Habitat, growth and life history of the goby *Parapocryptes serperaster*

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DECLARATION

I hereby declare that this thesis is based on my original work except for quotations and citations that have been duly acknowledged. I also certify that it has not been previously or currently published or submitted for a degree or diploma at any university.

ABSTRACT

Parapocryptes serperaster (Gobiidae) is a burrowing fish widely distributed in the Indo-West Pacific region, including the Mekong Delta. Although *P. serperaster* is commercially important, little is known of its habitat, growth pattern and reproduction. The thesis aims to provide fundamental information on (1) habitat use, (2) growth pattern and body condition, (3) food and feeding habit, (4) reproductive biology, and (5) population structure of this gobiid based on studies from June 2012 to March 2015 in the Mekong Delta, Vietnam. The results improve the understanding of the biology, fishery management and aquaculture of this gobiid fish.

The habitat of *P. serperaster* was investigated by examining the burrow morphology using resin castings *in situ* to obtain the physical structure and configuration of the burrow. The larger fish could make more sophisticated burrows and the burrow dimensions were positively correlated with fish size. Burrow structure and dimensions were similar between dry and wet seasons. In the laboratory, *P. serperaster* actively excavated burrows using body movement, which is an important adaptation for living in a shallow and muddy habitat. The burrow provides a retreat to protect gobies from predation, while its use for spawning and feeding were not found.

The morphometric and growth characteristics of *P. serperaster* were investigated in both dry and wet seasons. Both male and female gobies shared a similar growth pattern between the wet and dry seasons, and the slope of the length-weight regression was close to the threshold of isometric growth (i.e., 3). The body condition factor decreased as fish grew, and seasonal differences in the condition factor depended on the gender. The condition factor of male *P. serperaster* was greater than of females in the wet season, but similar in the dry season.

Diet composition and feeding habits of *P. serperaster* were investigated on different sizes of fish. The gut length was positively related to fish length and the relative gut length to total length ratio was 1.57±0.30, falling into the category of omnivorous fish. Its diet composition and diet breadth varied between seasons and fish sizes, and smaller fish had a wider diet breadth than larger individuals. This goby was a generalist feeder, mainly feeding on detritus, followed by diatoms. Its feeding intensity was higher in the wet season than in the dry season, but was not affected by fish size. *P. serperaster* fed *Navicula* spp. in the wet season, but *Nitzschia* spp. in the dry season.

P. serperaster was a multiple spawner as the ovaries contained oocytes at different developmental stages during the spawning season. The peak spawning period occurred in September when the gonadosomatic index was highest. Fish reproduction was characterised by high variation of fecundity and heterogeneous distribution of egg sizes in the ovary, indicating a possible adaptation of this goby species to the monsoonal climate.

The population biology and age structure of *P. serperaster* were studied based on monthly samples over a year. Fish age was determined using fish length distribution and otolith dimensions. The size of fish at first entry to the fishery catch was 14.6 cm and fish longevity was 4.05 yr. Fishing, natural and total mortalities were 1.57 yr^{-1}, 1.51 yr^{-1} and 3.07 yr^{-1} respectively. The goby stock was not subject to overfishing since the exploitation rate (0.49) was lower than the maximum exploitation yield (0.83). Proper otolith morphometry of female and male gobies was used for age identification, and the age obtained from reading otolith annual rings matched the age determination from length frequency distribution.

This thesis provides new knowledge of habitat use, growth and life cycle of *P. serperaster* in tropical regions. The burrowing activity is an adaptation of fish living in shallow and muddy habitats, and the growth pattern provides useful information to understand gobiid morphometric variation in areas with monsoonal seasonality. The results of food and feeding habits, spawning pattern and reproductive biology contribute to knowledge of feeding adaptation of small-bodied bottom dwelling gobies to the mudflat habitat. The biovolumetric method is considered a reliable approach to quantify dietary composition in gobiid fish, and the use of otolith morphometry is a useful tool for age determination for gobies in tropical areas.

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CHAPTER 1: GENERAL INTRODUCTION

1.1. Coastal and estuarine ecosystems and fisheries

Coastal and estuarine habitats are naturally dynamic and fertile areas occupied by estuarine-dependent species for spawning and nurturing (Blaber et al. 1995; Paterson and Whitfield 2000; Levin et al. 2001; Barbier et al. 2010; Sheaves et al. 2015). The muddy estuarine zones provide an ideal habitat for fish to reproduce, grow and forage (Elliott and Dewailly 1995; McLusky and Elliott 2004; Elliott et al. 2007; Elliott and Whitfield 2011). Tropical estuaries are highly productive due to the availability of vegetation such as seagrass and mangroves (Nagelkerken et al. 2008; Alongi 2014), and high nutrient input from rivers (Blaber 2000; Burford et al. 2011). These areas are suitable for fish and their progeny to dwell, reproduce (Robertson and Duke 1987; Elliott et al. 2007) and feed (Nagelkerken et al. 2002; Nagelkerken et al. 2015). However, the function of these areas can be impacted by change of the habitat's integrity due to resource exploitation, water quality degradation and exotic species invasion (Lotze et al. 2006).

