 = Station 1 to Station 38 labeled as open circle) around the Songkhla Lake, as well as canals and rivers running into the lake, and the coast around mouth of the lake.

Table 1. Sampling locations of *M. sallei*, *M. strigata* and *P. viridis* distribution around the Songkhla Lake, as well as canals and rivers running into the lake, and the coast around mouth of the lake.

No.	Station names	Co-ordinates
1	Samila beach	7°12'56.1"N 100°35'46.2"E
2	Song Thale Park	7°13'25.6"N 100°34'38.9"E
3	Red Rice Mill	7°11'52.9"N 100°35'17.1"E
4	Marine and Coastal Resources Research Center	7°10'10.9"N 100°35'20.3"E
5	Coastal Aquatic Animal Health Research Institute	7°09'51.5"N 100°34'43.7"E
6	Laem Kwan	7°09'42.7"N 100°34'21.3"E
7	General Prem Park 1	7°08'52.0"N 100°33'37.3"E
8	General Prem Park 2	7°08'41.0"N 100°33'31.0"E
9	Pawong canal 1	7°07'20.6"N 100°33'24.7"E
10	Pawong canal 2	7°06'52.1"N 100°33'23.7"E
11	Koh Yor (south)	7°09'07.0"N 100°32'48.4"E
12	Koh Yor (north)	7°11'00.3"N 100°32'28.4"E
13	Wat Tai Yor	7°09'21.5"N 100°31'56.7"E
14	Ror canal (water gate)	7°07'33.4"N 100°30'28.9"E
15	Wat Bang Nod	7°07'55.3"N 100°29'46.5"E
16	Bang Luek	7°08'05.1"N 100°29'21.1"E

17	Laem Po canal	7°08'47.4"N 100°28'01.4"E
18	Wat Ku Tao	7°06'30.1"N 100°27'57.0"E
19	Wat Tha Men	7°07'57.1"N 100°27'02.7"E
20	Wat Bang Yee	7°07'33.5"N 100°25'44.7"E
21	Wat Bang Riang	7°08'14.2"N 100°25'18.4"E
22	Kok Muang	7°10'14.4"N 100°25'04.4"E
23	Haad Pak Bang Pu Mee	7°11'07.6"N 100°24'54.3"E
24	Wat KhongKha Wadi	7°11'19.9"N 100°24'18.9"E
25	Tha Muang Canal	7°12'27.3"N 100°23'00.2"E
26	Wat Tha Muang	7°12'37.6"N 100°23'00.5"E
27	Ao Tueng	7°12'38.4"N 100°23'16.6"E
28	Pak Ja canal (water gate)	7°15'03.2"N 100°23'39.6"E
29	Wat Laem Jak	7°16'02.9"N 100°24'44.8"E
30	The Industrial Rehabilitation Centre Region 5	7°15'04.3"N 100°26'59.2"E
31	Wat Pa Khat Crematory	7°15'23.6"N 100°28'11.2"E
32	Wat Pa Khat	7°15'15.0"N 100°28'47.0"E
33	Wat Lokaram Reservoir	7°13'27.4"N 100°31'33.4"E
34	Sa Ting Mo	7°12'33.1"N 100°32'25.0"E
35	Laem Son	7°11'46.4"N 100°34'53.6"E
36	Mangrove Forest Conservation Club	7°12'06.8"N 100°34'40.8"E
37	Hua Khoa Dang	7°13'22.2"N 100°34'22.6"E
38	Haad Kaew Lagoon	7°14'19.8"N 100°33'38.9"E

Fig. 6. Potential substrates for bivalve attachment (A) mangrove roots; (B) submerged tree trunks, (C) fish nets, and (D) ropes; (E-F) wooden poles; (G) concrete walls (H) water gate.

Data collection

As distribution of the bivalves may vary due to the salinity gradient, survey of *M. sallei*, *M. strigata*, and *P. viridis* distributions were conducted twice to quantify the pattern of distribution of these three bivalves in Songkhla Lake and its tributaries. The first survey was conducted in June 2022, when salinity in the lake was relatively low due to rainfall during the southwest monsoon. The second survey was conducted in October 2022, which is a transition period between the southwest monsoon and the northeast monsoon season, salinity in the lake was relatively higher than in June. Bivalves were observed on the bottom of the water bodies and hard submerged substrates, such as mangrove roots, the walls of the water gate, concrete, or wooden poles (Fig. 6). Therefore, bivalve abundance was determined with a visual assessment due to the heterogeneity of habitat. At each station, the percentage cover of the bivalves was visually estimated within an area of 2 × 2 m and categorized into: absent (0%), rare (<10%), common (10–80%), and abundant (>80–100%) (Sa-nguansil & Wangkulangkul, 2020). In addition, the salinity was measured using a hand-held refractometer.

Results

The first survey of bivalve distribution in June 2022 (Fig. 7) when salinity in the Songkhla Lake was relatively low, showed that *M. sallei* were observed in the rivers and canals that running into the lake and some were observed on the shallow areas of the lake in a range of salinities from 0 to 7. *M. strigata* were observed from the southernmost part to the mouth of the lake at salinity of 6 to 27. While the native bivalves *P. viridis* were found on the coast around the mouth of the lake in marine condition at salinity 32. There was only one location where *M. sallei* and *M. strigata* co-occurred (ST6).

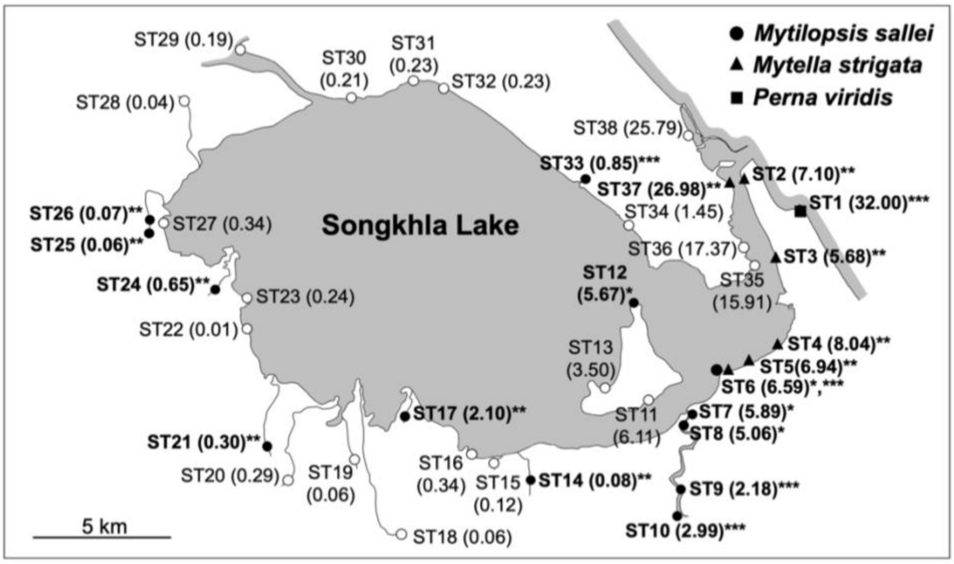

Fig. 7. Distribution pattern of *Mytilopsis sallei*, *Mytella strigata* and *Perna viridis* in Songkhla Lake and its tributaries in June 2022. ST1-ST38 = sampling stations 1-38. Stations are labeled as open circles except stations where bivalves were observed are marked by icons indicating each species (See the figure legend); Numbers in brackets = salinity; Asterisks = relative abundance of *Mytilopsis sallei*, *Mytella strigata* and *Perna viridis* (single asterisk = rare, double asterisks = common, triple asterisks = abundant).

Table 2. Relative abundance of *M. sallei*, *M. strigata* and *P. viridis* at 38 sampling locations in Songkhla Lake and its tributaries in June 2022. Number (0) and asterisks indicated relative abundance of the bivalves (0 = absent, * = rare, ** = common, *** = abundant).