The geomorphology of estuarine habitats has obvious influence on fish recruitment (Able 2005). The suitability of a habitat for fish is subject to sediment types, vegetation coverage, tidal range, slopes of intertidal and subtidal areas (Shahraki et al. 2014). The abundance and distribution of fish are affected by water currents that determine the larval dispersal and distribution of benthic communities (Costa et al. 2002). Cattrijsse et al. (2002) suggested that fish communities in the intertidal or subtidal areas are regulated by the complexity of habitat geomorphology. According to Chong et al. (1990), the intertidal mudflat community is largely transitory, and the mangrove and subtidal areas are the two main habitats for species living in the intertidal zone. Moreover, various inshore foragers utilize mangroves and mudflats in yearly flooded tides (Chong et al. 1990; Vaslet et al. 2012). Although estuarine areas are small, the economic value of the estuarine ecosystem far outweighs other global areas (Costanza et al. 1997; Nagelkerken et al. 2015).

DeMartini (1999) reported that migrants and intertidal residents are two basic functional groups of intertidal fishes. Most resident species and partial adult occupants spawn in the intertidal zone, whereas other species, including strictly subtidal species, move into the intertidal zone to spawn during high tide. Potter et al.

(2015) categorised estuarine fishes into fourteen functional groups including marine stragglers, marine estuarine-opportunist, marine estuarine-dependent, solely estuarine, estuarine and marine, estuarine and freshwater, marine migrant, anadromous, semi-anadromous, catadromous, semi-catadromous, amphidromous, freshwater straggler, and freshwater estuarine-opportunist. Peters and McMichael (1987) showed that the reproductive development and activity of estuarine species may be caused by the increase in temperature; however, the proximal stimulus for fish spawning may be related to factors like moon phase in some species (e.g. peak spawning of *Sciaenops ocellatus* always takes place in the full moon nights). Gibson (1999) also stated that virtually all estuarine spawners spend their whole life in estuaries or move into estuaries to spawn, and the demersal eggs can cling to vegetation or rocks to protect from being washed away by currents into the sea. In the intertidal zone, some estuarine species move into spring tides to deposit their eggs on vegetation or bury eggs in the substrate, and these eggs develop into larvae when flooded by the next set of spring tides (DeMartini 1999). The egg size, yolk reserve, and the time of planktonic phase are the main factors determining the fish size at recruitment (Pfister 1999).

Gobiidae is a large fish family including about 200 genera and 2,000 species (Nelson 2006), and mainly comprises small, demersal fishes residing in waters of estuarine regions (Horn et al. 1999; Thacker 2011). Most fishes of the subfamily Oxudercinae (Gobiidae) live in tidal flats along the coastline and near estuaries (Murdy 1989; Takita et al. 1999; Murdy 2011) and are mudskippers belonging to 10 genera and 33 species (Murdy 1989). The subfamily Oxudercinae consists of 10 genera and 40 species that are elongate gobiid fishes compressed posteriorly (Murdy 2011). The genus *Parapocryptes* has two species which are *P. rictuosus* (Valenciennes, 1837) and *P. serperaster* (Richardson, 1846), both occurring in muddy areas (Murdy 1989; Murdy 2011). However, there is little research on fish in Oxudercinae, especially *P. serperaster*, an important potential fish for the economy in the Mekong Delta.

1.2. Coastal and estuarine fisheries in the Mekong Delta

The Mekong River is the 10th largest river in the world and is the greatest river in the Southeast Asia about 4,909 km long, roughly 505 km^3 in annual water discharge, and approximately 816,000 km^2 of drainage area (Milliman and Meade 1983; Liu et al.

2007; Kummu et al. 2010). Ta et al. (2002) also revealed that an enormous delta is formed by a few main channels and a large number of smaller channels of the estuary. The Mekong Delta is situated in the southern Vietnam between 8º30’ to 11º00’N and 10º30’ to 106º50’E, and its subaquatic topography is formed by two parts that are the subtidal flats and the front of Delta (Nguyen et al. 2000). The subtidal flats can expand to 6 m deep and 5–20 km wide on the right site of the river’s mouth due to the monsoonal long-shore currents affecting the predominantly south-westward transport of sediment (Nguyen et al. 2000; Ta et al. 2002). The coastal waters are shallow with a 20-m deep contour located from the coast (Wolanski et al. 1996). The mudflat area plays a crucial role as a spawning ground for various aquatic organisms (Nedeco 1993; Walton et al. 2006).