No.	Station names	Salinity	Relative abundance		
			M. sallei	*M. strigata*	*P. viridis*
1	Samila beach	32.00	0	0	***
2	Song Thale Park	7.10	0	**	0
3	Red Rice Mill	5.68	0	**	0
4	Marine and Coastal Resources Research Center	8.04	0	**	0
5	Coastal Aquatic Animal Health Research Institute	6.94	0	**	0
6	Laem Kwan	6.59	*	***	0
7	General Prem Park 1	5.89	*	0	0
8	General Prem Park 2	5.06	*	0	0
9	Pawong canal 1	2.18	***	0	0
10	Pawong canal 2	2.99	***	0	0

11	Koh Yor (south)	6.11	0	0	0
12	Koh Yor (north)	5.67	*	0	0
13	Wat Tai Yor	3.50	0	0	0
14	Ror canal (water gate)	0.08	**	0	0
15	Wat Bang Nod	0.12	0	0	0
16	Bang Luek	0.34	0	0	0
17	Laem Po canal	2.10	**	0	0
18	Wat Ku Tao	0.06	0	0	0
19	Wat Tha Men	0.06	0	0	0
20	Wat Bang Yee	0.29	0	0	0
21	Wat Bang Riang	0.30	**	0	0
22	Kok Muang	0.01	0	0	0
23	Haad Pak Bang Pu Mee	0.24	0	0	0
24	Wat KhongKha Wadi	0.65	**	0	0
25	Tha Muang Canal	0.06	**	0	0
26	Wat Tha Muang	0.07	**	0	0
27	Ao Tueng	0.34	0	0	0
28	Pak Ja canal (water gate)	0.04	0	0	0
29	Wat Laem Jak	0.19	0	0	0
30	The Industrial Rehabilitation Centre Region 5	0.21	0	0	0
31	Wat Pa Khat Crematory	0.23	0	0	0
32	Wat Pa Khat	0.23	0	0	0
33	Wat Lokaram Reservoir	0.85	***	0	0
34	Sa Ting Mo	1.45	0	0	0
35	Laem Son	15.91	0	0	0
36	Mangrove Forest Conservation Club	17.37	0	0	0
37	Hua Khoa Dang	26.98	0	**	0
38	Haad Kaew Lagoon	25.79	0	0	0

The second survey of bivalve distribution in October 2022 (Fig. 8) when the salinity was relatively higher, showed that bivalves were observed in the same pattern but expanded their ranges. *M. sallei* expanded toward the mouth of the lake (ST5-4) which were observed in Songkhla Lake and some rivers or canals that running into the lake in a wide range of salinities from 0 to 32. However, high abundance of *M. sallei* was mostly observed in the rivers or canals where salinity is relatively lower, largely lower than 20. *M. strigata* were observed near the mouth of the lake and expanded its ranges into Pawong canal (ST7-9) at salinities ranging from 15 to 32. There were 7 locations where *M. sallei* and *M. strigata* co-occurred. The native bivalves

P. viridis were found at the mouth of Songkhla Lake (ST2) at salinity 32, where it was not observed before.

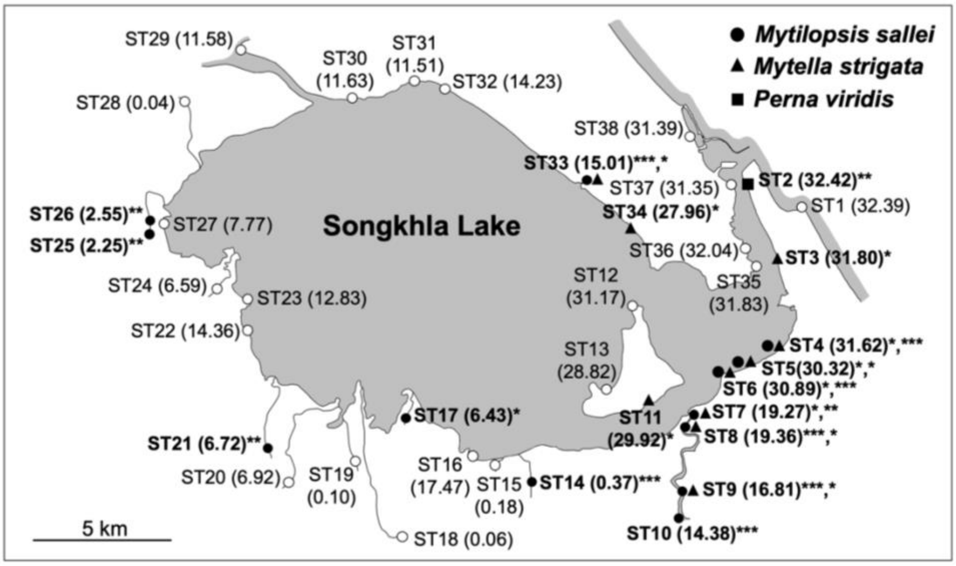

Fig. 8. Distribution pattern of *Mytilopsis sallei*, *Mytella strigata* and *Perna viridis* in Songkhla Lake and its tributaries in October 2022. ST1-ST38 = sampling stations 1-38. Stations are labeled as open circles except stations where bivalves were observed are marked by icons indicating each species (See the figure legend); Numbers in brackets = salinity; Asterisks = relative abundance of *Mytilopsis sallei*, *Mytella strigata* and *Perna viridis* (single asterisk = rare, double asterisks = common, triple asterisks = abundant).

Table 3. Relative abundance of *M. sallei*, *M. strigata* and *P. viridis* at 38 sampling locations in Songkhla Lake and its tributaries in October 2022. Number (0) and asterisks indicated relative abundance of the bivalves (0 = absent, * = rare, ** = common, *** = abundant).

No.	Station names	Salinity	Relative abundance		
			M. sallei	*M. strigata*	*P. viridis*
1	Samila beach	32.39	0	0	0
2	Song Thale Park	32.42	0	0	**
3	Red Rice Mill	31.80	0	*	0
4	Marine and Coastal Resources Research Center	31.62	*	***	0
5	Coastal Aquatic Animal Health Research Institute	30.32	*	*	0
6	Laem Kwan	30.89	*	***	0
7	General Prem Park 1	19.27	*	**	0

8	General Prem Park 2	19.36	***	*	0
9	Pawong canal 1	16.81	***	*	0
10	Pawong canal 2	14.38	***	0	0
11	Koh Yor (south)	29.92	0	*	0
12	Koh Yor (north)	31.17	0	0	0
13	Wat Tai Yor	28.82	0	0	0
14	Ror canal (water gate)	0.37	***	0	0
15	Wat Bang Nod	0.18	0	0	0
16	Bang Luek	17.47	0	0	0
17	Laem Po canal	6.43	*	0	0
18	Wat Ku Tao	0.06	0	0	0
19	Wat Tha Men	0.10	0	0	0
20	Wat Bang Yee	6.92	0	0	0
21	Wat Bang Riang	6.72	**	0	0
22	Kok Muang	14.36	0	0	0
23	Haad Pak Bang Pu Mee	12.83	0	0	0
24	Wat KhongKha Wadi	6.59	0	0	0
25	Tha Muang Canal	2.25	**	0	0
26	Wat Tha Muang	2.55	**	0	0
27	Ao Tueng	7.77	0	0	0
28	Pak Ja canal (water gate)	0.04	0	0	0
29	Wat Laem Jak	11.58	0	0	0
30	The Industrial Rehabilitation Centre Region 5	11.63	0	0	0
31	Wat Pa Khat Crematory	11.51	0	0	0
32	Wat Pa Khat	14.23	0	0	0
33	Wat Lokaram Reservoir	15.01	***	*	0
34	Sa Ting Mo	27.96	0	*	0
35	Laem Son	31.83	0	0	0
36	Mangrove Forest Conservation Club	32.04	0	0	0
37	Hua Khoa Dang	31.35	0	0	0
38	Haad Kaew Lagoon	31.39	0	0	0

Discussion

This study showed that three byssally-attached bivalve species occupied areas with different salinity ranges in the Songkhla Lake and its tributaries. Adults of *M. sallei* were found in Songkhla Lake and rivers or canals that running into the lake at salinities ranging from freshwater to high salinity, but most abundant at low salinity. *M. strigata* were found along the southernmost part to the mouth of Songkhla Lake at mid to high salinity. While adults of *P. viridis* were found at the mouth of the lake and on the coast of the Gulf of Thailand where salinity was high. Seasonal variation in adult distribution was detected. In October 2022, bivalves were observed at stations where they had not been observed before, and the salinity at the stations was relatively higher than where they were found previously. *M. sallei* were found at more stations on the bank of the lake. *M. strigata* were found in Pawong canal where they have not been observed in the first survey. *P. viridis* were found on the bank of a channel connecting Songkhla Lake with the sea, while they were present only at on the coast of the open sea previously. The occurrence of these bivalves in new areas might result from their wide salinity tolerance as they are generally considered as euryhaline species (Segnini De Bravo et al., 1998; Yuan et al., 2010; Sa-nguansil & Wangkulangkul, 2020), and bivalves can also migrate onto different substrates or to new locations many times before they permanently enter the adult population (Bownes & McQuaid, 2009). In addition, for *M. sallei*, it is possible that a massive recruitment event that occurred in August 2022 (see also Chapter 3) before the second survey in October 2022 might also have contributed to the presence of the species in new areas.

Furthermore, the salinity range where the bivalves were observed in this present study is similar to previous studies in other invaded areas in the Indo-Pacific region. Tan & Morton (2006) reported that *M. sallei* was found in the tropical monsoon drains of Singapore, and the species was also found in the estuary of Malaysia in a wide range of salinities between 2 and 22 ppt. In Vietnam, *M. sallei* was found in the shallow Bai Tai Lake, where the salinity ranged from 15 to 20 ppt (Lutaenko et al., 2019). Huang et al. (2021) reported that *M. strigata* was found abundantly on the concrete riverbanks of estuaries in Taiwan between salinities 15 and 42 ppt, and the species was reported in Manila Bay, Philippines, at salinities ranging from 21.8 to 31.4 ppt (Vallejo et al., 2017). While the native bivalve *P. viridis* generally inhabits the intertidal and subtidal zones of marine environments and estuaries in areas with relatively high salinity (Rajagopal et al., 2006).