The rainfall and diurnal tides in the Gulf of Thailand are two main factors influencing the flood in the Mekong Delta, and the average tide range decreases with the distance upstream (Thuy 1988). The mean tidal range is 2.6±0.1 m, and the maximum tidal range is 3.2–3.8 m (Wolanski et al. 1996; Nguyen et al. 2000). Flood water depth varies 1–4 m and can persist for 2–6 months, depending on locations (Nguyen et al. 1998). Humid tropical climate dominated by a monsoon is typical for the Mekong Delta (Nedeco 1993; Nguyen et al. 2000). The wet (June–December) and the dry (January–May) periods are two main seasons in the Mekong Delta, and the annual temperature is ~27°C (Nguyen et al. 2000). The annual rainfall is approximately 1,700 mm, but the distribution of the rainfall varies depending on geography and seasons, e.g., in the western part rainfall can reach 2,500 mm (Nguyen et al. 1998; Nguyen et al. 2000).

The wetlands, which are created by seasonal or permanent inundation, occupy a large area in the Mekong Delta, and are divided into four groups including the coastal wetland ecosystem covered mainly by mangroves, the inland wetland ecosystem covered mostly by *Melaleuca* forests, the coastal estuarine ecosystem and special ecosystem in Dong Thap Muoi (Mai et al. 2013). The Mekong Delta wetlands are previously divided into two main groups comprising the inland wetlands dominated by flooded grasses and *Melaleuca* forests, and the coastal wetlands dominated generally by mangrove forests (Torell et al. 2001). The wetlands are productive natural systems and are fundamentally important in ecology and economy by providing habitats for various wildlife species to spawn, shelter and feed (Torell et al. 2001; Do and Benett 2009; Safford et al. 2009; Mai et al. 2013).

The mangrove ecosystem in the Mekong Delta, comprising intertidal flora and fauna of tropical and subtropical regions, is a transitional forest between marine and terrestrial environments (Nedeco 1993), and its physical characteristics are found in the coastal fringe on saline mudflats where the land and sea meet (Nedeco 1993; Phan et al. 2014). The Mekong Delta mangrove forests and their waterways provide organic matter and detritus as a food-supply base for marine and brackish animals and are also used extensively for artisan fisheries (Binh et al. 1997; Tong et al. 2004; Walton et al. 2006; FAO 2007). The mangrove forests have been created through the interaction of fluvial deposits and tidal influence, and cover almost all the coast in the Mekong Delta. However, the mangrove forests have been lost due to war, the fire of forest, and deforestation for shrimp culture and cropping systems (Minh et al. 1998; Thu and Populus 2007; Phan et al. 2014; Van et al. 2015).

The Mekong River generates large marine, coastal and inland water bodies forming the basis for fisheries and aquaculture, playing a crucial role in the local economy and being engaged with different fishing gears and methods to target economically important species from small to large scales (Wilder and Nguyen 2002). Aquaculture development is necessary to a precipitous increase in fish production because the natural fish supply cannot meet the demand for seafood consumption (Akpaniteaku et al. 2005). In the Mekong Delta, the production of freshwater aquaculture has played an increasingly important role in the development of the regional economy due to the favourable natural condition for fish growth, and extensive muddy tidal flats also provide promising environments for the development of coastal aquaculture in shrimp, mud crab and fish (Tien 1993; Sverdrup-Jensen 2002; Tran 2008). However, climate can moderately influence the coastline in the Mekong Delta by degradation of shoreline and saltwater intrusion (Le et al. 2007; Vastila et al. 2010; Takagi et al. 2015).

Matics (2000) indicated that the gobiid fauna in Mekong Delta is very diverse with about 200 gobiid species that are targeted with special gears and sold in large quantities in markets. *P. serperaster* (Fig. 1.1) is a popular species in commercial fishing in the Mekong Delta, but there are a few studies on its ecology, environment requirement, disease diagnosis, diet, growth and distribution (Murdy 1989; Rainboth 1996; Khaironizam and Norma-Rashid 2000; Riede 2004; Nguyen and Nguyen 2006).

Figure 1.1 ***Parapocryptes serperaster*** **(Photo by QM Dinh)**

P. serperaster is a burrowing fish in the Oxudercinae subfamily and distributed in the Indo-West Pacific regions including mainland China, India, Taiwan region, Malaysia, Myanmar, Singapore, Thailand and Indonesia (Murdy 1989; Talwar and Jhingran 1991; Kottelat et al. 1993; Khaironizam and Norma-Rashid 2000). This species lives in muddy coastal areas, and also resides in burrows situated in estuarine mudflats (Murdy 1989; Clayton 1993). *P. serperaster* has various synonyms given in Table 1.1 (Murdy 1989).

Table 1.1 Synonyms of ***P. serperaster***

Synonym	Authors
Apocryptes henlei	Bleeker, 1849
Apocryptes macrolepis	Bleeker, 1851
Apocryptes serperaster	Richardson, 1846
Boleopthalmus smithi	Fowler, 1934
Parapocryptes cantonenesis	Herre, 1932
Parapocryptes macrolepis	Bleeker, 1851

Rainboth (1996) indicated that this species resides in bays, estuaries and the freshwater tidal area of the Mekong Delta. Nguyen and Nguyen (2006) stated that this species also occupies the coral reef along the estuaries in Vietnam. The maximum total length of *P. serperaster* ranges from 17.3 cm (Murdy 1989) to 23 cm (Rainboth 1996). The external morphology of this goby is described by Murdy (1989) and Khaironizam and Norma-Rashid (2000) as follows: it has an elongated body with small, cycloid scales arranged in lateral series. Its eyes are located at or below the head's dorsal profile, absent lower eyelid. Lower jaw's teeth are pointed. Dorsal fin 1 (D1) and dorsal 2 (D2) are moderately high but not contiguous. The first spine of D2 and D1's spines is not branched, whereas the rest of D2 are branched. Pelvic fins, which are united by a strong freum, look like a round disk, and caudal fins are not strengthened. Khaironizam and Norma-Rashid (2000) mentioned that this goby is omnivorous and feeds on phytoplankton, mainly diatoms, and plant detritus with a few animal components (nematode and polychaeta). Its taxonomical classification is provided by Murdy (1989) as follows:

Phylum: Chordata

Class: Actinopterygii

Order: Perciformes

Family: Gobiidae

Genus: *Parapocryptes*

Species: *Parapocryptes serperaster* (Richardson, 1846)

However, there is little knowledge of the burrow morphology and utilisation, growth pattern and body condition, food and feeding habit of *P. serperaster* in the Mekong Delta. Moreover, the reproductive biology of this species such as sex ratio, gonadal development, gonadosomatic index, egg size, length at first maturity, spawning seasons and batch fecundity has not been studied. Furthermore, there is no published literature on population and age structure of *P. serperaster*. The age determination is one of the most important and fundamental steps in fishery management since this information can contribute to evaluating fish growth, age at spawning and population structure of the targeted fish in fisheries. Therefore, there is a need to study habitat, growth pattern and body condition variation, food and feeding habit, reproductive biology, and population structure of *P. serperaster* to improve understanding of the life history of this goby for future fishery management.

1.3. Habitat use and features

Most gobies build burrows with long, wide and multiple chambers as a shelter to protect from predation, desiccation, and extreme temperature, and facilitate reproductive activities and egg incubation (Atkinson and Taylor 1990; Takegaki and Nakazono 1999; Takegaki 2001; Itani and Uchino 2003). Clayton (1993) revealed that mudskippers are poor swimmers and use burrows as refuge to avoid predation or being washed away by the high tide. For example, *Amblygobius nocturnus* excavates burrow, which comprises multi-tunnels with a 3 cm diameter entrance, 5–7 cm deep, 6 cm long, and 4 cm wide, underneath dead and rubble corals (Mazzoldi 2001). The burrows are built by mouth excavation or body movements for the horizontal or vertical channels, and burrow shapes vary from U-shaped or J-shaped or Y-shaped (Atkinson and Taylor 1990; Ishimatsu et al. 1998; Ishimatsu et al. 2007). However, some gobies like the eel goby *Odontamplyopus lacepedii* have burrows without a definite shape (Gonzales et al. 2008).

After burrow observations, Gonzales et al. (2006) found a possible relationship between burrowing behaviour and reproduction of eel goby *Odontamplyopus lacepedii*. Usually, the water in a burrow is hypoxic leading to abnormal development of eggs due to inadequate oxygen supply, and air breathing benefits respiratory requirements of both embryos and adults when a fish spawns in the chamber (Gonzales et al. 2006; Gonzales et al. 2008). However, little is known of the burrow morphology and utilisation in the commercial gobiid, *P. serperaster*, especially in the Mekong Delta. Therefore, there is a need to explore the morphological structure and burrow use by *P. serperaster*, and fish burrowing behaviour and burrow utilisation at different fish sizes between wet and dry seasons. The results will contribute to the understanding of fish adaptation in muddy habitats and the function and structural diversity of burrows used by gobiid fish in tropical regions.