CHAPTER 3

Spatial and temporal variation in recruitment of *Mytilopsis sallei*, *Mytella strigata* and *Perna viridis* in Songkhla Lake

Introduction

This chapter aims to investigate spatial and temporal variation in recruitment of *M. sallei*, *M. strigata* and *P. viridis* by monitoring recruitment for thirteen months at the stations that were located along a salinity gradient. Dissolved oxygen, pH, salinity, water temperature, and chlorophyll-*a* concentration were measured as environmental parameters, which may affect the variability of recruitment. Therefore, this chapter also investigated the relationship between environmental parameters and the density of bivalves recruitment. Furthermore, to correctly identify the recruits, an identification key for recruits of *M. sallei*, *M. strigata*, and *P. viridis* was constructed in this study.

Research Methodology

Study site

Sampling were carried out for thirteen months from December 2021 to December 2022 at 8 stations along a salinity gradient from high to low range salinity from the mouth of Songkhla Lake to the inner part of Pawong canal (Fig. 9). Pawong canal (7°14′N, 100°34′E, Fig. 9) is one of the canals that drain into the Songkhla Lake. In this area, the tide is classified as microtidal, and salinity generally decreases from the mouth of the lake toward the inner portion of Pawong canal.

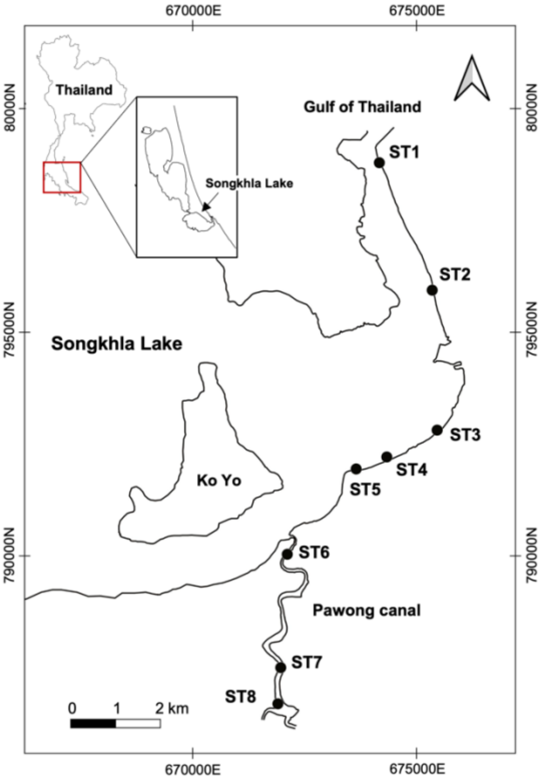

Fig. 9. Location of sampling stations (ST1-ST8 = Station 1 to Station 8) labeled as black dot from the mouth of Songkhla Lake along to Pawong canal.

Data collection

To determine spatial and temporal variations in the recruitment of *M. sallei*, *M. strigata* and *P. viridis*. In this study, scouring pads (14 × 16 × 0.5 cm) were used as artificial filamentous substrates to facilitate the settlement of the bivalve. At each station, a set of pads was installed and suspended at a depth of 0.5 m below the water surface to ensure that the pads were submerged all the time of tidal range. Each set of pads consists of 5 scouring pads that were tied randomly to the polyester rope and attached to concrete poles. Some of these poles are parts of the structure of piers, and some are parts of bridges. Scouring pads were collected and replaced on a monthly interval. After the pads were collected, they were taken to the laboratory for estimation of the density of the bivalves. During sampling, dissolved oxygen, pH, salinity, and water temperature were measured at each station using a multiparameter digital water quality meter (YSI ProQuatro). Moreover, water samples were collected for chlorophyll-*a* analysis. In the laboratory, scouring pads from each station were kept separately and placed into a glass aquarium (22 × 45 × 28 cm) filled with artificial seawater. Artificial seawater was prepared from distilled water and artificial sea salt. The salinity of the water in each container is the same as the salinity of the natural habitat where the pads are collected. The pads with recruits were kept for 14 days to allow the recruits to grow until they are identifiable. Afterwards, the total number of settled individuals on each pad was identified to the species, counted, and recorded as the density of the recruits. Bivalves were fed daily with *Nannochloropsis* sp. This microalgae was chosen because it was readily available from the Coastal Aquaculture Research and Development, which is one of the diets generally utilized in bivalves culture (Okumuş et al., 2002). Solution containing the algae was poured into the containers with bivalves to meet the concentration of 4×10^4 cells ml^{-1} (Wang et al., 2011) and the artificial seawater was changed every 2 days for all containers.

For chlorophyll-*a* analysis, the water sample was filtered through a GF/C membrane filter (pore size = 0.7 µm) with a vacuum pump. The filter membranes were subjected to chlorophyll-*a* analysis following the method of Jeffrey & Humphrey (1975). Chlorophyll-a was extracted with 90% acetone for 24 h at 4 °C in the dark, and the absorbance of the extracts was measured by using a UV-vis spectrophotometer.

Recruitment identification

In Songkhla Lake, there are many species of bivalve that can attach to the collecting panel (scouring pads). There are four species of bivalves with mytiliform shells present at Songkhla Lake: *Mytilopsis sallei*, *Mytella strigata*, *Perna viridis*, and *Parabrachidontes cochinensis.*

Recruits of these bivalves with mytiliform shells are morphologically similar (e.g., shells shape, colors). Therefore, a key to the species of recruits of these bivalves was constructed. Recruits were identified by following this key, and only recruits of *M. sallei*, *M. strigata* and *P. viridis* were counted.

Key to species

1a. The external surface of shell is rough. The shell is thin, and easy to crush. The left valve is slightly smaller than the right valve. Septum and an apophysis are located on the anterior end of each valve…...……………………………………..***Mytilopsis sallei***
1b. Shells not as above………………………………………………………………(2)

2a. The anterior margin of valves clearly extends forward beyond the umbones. The ventral margin is concave. No resilial pits on the hinge plate…………………………..
……………………………………………………….....***Parabrachidontes cochinensis***
2b. Shells not as above. Resilial pits are presence on the hinge plate…………………(3)

3a. The external shells have light brownish patterns, starting from posterior end at the shell. The color ranges from brown, dark brown and (rarely) green……***Mytella strigata***
3b. The shells are tapering to form a sharp downturned beak. The shell is bright green in juvenile…………………………………………………………... ***Perna viridis***

Description of the three bivalves

***Mytilopsis sallei* (Recluz, 1849)**

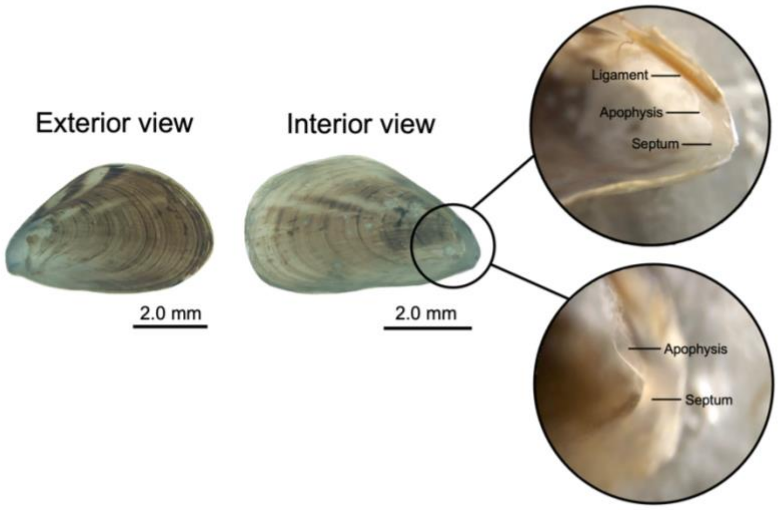

Fig. 10. Exterior and interior view of left valve of *M. sallei*.

Description: *M. sallei* is a relatively small mussel. The average ratio of length to width is 2.45 to 1 (Personal observation). The external shell is varied in color and pattern. It can be white, cream-colored or grey. Some small individuals may have concentric lines or zig-zag pattern (Marelli & Gray, 1985; MPSC, 2015). The shell is rough, thin and easy to crush. The shell valves are slightly unequal in size. The left valve is slightly smaller than the right valve (Morton, 1981). The internal shell is dull with white or bluish-white, not pearly. The septum, and apophysis are located on the anterior end of each valve (Morton, 1981). *M. sallei* can be recognized by the presence of an apophysis, a hook shaped-like structure that located lateral to the septum near the dorsal margin of the shell (MPSC, 2015).