1.4. Growth pattern and condition factor variation

The information on the length-weight relationship (LWR) of fish can be used as a guide for fishery management (Froese and Pauly 2000; Gonzalez Acosta et al. 2004; Mahmood et al. 2012). Moreover, the fish length can be converted into weight estimation and indirect measurement of fish biomass using the LWR (Froese 1998); and fish growth and wellbeing conditions can be predicted using the slope value of the LWR regression (Froese 2006). In addition, Abdoli et al. (2009a) and Abdoli et al. (2009b) use the fish condition factor as an indicator to compare fish wellbeing between locations and stocks, while this factor was used by Le Cren (1951) to assess the effects of food availability, parasitic infection and physiological stress. The fish condition factor is also influenced by fish size and seasons (Froese 2006), for example, *Rhinogobius giurinus* body condition varied significantly in summer, autumn and winter (Guo et al. 2012), but the annual change in condition factor in females is much more pronounced than in male fish due to different strategies of energy investment in gonadal development (Mahmood et al. 2012). However, little information is available on growth pattern and body condition variation that greatly hinders fishery management in benthic goby species including *P. serperaster*. Therefore, there is a need to use body morphometry and condition as dependent variables to evaluate the fish growth pattern in different environmental conditions for *P. serperaster*. Such knowledge will contribute to fisheries management for burrow dwelling fishes.

1.5. Food and feeding habit

The seasonal changes of food and feeding habit of fish are strongly related to the amount of precipitation and can influence the composition and abundance of food organisms (Blaber 2000; Manon and Hossain 2013). The understanding of fish biology is incomplete without knowing fish diet and feeding habit (Blaber 2000; Gumus et al. 2002; Azadi et al. 2009). The food types of fish in nature are categorised as main food, occasional food and emergency food; and fish feeding ability is related to environmental factors such as season, time of a day, temperature, and internal factors such as sensitivity of taste buds, visual ability and the lateral line system that releases and controls the momentary feeding activities (Contente et al. 2012; Manon and Hossain 2013). Stomach contents reflect habitat separation in fish and the analysis of stomach content can reveal the habitat where fish feed (Gumus et al. 2002; Bayhan et al. 2013). Furthermore, the knowledge of food and feeding habit of fish provides a key to select species for aquaculture, and the information on dietary requirement is necessary for the improvement of fish farming efficiency (Azadi et al. 2009; Manon and Hossain 2013).

The variations of feeding habit and food selectivity indicate how many kinds of food item are preyed on by fish and which prey is the most common item in the fish stomach, enabling to choose suitable food for fish farming (Bakhoum and Fatas 2003). However, little is known of food and feeding habits of fish species from fresh to marine environments in the Mekong Delta where the fish composition is highly diversified, especially the gobiids living in the estuary and muddy regions. Therefore, the food and feeding habits of *P. serperaster* need to be studied to fill the knowledge gap on food and feeding habits of burrow dwelling fishes.

1.6. Reproductive biology

Miller (1984) stated that iteroparity and semelparity are the main reproductive modes of fishes. Semelparity refers to individuals spawning once and die (Caputo et al. 2003; Keith 2003), whereas iteroparity covers individuals having several sexual maturation cycles in their life history (Plaza et al. 2007; Yamasaki et al. 2011). Gobiid species are repetitive spawners and virtually all gobies release benthic eggs, and the male cares for the eggs during the spawning season (Miller 1984; Takahashi and Yanagisawa 1999; Gaisner 2005).

Caputo et al. (2001) showed that the goby *Aphia minuta*'s mature ovary contains numerous oocytes at various vitellogenic phases during the spawning season, indicating that this species can spawn during a lengthy period with a minimum of two peaks. Tran et al. (2007) revealed that the goby *Pseudapocryptes elongatus* annually recruits two times in July and October. Similarly, the winter and early spring seasons are two annual recruitment cycles of the rock goby *Gobius paganellus* (Azevedo and Simas 2000). According to Healey (1971), the female sand goby *Pomatoschistus minutus* can spawn at least three batches in the spawning period.

Water temperature determines the time of embryonic development of goby eggs, and more egg batches might be produced in a warmer summer, suggesting that there is a positive correlation between temperature and the production of goby larvae (Parmanne and Lindström 2003). Blaber (2000) also reported that the species in the genus *Acentrogobius* spawn in the monsoon period when salinity is low but turbidity, temperature and plankton biomass are high. Keith (2003) indicated that the seasonal variables such as rainfall, drought, floods and typhoons influence fish survival, reproduction and the dispersal of larvae. Tran et al. (2007) showed that the commercial goby *Pseudapocryptes elongates* spawns from June to November in the wet season in the Mekong Delta. The spawning cycles in some gobies was species specific, for instance, the goby *Rhinogobius cliffordpopei* spawned during a period from February to June, whereas the goby *R. giurinus* released eggs from April to August in Lake Erhai, China (Guo et al. 2013).

However, there has been little published information on batch fecundity, egg size, size at maturity, spawning season and gonadal development of *P. serperaster* despite its importance in contributing to fisheries. Therefore, there is a need to further explore the reproductive biology of *P. serperaster* to understand the reproductive biology of burrow dwelling fishes to manage the fishery catch and resource sustainability.