Mytella strigata **(Hanley, 1843)**

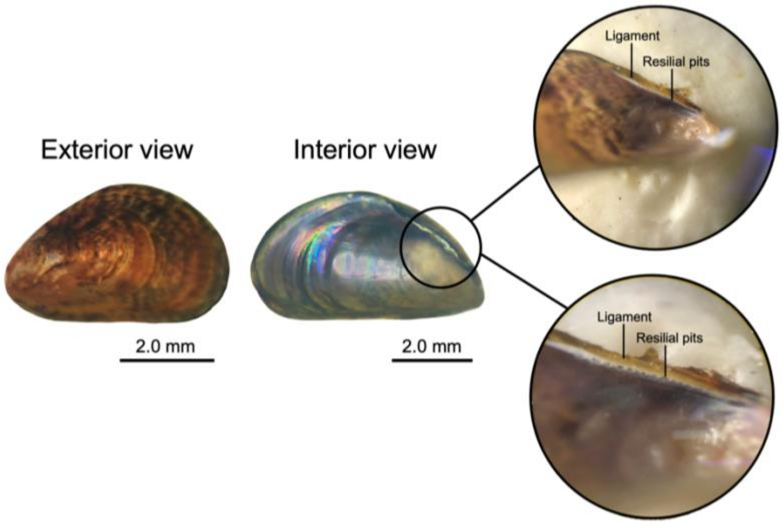

Fig. 11. Exterior and interior view of left valve of *M. strigata*.

Description: *M. strigata* is a moderately large-sized mussel. The shell is elongated and symmetrical between the left and right valve (Mediodia et al., 2017). The average ratio of length to width is 3.20 to 1 (Personal observation). The external shell is smooth and shiny that has varied in color and pattern. The color ranges from brown, dark brown and (rarely) green. The pattern ranges from zig-zags, spots, or concentric lines (Lim et al., 2018). In juveniles, light brownish patterns gradually appear, starting from the posterior end of the shell (Tay et al., 2018). The internal shell is pearly. The presence of resilial pits which tiny pores or pits on the hinge plate (Lim et al., 2018). It allows us to separate *M. strigata* from *A.* cf. *cochiensis*, as the shell shape and external morphology look-alike in the juvenile stage.

***Perna viridis* (Linnaeus, 1758)**

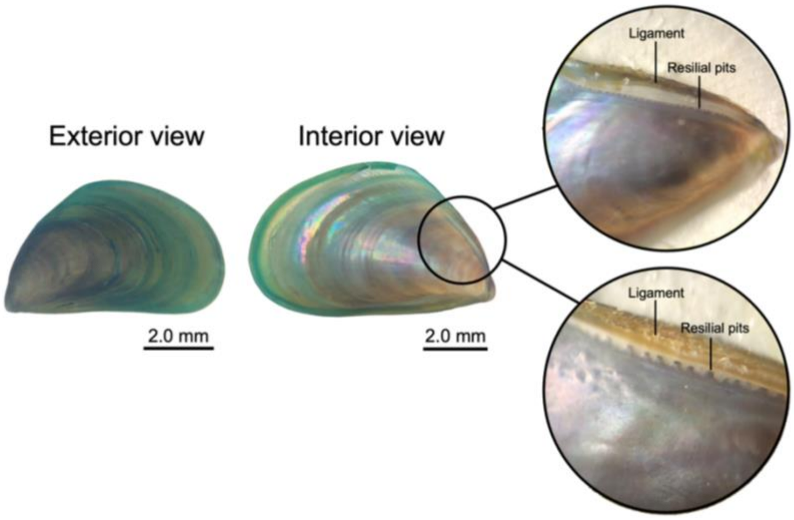

Fig. 12. Exterior and interior view of left valve of *P. viridis*.

Description: *P. viridis* is a large-sized mussel. The shell is elongated, roughly ovate, and tapering to form a sharp downturned beak (Carpenter & Niem, 1998). The ventral margin of the shell is straight or slightly concave (NIMPIS, 2002). The average ratio of length to width is 2.57 to 1 (Personal observation). The external shell color ranges from bright green to dark green or brownish. The shell is bright green color in juveniles and fading to brown with green edges in older individuals (Rajagopal et al., 2006). The internal shell is pearly with shiny blue green. *P. viridis* also has resilial pits on the hinge plate.

Data analysis

One-way analysis of variance (ANOVA) was performed to test the differences of all environmental parameters between stations (fixed factor, 8 levels, monthly data was used as replicates) and between months (fixed factor, 13 levels, data from 8 stations was used as replicates). Multiple comparisons of levels within significant factors were made using Student Newman Keuls (SNK) tests. Cochran's C test was performed to test for the heterogeneity of variance. Transformation was carried out only where heterogeneity of variance was detected.

Two-way permutational multivariate analysis of variance (PERMANOVA) with pairwise comparison were performed to test the differences of

M. sallei, *M. strigata* and *P. viridis* recruitment density between stations (fixed factor, 8 levels) and months (fixed factor, 13 levels). Euclidean distance resemblance matrices (with a dummy variable added) were constructed from untransformed data.

Multiple regression analysis was performed to access the relationship between environmental parameters with density of *M. sallei*, *M. strigata* and *P. viridis* recruitment.

One-way PERMANOVA with pairwise comparison were performed to test the differences of *M. sallei* and *M. strigata* recruitment density between station (fixed factor, 8 levels). As *P. viridis* recruits were found only at one station in one month, these analyses were not performed for this species. Euclidean distance resemblance matrices (with a dummy variable added) were constructed. The data of recruitment density were pooled across months.

One-way ANOVAs were done using the GMAV5 program. Univariate analyses were performed in PRIMER v7 add-on package PERMANOVA+ (Anderson et al., 2008). Multiple regression was performed in R Studio.

Results

During the study period (December 2021-December 2022), The magnitude of all parameters was fluctuated except for water temperature and pH. Water temperature ranged from 27.10 to 34.63 °C. Temperature was higher than most of other months in April 2022 and temperature was lower than most of other months in December 2022 (Fig. 13. C., Table 4, pairwise comparison, $p<0.01$). In addition, temperature was higher than most of other stations at station 8 (Fig. 13. C., Table 4, pairwise comparison, $p<0.05$). pH ranged from 6.58 to 10.30. pH was highest in May 2022 and pH was lower than most of other months in February 2022 (Fig. 13. B., Table 4, pairwise comparison, $p<0.01$). Dissolved oxygen varied between 2.30 to 11.10 mg/L. Dissolved oxygen was higher than most of other months in September 2022 (Fig. 13. A., Table 4, pairwise comparison, $p<0.01$). Salinity varied from freshwater to saline water ranging from 0 to 32.71. Salinity was higher than most of other months in September 2022 (Fig. 13. D., Table 4, pairwise comparison, $p<0.01$). Chlorophyll-*a* concentration varied between 0.24 to 216.58 µg/L. Chlorophyll-*a* concentration at station 8 was higher than at station 1 and station 2 and Chlorophyll-*a* concentration at station 1 was lower than most of other stations (Fig. 13. E., Table 4, pairwise comparison, $p<0.01$). Annual rainfall accumulated of Songkhla province is 3,284.9 mm. The highest rainfall accumulated is in December 2022 (1,052.9 mm) and the lowest rainfall accumulated is in August 2022 (42.9 mm) (Fig. 13. F.) (Meteorological Department, unpublished data).

During the study period, the density of *M. sallei* recruitment ranged from 0 to 9,743.6 individuals per pad. There were 2 peaks of *M. sallei* recruitment at station 7 and station 8 which were peak in February 2022 and August 2022, respectively (Fig. 13. G., Table 5, pairwise comparison, $p<0.05$) and at station 7, recruitment remain until October 2022. In contrast, recruitments at other stations were scanty. The density of *M. strigata* recruitment ranged from 0 to 2,017 individuals per pad. Recruitment of *M. strigata* were peak at station 3 and station 4 in February 2022 (Fig. 13. H., Table 5, pairwise comparison, p<0.05) and few recruitments were present at station 2 in July 2022. Recruitment of *P. viridis* was very few which present only at station 3 in February 2022 (Fig. 13. I.).

The multiple regression analysis revealed that the density of *M. sallei* recruitment was negatively related to pH whereas it was positively related to temperature (Table 6, $R^2 = 14.1\%$, $p<0.05$). While the density of *M. strigata* and *P. viridis* recruitment was not related to any environmental parameters (Table 6).

Table 4. *p-value* of one-way ANOVA testing the effect of month and station on environmental parameters.

Source of Variation	**Month**	**Station**
Dissolved oxygen (mg/L)	**<0.01****	0.8279
pH	**<0.01****	0.9795
Temperature (°C)	**<0.01****	**<0.05***
Salinity	**<0.01****	0.1004
Chlorophyll-a (µg/L)	0.3031	**<0.01****

Table 5. Two-way PERMANOVA testing the effects of months and stations on density of recruitment.

Source of Variation	**df**	**MS**	**Pseudo-F**	***p* (perm)**
M. sallei				
Month (Mo)	12	1.13×10^7	92.534	**<0.05***
Station (St)	7	2.10×10^7	172.81	**<0.05***
Mo × St	83	5.72×10^6	47.01	**<0.05***
Residual	406	1.22×10^5		
Total	508			
M. strigata				
Month (Mo)	12	7.78×10^5	295.76	**<0.05***
Station (St)	7	2.32×10^5	88.293	**<0.05***
Mo × St	83	2.44×10^5	92.619	**<0.05***
Residual	406	2631.2		
Total	508			

Table 6. Multiple regression analysis testing the relationship between environmental parameters with recruitment density.

	M. sallei		***M. strigata***		***P. viridis***	
	Estimate	Pr(>|t|)	Estimate	Pr(>|t|)	Estimate	Pr(>|t|)
(Intercept)	-1055.298	0.669	896.158	0.089	1.058	0.214
DO	0.169	0.998	5.287	0.676	0.003	0.886
pH	-439.251	**<0.01****	-54.427	0.112	-0.060	0.274
Salinity	14.309	0.214	-2.208	0.366	-0.002	0.546
Temperature	157.188	**<0.05***	-13.594	0.343	-0.017	0.458
Chlorophyll-*a*	2.203	0.141	-0.316	0.667	-0.000	0.899
R^2	0.141		0.055		0.025	
p-value	**<0.05***		0.352		0.772	

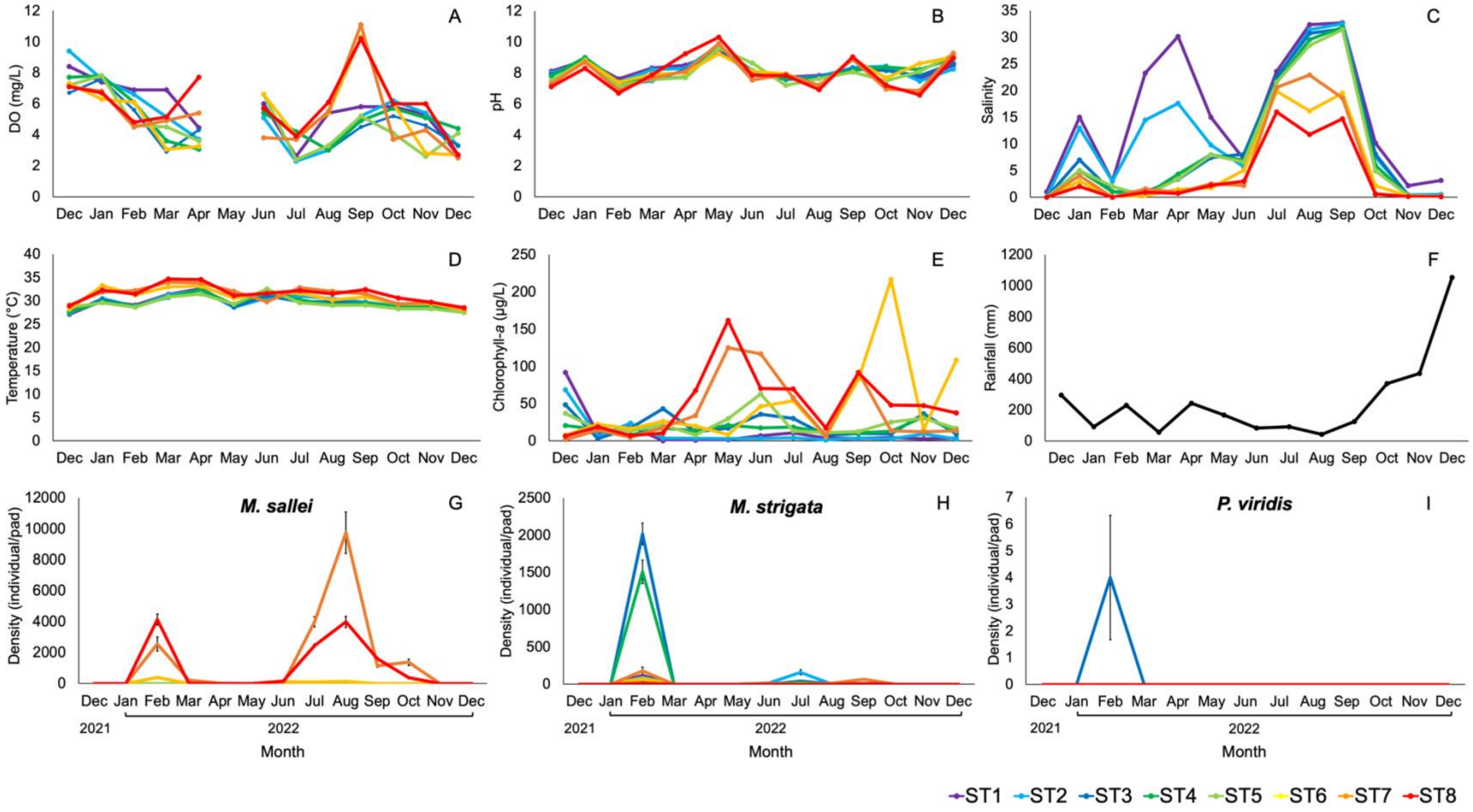

Fig. 13. Environmental parameters and density of bivalves recruitment from December 2021 to December 2022 at station 1 to station 8 (ST1-ST8). (A) Dissolved oxygen (The data in May 2022 was missing.); (B) pH; (C) Water temperature; (D) Salinity; (E) Chlorophyll-a concentration; (F) Rainfall accumulation in Songkhla province; (G) Density of *M. sallei* recruitment; (H) *M. strigata* recruitment; (I) *P. viridis* recruitment.

Spatial variation of bivalve recruitment showed that recruits of *M. sallei* were found at all stations except station 2. The highest density of *M. sallei* recruitment was 1463.77 individuals per pad which were found at station 7 (Fig. 14., Table 7, pairwise comparison, p<0.05). In addition, the density of *M. sallei* recruitment was higher than 900 individuals per pad at station 7 and station 8 and few recruitments were present at station 6 where salinity is relatively low most year-round. Recruits of *M. strigata* were found at all stations. The density of *M. strigata* recruitment was higher than 100 individuals per pad at station 3 and station 4 (Fig. 14., Table 7, pairwise comparison, p<0.05) where is mid to high-range salinity. Recruits of *P. viridis* were found very few only at station 3.

Table 7. One-way PERMANOVA for the effects of stations on recruitment density. The density of recruitment was pooled across months.

Source of Variation	**df**	**MS**	**Pseudo-F**	***p* (perm)**
M. sallei				
Station (St)	7	2.14×10^7	16.227	**<0.05***
Residual	501	1.32×10^6		
Total	508			
M. strigata				
Station (St)	7	2.34×10^5	3.8196	**<0.05***
Residual	501	61201		
Total	508			

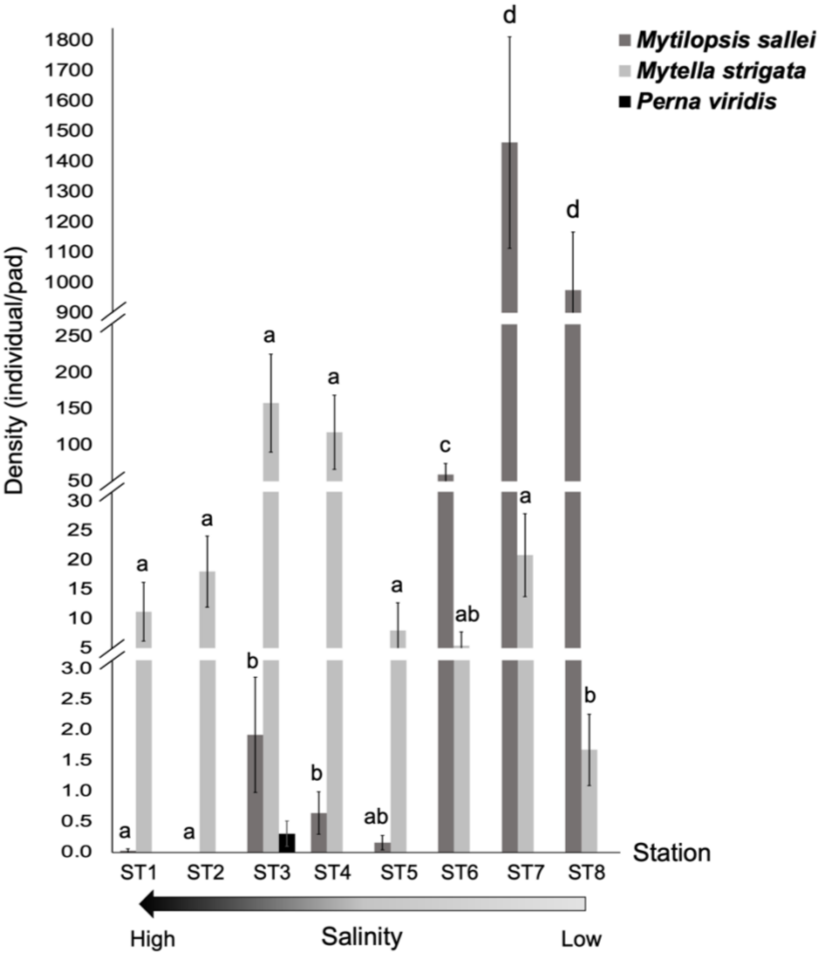

Fig. 14. Mean density of recruitment (Mean ± SE) at station 1 to station 8 (ST1-ST8) (Different letters above the bars indicate differences between stations).