1.7. Population and age structure

Fish age determination is fundamental for fishery managements as age is a key variable to evaluate fish growth, predict the fish age at spawning, and identify population structure (Beatriz 1992; Cardinale et al. 2000). Devries and Frie (1996) reported that three methods to determine fish age, i.e., direct observation, length-frequency analysis and reading the annual or daily rings on the hard parts of fish. The direct observation of individual fish under captivity is one of the most accurate methods; however, it is just suitable in an aquaculture situation, and is a time-consuming and costly way to determine fish age. Regarding the length-frequency analysis, it is a beneficial method and less expensive compared with direct observation of individuals. Nevertheless, it is not a suitable and useful technique for older fish. With regard to reading the annual or daily rings on the hard parts of fish, it is hard to apply for tropical species due to less pronounced seasons in tropical regions (Pilling et al. 2003). The use of annual or daily rings on the hard parts and length-frequency analysis is more practical for age determination. The translucent and opaque annuli or rings are usually found on the scale, opercular bone, otoliths, vertebra and fin rays (Gaamour and Khemiri 2005). The rings on otolith and otolith weight have a close relationship with fish age, length and weight (Worthington et al. 1995; Pilling et al. 2003; Cardinale and Arrhenius 2004; Lou et al. 2005).

The size structure and length frequency distribution of a fish population contain the information on individual growth, mortality and recruitment. The length-frequency analysis is based on the assumption that recruitment is seasonal with one or two peaks per year (Sparre and Venema 1992). The fish population structure can be analysed using various models such as surplus yield models, yield per recruit models, biomass models and simulation models (King 2007). The yield per recruit model was used by Etim et al. (2002) to analyse the yield of *Periophthalmus barbarous*. The yield per recruit model is a steady model that describes the state of stock when fishing pattern remains unchanged for a long time so that all fish are vulnerable to capture after recruitment. The major assumptions underlying the yield per recruit model are that recruitment is constant, yet not specified; recruitment and selections are "knife-edge"; fishing and natural mortalities are constant from the entry to the exploited phase; and there is a complete mixing within the stock (Sparre and Venema 1992).

However, there is a lack of knowledge of age and population structure on the burrowing gobiid especially in the Mekong Delta. Therefore, it is necessary to study the age structure of gobiids to develop sustainable models to guide fishery management.

1.8. Objectives of the study

This study generally aims to understand how *P. serperaster* has adapted to the mudflat habitat and how the environmental conditions influence its growth pattern, food and feeding habit that are important characteristics for future aquaculture of this species. Moreover, this study also explores the reproductive biology of this goby species towards the practice of artificial reproduction of gobiids. Furthermore, the population and age structure of *P. serperaster* are examined to understand its current status of fishing exploitation to establish policies for sustainable fishery development. The results of this study would contribute to the improved understanding of the biology of fishes and especially for gobies and other demersal species. Specifically, this study aims to:

(1) examine the burrow morphology, activities and utilisation of *P. serperaster* in the dry and wet seasons.

(2) understand the growth pattern and condition factor variation of male and female *P. serperaster* at different size classes between the wet and dry seasons.

(3) understand the seasonal and intraspecific impacts on food and feeding habits of *P. serperaster*.

(4) investigate the reproductive biology of *P. serperaster* in the dry-wet season pattern.

(5) understand the population and age structure of *P. serperaster*.

1.9. Thesis organisation

The thesis consists of seven chapters. Chapter 1 provides general background information, identifies the knowledge gaps and presents the study objectives of the thesis. Chapters 2–6 address five study objectives of the thesis, respectively. Each chapter is presented as an independent manuscript suitable for publication in peer-reviewed scientific journals, which may result in some repetition of background and methods among chapters. All experiments in this thesis were performed by the PhD candidate under the supervision of his supervisors. The supervisors are included as co-authors in most of the manuscripts for publication. All chapters have been reformatted in this thesis as different formats were used in published papers.

Chapter 2 addresses burrow morphology and behaviour of *P. serperaster* and the differences in burrow structure and function between fish sizes and seasons (dry and wet), which differ from other bottom dwelling fish species. Meanwhile, the information on growth pattern and condition factors between different fish sizes and between dry and wet seasons are presented in Chapter 3. In Chapter 4, natural food item diversity of this goby at different fish sizes and seasons, and feeding habits of *P. serperaster* are documented. The reproductive biological parameters including batch fecundity, egg size, size at first maturity, spawning season and gonad development of *P. serperaster* are given in Chapter 5, while the information on age and population structure using length-frequency distribution was used to test if otolith morphometry is related to fish growth and aging of this goby in Chapter 6. The results of five independent chapters were combined to discuss the implementation of major objectives of this thesis. The general conclusions and future research are represented in Chapter 7.