Discussion

Variation in environmental parameters showed that temporal variation was found for almost all parameters except chlorophyll-*a*. Whereas spatial variation was found for water temperature and chlorophyll-*a*. Generally, the Songkhla Lake Basin has two heavy rainfall periods, including the southwest monsoon season from May to September and the northeast monsoon season from October to January (Pornpinatepong et al., 2016). At this time of the year, heavy rainfall can increase freshwater runoff and freshwater discharge that intensively flow into the system with high concentrations of nutrients (Groß et al., 2022). Nutrient enrichment in the system, especially nitrogen (N) and phosphorus (P), can consequently enhance phytoplankton growth and increase in biomass (Xu et al., 2010). Salinity decreased at all stations caused by heavy rainfall at the end of 2022. High chlorophyll-*a* concentrations at the upstream stations during September and October 2022 might result from the nutrient enrichment in the system at this time of the year.

A multiple regression analysis showed that only pH and temperature had a significant relationship with *M. sallei* recruitment. Whereas, recruitment of *M. strigata* and *P. viridis* was not related to any environmental parameters. There may be a gap between changes in environments and responses of organisms. Moreover, reproductive traits are difficult to detect because they are cumulative responses to environmental variations (Mazzuco et al., 2015).

The observation showed that recruitment of the three bivalves had a strong temporal variation throughout the year. *M. sallei* had two peaks of recruitment, a minor peak was in February 2022, and a major peak was in August 2022. They intensively recruited at stations in Pawong canal where salinity is relatively low. Recruitment of *M. strigata* peaked in February 2022 and a few recruitments were found in July and September 2022. They were found at station close to the mouth of Songkhla Lake having mid to high-range salinity compared to other stations. While a few recruits of *P. viridis* were found at high salinity near the mouth of Songkhla Lake only in February 2022. Previous studies suggested that salinity is one of the main factors affecting recruitment of marine organisms because it influences the survival of larvae and settlers (Rodríguez et. al., 1993). Morton (1989) suggested that low salinity can induce spawning in bivalves. This is supported by the study of Stephen and Shetty (1981) which reported that under laboratory conditions rapid salinity changes from 34 ppt to 24 ppt can stimulate bivalves to spawning. This might also true for *M. sallei* and *M. strigata* as the high recruitment in February 2022 coincided with a drop in salinity. Furthermore, the spawning of aquatic invertebrate has been coupled with nutrient availability and phytoplankton blooms (Starr et al. 1990; Bertness et al., 1991). Food availability conditions supported growth and gonadal development of bivalves (Galbraith & Vaughn, 2009; Arapov et al., 2013). High chlorophyll-*a* concentration which is a proxy of food for filter-feeding bivalves (Gosling, 2003) might trigger bivalves to spawning, then resulting in a high density of recruitment. For *M. sallei*, the February peak was likely to be a result of high food supply around the end of the previous year and the August peak might result from the high supply around May. For

M. strigata, high recruitment in February seemed to follow the high chlorophyll content in the lake seen in December 2021.

The present study showed that spatial variation in recruitment of *M. sallei*, *M. strigata*, and *P. viridis* was observed and recruits of different species were found in different salinity ranges. Recruitment of *M. sallei* was found in greater density in the upstream areas with a relatively low salinity. *M. strigata* recruits were highest in areas with mid-range salinity. While recruitments of *P. viridis* were found at the station with relatively high salinity. For *M. sallei*, high salinity at downstream stations might kill the larvae before recruitment. This is supported Sa-nguansil and Wangkulangkul (2020) who investigated the effect of salinity on the different life history of *M. sallei*, the study reported that high survival of *M. sallei* larvae was observed in low to mid-range salinity (5-20 ppt). As a result, recruits of *M. sallei* were found in most areas of the system at salinities ranging from freshwater to mid-range salinity. Moreover, density of *M. sallei* recruits was higher than 50 individuals per pad only at stations in Pawong canal where salinity was low. For *M. strigata*, there is no report on the salinity tolerance of their larvae. However, it is likely that its optimum salinity might be higher than *M. sallei*'s. *M. strigata* populations in natural habitats were observed in salinities ranging from 5 to 30 ppt (Rice et al., 2016), 3 to 25 ppt (Mediodia et al., 2017), and 7 to 35 ppt (Sanpanich & Wells, 2019). Unlike *M. sallei*, *M. strigata* have never been observed in fully freshwater. This might reflect that larvae of *M. strigata* can survive in these salinity ranges, then settle, develop into recruits, and finally becoming part of the adult population. For *P. viridis*, recruitment observed at the mouth of the lake and on the coast of the Gulf of Thailand suggest that the larvae must survive in higher salinity condition compared to other species. This is supported by Tan (1997). His study reported that *P. viridis* larvae survived until they settled at salinities of 18, 24, and 30 ppt, with the highest survival occurring at 24 ppt.

Since the mussel beds of *P. viridis* that might be an important source of larvae for *P. viridis* in the Songkhla Lake were abundantly found on the coast around the mouth of the lake, this prompted the question of why *P. viridis* has rarely been found in the lake at the section where salinity might be as high as the open sea. According to personal observation in 2022, a bed of *P. viridis* on the coast around the mouth of the lake were found in January. Therefore, recruitment of the species may occur around

October to December 2021. At this time, there is an intensive rainfall due to the influence of the northeast monsoon, and. freshwater runoff dominated the hydrodynamics of the lake. The water current flows towards the mouth of the lake and the Gulf of Thailand. It is possible that the current that flows from the lake towards the sea probably reduces the chance of mussel larvae migrating into the lake.

CHAPTER 4

Salinity tolerance of *Mytilopsis sallei*, *Mytella strigata* and *Perna viridis*

Introduction

This chapter aims to examine the role of the bivalves' salinity tolerance as part of post-recruitment process that might determine the distribution pattern of bivalves by conducting laboratory experiments to investigate the effects of salinity on survival, growth, and condition of juvenile bivalves at different levels of salinity (0, 20, and 35).

Research Methodology

Juveniles of each bivalve species were used in the experiment as the growth rate of juveniles are generally the fastest and they are relatively more active than adults. The sexual maturity is at 8 mm for *M. sallei* (Morton, 1989), 12.5 mm for *M. strigata* (Stenyakina et al., 2010) and 15-30 mm for *P. viridis* (Siddall, 1980). Therefore, the experiment was carried out with specimens having shell lengths 5-8 mm for *M. sallei*, 9-12.5 mm for *M. strigata*, and 12-15 mm for *P. viridis*. The minimum lengths are determined as the bivalves of these sizes are not too difficult to handle.

All bivalves were collected by hand in March 2022. Juveniles of *M sallei* were collected from Songkhla Lake (salinity = 0). *M. strigata* specimens were retrieved from Pak Panang estuary, located around 70 km north from the northern part of the Songkhla Lagoon System (salinity = 8). While *P. viridis* were collected at Kao Seng beach and Sakom beach, located on the coast of the Gulf of Thailand, 6 and 20 km south from the mouth of the Songkhla Lake (salinity = 32). After collection, specimens of each species were transported to the laboratory, cleaned of any epibionts from shells by scrubbing and rinsing, and separately acclimated to laboratory condition for 7 days in artificial seawater. The salinitys that were used for acclimating is the same as the salinity of the natural habitat where bivalves were collected.

Before the onset of the experiment, each bivalve was labeled with an individual number and measured for wet weight then individuals was randomly assigned into the treatments. Each treatment consisted of twelve individuals with three replications. The experiments were performed in a set of 900 mL cylindrical plastic containers with varying salinities of artificial seawater. Artificial seawater was prepared from distilled water with artificial sea salt to salinity 0, 20 and 35. All experiments were conducted in a laboratory for 5 weeks to determine the response of two invasive mussels *Mytilopsis sallei* and *Mytella strigata* and indigenous mussel *Perna viridis* under different salinities. During the experiment, bivalves were fed daily with *Nannochloropsis* sp. This microalgae was chosen because it was readily available from the Coastal Aquaculture Research and Development, which is one of the diets generally utilized in bivalves culture (Okumuş et al., 2002). Solution containing the algae was poured into the containers with bivalves to meet the concentration of 4×10^4 cells ml^{-1}(Wang et al., 2011). Survival of bivalves were checked daily. Dead bivalves (having opened valves with on movement when touch) were removed from the treatments.

At the end of the experiment, growth rate, survival rate and condition indices were measured. All surviving bivalves were counted, and calculated for survival rate as [% Survival = (number of surviving bivalves/total number of bivalves at onset of the experiment) × 100]. Wet weight of all surviving bivalves were measured by using a digital weighing scale then calculated for growth rate as [Growth rate (mg d^{-1}) = (wet weight of surviving bivalves at the end of the experiment (g) – wet weight of surviving bivalves before onset of the experiment (g)) × 1000/total days of the experiment]. To analyze the condition index (CI) of bivalves, the shells and tissue samples were oven dried at 60° C for 48 h then dry mass (g) were measured to the nearest 0.001 g using a digital weighing scale. The condition index was calculated as [CI = tissue dry mass (g) × 100/dry shell weight (g)] (Davenport & Chen, 1987).