In summary, the thesis is organized in the following manner:

- Chapter 1: General introduction

- Chapter 2: Habitat use and features

This chapter has been published as:

Dinh QM, Qin JG, Dittmann S, Tran DD (2014) Burrow morphology and utilization of the goby (*Parapocryptes serperaster*) in the Mekong Delta, Vietnam. Ichthyological Research, 61:332–340.

- Chapter 3: Growth pattern and condition factor variation

This chapter has been published as:

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- Chapter 4: Food and feeding habits

This chapter has been submitted to Ichthyological Research as:

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- Chapter 5: Reproductive biology

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- Chapter 7: General discussion

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CHAPTER 2: BURROW USE AND FEATURES

This chapter has been published in Ichthyological Research as:
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2.1. Abstract

Some fish species living in mudflats construct burrows for dwelling and hiding. *Parapocryptes serperaster* is a burrowing fish in mudflats of many estuaries in South East Asia. This study was carried out in the Mekong Delta, Vietnam, to examine burrow morphology and usage by this species. Morphology of the burrows constructed by *P. serperaster* was investigated by resin castings *in situ* to obtain the physical structure and configuration of each burrow. Fish from the burrows were caught and measured before burrow casts were made. Fish burrows comprised several openings, a few branching tunnels and multi-bulbous chambers. The surface openings were circular, and the shapes of branching tunnels were nearly round. The burrows had interconnected tunnels, and various short cul-de-sac side branches. The burrow structure differed between fish sizes but burrow dimensions were positively correlated with fish size, indicating that larger fish can make larger and more sophisticated burrow. The burrow structure and dimensions were not different between dry and wet seasons. The laboratory observations showed that *P. serperaster* used body movements to dig burrows in the sediment. Burrows could provide a low tide retreat to prevent predation, but were not used for spawning and feeding for this goby species. This study indicates that the burrowing activity of gobies is an important adaptation for living in shallow and muddy habitats.

Keywords: *Parapocryptes serperaster*, burrow structure, mudflat, resin cast

2.2. Introduction

Some fishes have the ability to construct burrows in habitats ranging from fresh to salt water and from mudflats to the deep sea (Jones et al. 1989; Atkinson et al. 1998; Clark et al. 2000; Gonzales et al. 2008; Takeda et al. 2012). Fish construct a burrow through twisting the body and ejecting mud pellets from the mouth (Atkinson and Taylor 1990). Burrow construction is an individual behaviour without cooperation of other organisms in most fish species such as the goby *Pseudapocryptes elongatus* (Tran 2008), air-breathing eel goby *Odontamblyopus lacepedii* (Gonzales et al. 2008) and the goby *Taenioides cirratus* (Itani and Uchino 2003). However, some other fishes create burrows together with other organisms living in the same habitat. For example, the goby *Cryptocentrus cryptocentrus* works together with the pistol shrimp *Alpheus djiboutensis* to dig burrows (Karpulus et al. 1972).

Burrows can be used for predator protection, feeding, spawning and egg incubation (Atkinson and Taylor 1990). For instance, the gobiid fish *Signigobius biocellatus* (Hudson 1977) use burrows for living and spawning, while the goby *Taenioides cirratus* (Itani and Uchino 2003) use burrows mainly for living. In addition, the mudskipper *Periophthalmus modestus* (Ishimatsu et al. 2007) uses burrows for spawning and egg care. Moreover, burrows have also been used as a refuge to hide from predators and a foraging place in *Boleophthalmus boddarti* (Clayton and Wright 1989). Furthermore, burrows can be used to store oxygen for survival of eggs, as found in some mudskippers species (Ishimatsu et al. 1998; Ishimatsu et al. 2007).

The burrow structures of gobies and mudskippers have been categorised as U-shape, J-shape, I-shape and Y-shape based on morphological configuration (Atkinson and Taylor 1990). For example, the mudskipper *Pseudapocryptes elongatus* creates burrows in a Y-shape (Tran 2008), while *Periophthalmodon schlosseri* creates J-shaped burrows (Ishimatsu et al. 1998), and *Boleophthalmus boddarti* makes U-shape burrows (Clayton and Wright 1989). In contrast, burrows of some fishes such as the eel goby *Odontamblyopus lacepedii* have no clearly defined shapes (Gonzales et al. 2008). Although the shape, structure, and configuration of burrows may have important implications for the life strategy of demersal fishes (Atkinson and Taylor 1990), little is known of structural variation in burrows of fishes. In the Mekong Delta region where dry and wet seasons are distinctive and fish habitats are severely affected by heavy floods (Le et al. 2007), adaptation of fish burrows to flood change is not known.