Data analysis

One-way PERMANOVA with pairwise comparison were performed to test the differences of growth rate, condition index, and survival rate of juvenile bivalves that exposed to different salinities (fixed factor, 3 levels). Data of growth rate and condition index of *M. strigata* and *P. viridis* was not obtained from salinity 0 as all specimens died, therefore, effect of salinity was tested only between salinity 20 and 35. For univariate PERMANOVA, Euclidean distance resemblance matrices (with a dummy variable added) were constructed from untransformed data. Univariate analyses were performed in PRIMER v7 add-on package PERMANOVA+ (Anderson et al., 2008).

Results

At the end of the experiment, living specimens of *M. sallei* were found in all treatments and the species had a high survival rate across all treatments (Fig. 15). All *M. sallei* specimens survived at salinity 0. *M. strigata* and *P. viridis* survived only at salinity 20 and 35 (Fig. 15). Approximately 80% of *M. strigata* survived at salinity 20 and around 40% survived at salinity 35, however difference between treatments was not detected. More than half of *P. viridis* survived at salinity 20 but the survival rate was lower than 20% at salinity 35 (Fig. 15).

The growth rate of *M. sallei* at salinity 35 was higher than salinity 20 and 0, respectively. *M. strigata* and *P. viridis* had a growth rate at salinity 35 higher than salinity 20. However, the growth rates of these three bivalves were not different between treatments (Fig. 15).

The condition index of *M. sallei* was different between all treatments. *M. sallei* have the lowest condition index at salinity 0 and the highest at salinity 35 (Fig. 15., Table 8, pairwise comparison, $p<0.05$). While the condition index of *M. strigata* and *P. viridis* were not different between treatments (Fig. 15).

From fig. 16 at salinity 0, the survival rate of *M. sallei* was 100% throughout the 35 days experiment. While the survival rate of *M. strigata* and *P. viridis* rapidly decreased. Most of the bivalves died within 10 days after the onset of the experiment. In addition, the byssus formation was not observed in both species. When specimens of both species were still alive, they closed their shells, not attached to the aquarium, and secreted the mucus cover around themselves (Fig. 17). At salinity 20, the survival rate of *M. sallei* and *M. strigata* were high, however the survival rate of *M. strigata* slightly decreased after day 31 of the experiment. While the survival rate of *P. viridis* gradually decreased and the survival rate was 58% at the end of the experiment. At salinity 35, the survival rate of *M. sallei* was high with 97% throughout the experiment. While the survival rate of *M. strigata* and *P. viridis* gradually decreased and lower than 50% at the end of the experiment.

Table 8. *p*-values from PERMANOVA testing the effect of salinity on survival rate, growth rate and condition index of bivalves.

Source of Variation	***M. sallei***	***M. strigata***	***P. viridis***
Survival rate	0.46	**<0.01***	**<0.01***
Growth rate	0.99	0.17	0.50
Condition index	**< 0.01***	0.71	0.79

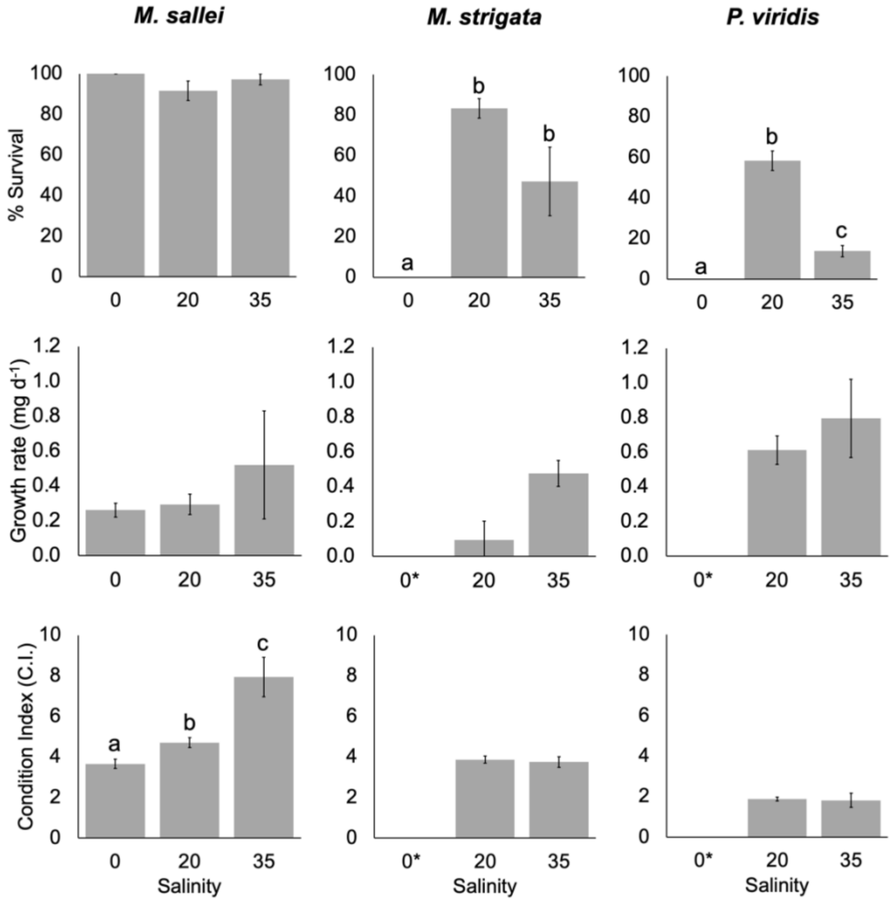

Fig. 15. Percentage of survival, growth rate and condition index of *M. sallei*, *M. strigata*, and *P. viridis* at different salinities (Different letters above the bars indicate differences between treatments, 0* = no data).

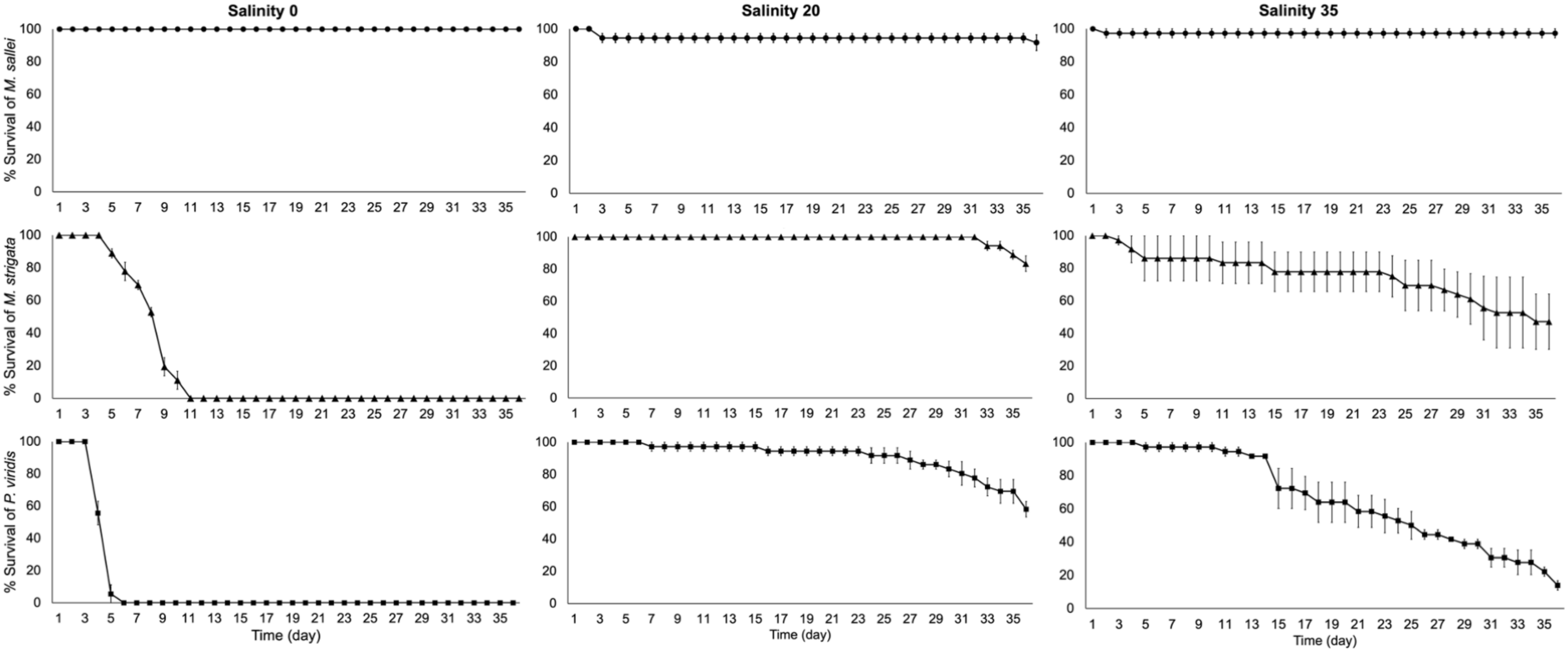

Fig. 16. Percentage of survival (Mean ± SE) of *M. sallei*, *M. strigata*, and *P. viridis* at different salinities from day 0 to day 35.

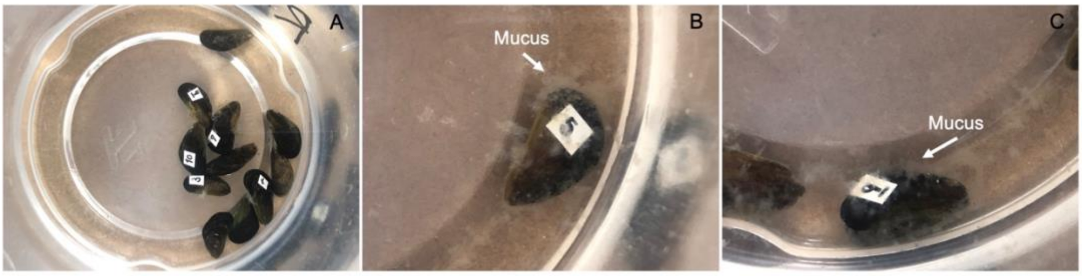

Fig. 17. *M. strigata* at salinity 0, (A) The byssus formation was not observed, and bivalve remained closed their valves, and not attached to the aquarium; (B-C) Bivalve secreted the mucus cover around themselves.

Discussion

In salinity tolerance experiment, *M. sallei* showed a high survival rate at all salinities. While *M. strigata* and *P. viridis* at salinity 0 died within 10 days after the onset of the experiment and survived only at salinity 20 and 35 at the end of the experiment. There was no difference in the growth rates of all bivalves species between treatments. The condition index of *M. sallei* increased as salinity increased with the highest condition index at salinity 35. While the condition index of *M. strigata* and *P. viridis* did not show a difference between treatments.

Salinity is one of the key environmental factors that affects growth, survival, activity, and physiology of marine organisms (Vernberg & Vernberg, 1972; Picoy Gozales & Laureta, 2022;). The responses of bivalves to varying salinities that were found in the present study were also reported in previous studies. For *M. sallei*, Sa-nguansil & Wangkulangkul (2020) reported that juveniles of *M. sallei* survived in a wide range of salinities from 0 to 40 ppt, which is consistent with the present study in which *M. sallei*'s juveniles had a high survival rate at all salinities (0, 20 and 35). In the present study, *M. strigata* survived only at salinity 20 and 35. While the study of Rice et. at. (2016) found that juveniles of *M. strigata* survived in salinities ranging from 5 to 30 ppt. Moreover, there was no record of *M. strigata* inhabiting fully freshwater condition. For *P. viridis*, the species survived only at salinity 20 and 35 in the present study. *P. viridis* was able to survive at as low as 6 ppt when it was exposed to a gradual salinity change (McFarland et al. (2015) and the species has never been found in freshwater habitat.

Generally, bivalves respond to salinity fluctuations by closing their valves to separate the internal fluid from the external environment, which protects them from osmotic stress (Pourmozaffar et al., 2020). However, this behaviour is a short-term response; if changes in salinity remain, bivalves would open their valves due to oxygen and food demand, then die because the cell osmolarity and cell volume can't be regulated (Gosling, 2003). Previous studies observed bivalve behavior that were similar to my experiment. *M. strigata* (Rice et. al., 2016) and *P. viridis* (Picoy-Gonzales & Laureta, 2022) showed no byssus formation, closed their valves, and were not attached to the aquarium when exposed to the salinity outside their optimum range (Fig. 17). When the valves were closed, bivalve activities such as respiration, feeding, and filtration may be stopped, thus the organism can't exchange gas and/or uptake food, resulting in anoxic respiration and the reduction of energy input (Navarro, 1988; Picoy-Gonzales & Laureta, 2022). Moreover, Maar et al. (2015) suggested that bivalves utilize more energy to maintain their cell volume and prevent osmotic stress in unfavorable salinity conditions.

Bivalves are osmoconformers that have little capability for osmotic regulation of their extracellular fluids (haemolymph) (Gosling, 2003). Generally, the cellular osmotic pressure of bivalves is in line with the external environment, but exposure to salinity fluctuations led to change in the osmolarity of their hemolymph (Pourmozaffar et al., 2020). Previous research proposed that bivalves can maintain cell osmolarity and cell volume by regulating the concentration of free amino acids (FAAs) and inorganic ions (Garton & Berg, 1989; Höher, 2013; Solan & Whiteley, 2016). The study of Banye (1975) found that when exposed to salinity stress, FAAs concentration increases rapidly and then decreases. Hawkins and Hilbish (1992) suggested that the primary source of FAAs comes from the breakdown of whole-body protein and diet. Therefore, an increase in FAAs concentration may result from the metabolism of protein during periods of limited food intake, which occur in unfavourable conditions (Navarro, 1988). Moreover, when exposed to salinity stress, channels and transporters of inorganic ions (Na^+, K^+, and Cl^-) are active to exchange and balance the concentration of ions between the cells and extracellular fluids for regulation of cell osmolarity and cell volume (Höher, 2013).

Generally, condition index reflects nutritional state of the bivalves as well as reproductive activities (Nalepa et al., 2010). Among all three species, there was only *M. sallei* that showed difference in CI between salinities. As *M. sallei* in the Songkhla Lake are most abundant in freshwater to low salinity conditions (salinity lower than 30), the result obtained from the experiment was somehow contrary to the distribution pattern observed by which highest CI was measured from salinity 35. It is impossible to distinguish whether low CI of *M. sallei* in salinity 0 was due to poor condition or release of gametes. However, settlement of larvae was not observed in the aquarium and the CI might actually be an indication of the health of the bivalves. In Sanguansil and Wangkulangkul (2020), *M. sallei* individuals that survived at salinity 40 did not showed a decrease in CI compared to those reared in optimal salinity. It seemed that once the bivalves managed to survive, they could maintain fine health condition.

CHAPTER 5

General conclusion

What are the mechanisms that shape the pattern of distribution of *M. sallei*, *M. strigata* and *P. viridis* in Songkhla Lake and its tributaries?

The preliminary investigation of the distribution patterns of alien invasive bivalves *M. sallei* and *M. strigata* as well as the native bivalve *P. viridis* in Songkhla Lake showed that the three bivalves seemed to occupy areas with different salinity ranges. This leads to the objective of my study, to investigate the mechanisms that shape the pattern of distribution of *M. sallei*, *M. strigata*, and *P. viridis* by focusing on role of salinity as a main driver that regulates the distribution.

In this study, I found that spatial patterns of bivalves' recruitment and adult distributions were congruent, and different species were prominent in different areas along the salinity gradient. Furthermore, the result of the experiment showed that salinity has an impact on the survival and condition of juvenile bivalves. Generally, the bivalves recruited at salinity ranges where adults were abundant.

Both recruits and adults of *M. sallei* was found at locations where salinities ranging from freshwater to low salinity. Most recruits (more than 50 individuals per pad) were found at station 6-8 where salinity never exceed 25. Result from the survey for distribution showed that adults of *M. sallei* were abundant at locations where salinity was lower than 20 which are further from the mouth of the lake where it connects to the sea. For *M. sallei* all individuals survived at salinity 0 and more than 90 % can survive in salinity 25 and 30. Condition index of *M. sallei* juvenile was unexpectedly highest at salinity 35. This suggests that, for *M. sallei*, once the larvae recruit, they can survive at almost any level of salinity and recruitment is the key process determining adult distribution.

From the survey, adult of *M. strigata* was found abundantly at salinity ranging from 31.62 to 6.59 at locations closer to the mouth of the lake compared to *M. sallei.* Although recruitment of *M. strigata* could be observed at all stations, recruitment peaked at station 3-4 where salinity was relatively higher than where most *M. sallei* recruited. It is possible that mortality of *M. strigata* recruits might occur at low salinity.

Therefore, adults were not found at the upstream stations where average salinity was low. It is likely that post-recruitment mortality shaped the distribution pattern of adults to some extent. This is supported by the result of the experiment showing that all *M. strigata* juveniles did not survive at salinity 0.

For *P. viridis*, the adults were observed only on the coast (open sea) when salinity in the lake was low, but a few individuals were found at the mouth of the lake when salinity was higher. A very few recruits of *P. viridis* were found at station 3 but the number was perhaps too low to draw a link between distribution of adults and recruits. Nonetheless, the result of experiment showing that *P. viridis* died at salinity 0 within a few days, suggests the bivalves cannot tolerate freshwater condition.

Our research provides an insight into the mechanisms regulating the distribution of alien invasive and a native bivalve in the Songkhla Lagoon System. Information obtained through the present study about the reproduction of alien invasive bivalves is also useful for species management. Populations in other estuaries where the species co-exist should be considered in future research. Furthermore, experiments on salinity tolerance of *M. strigata* and *P. viridis* at different life-history stages will also provide more understanding of how salinity limits the distribution of the species.

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