P. serperaster is an amphibious fish (Murdy 2011) with an elongated and round body living in estuary (Khaironizam and Norma-Rashid 2000). In the past, research on this fish has been focused on its morphology and diet preference (Khaironizam and Norma-Rashid 2000), habitat use (Takita et al. 1999), taxonomic characteristics (Murdy 2011), and geographic distribution (Talwar and Jhingran 1991; Kottelat et al. 1993; Rainboth 1996; Tran et al. 2013). However, little is known of the burrow morphology and utilisation by this goby species, and how a *P. serperaster* builds its burrows. Therefore, this study aimed to understand (1) the morphological structure and burrow utilisation by *P. serperaster* at different sizes between rainy and dry seasons, and (2) fish burrowing behaviour and burrow utilisation. This study thus seeks to contribute to the understanding of fish adaption in muddy habitats and the function and structural diversity of burrows used by gobiid fish in tropical tidal estuary.

2.3. Materials and methods

2.3.1. Study site

This study was carried out in intertidal mudflats in Kinh Ba river, Cu Lao Dung district, Soc Trang Province, Mekong Delta, Vietnam (9°26'3"N, 106°13'28"E), from June 2012 to May 2013 (Fig. 2.1). The intertidal flat was mainly characterized by mud and muddy sand sediments. Soc Trang is a typical Province in the Mekong Delta with a long coastline and large areas of mudflats. The region has a typical dry (January to May) and wet (June to December) seasonal cycle with an average annual temperature of ~27 °C (Soc Trang Statistical Office 2012).

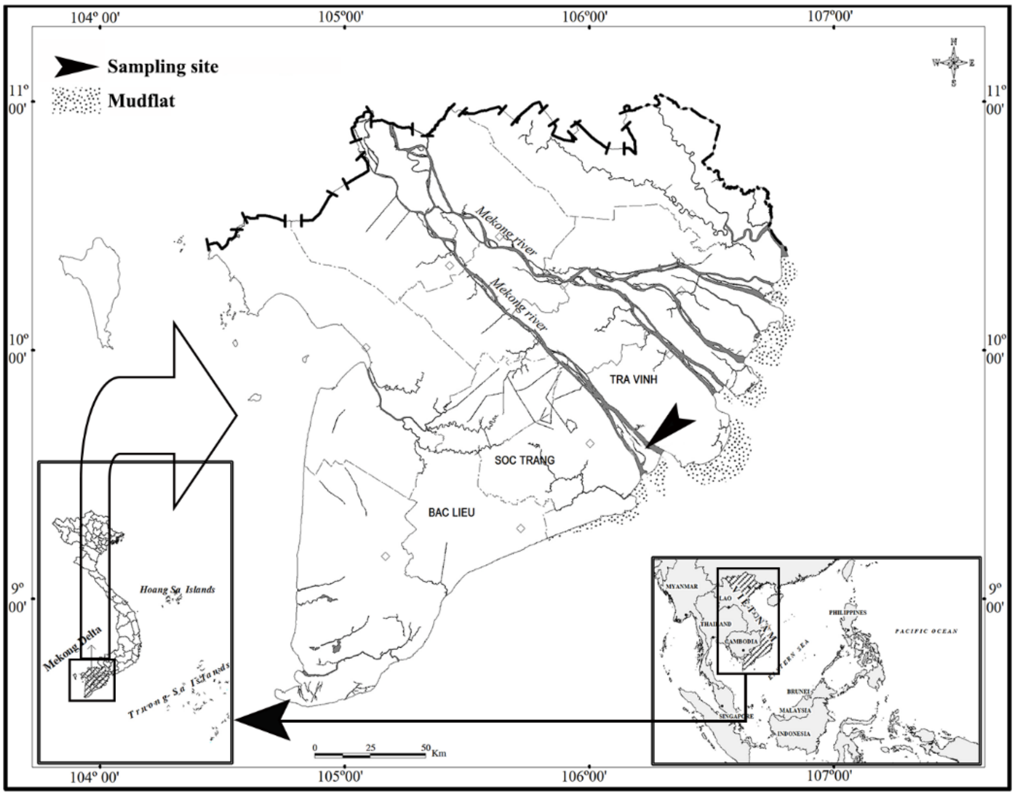

Figure 2.1 The study site located in Soc Trang, Mekong Delta, Vietnam (arrow head: sampling site)

The distance from the riverbank to riverbed of the mudflat was nearly 3 m at the lowest tide. Tides are semi-diurnal with a spring tidal range of ~0.7 m. Most field work was conducted on low tide in the early afternoon. An area of 10 m^2 (5 m along the riverbank and 2 m from the riverbank to the riverbed) was chosen for monthly investigations of burrow morphology, activities and utilisation by *P. serperaster*. The study area included 17–18 burrows at each time of observation during the study period.

2.3.2. Burrow casting and analysis

The burrow structure of *P. serperaster* was studied monthly by casting burrows in the field and analysing burrow casts in the laboratory. Each month, two burrows were randomly selected from the 10 m^2 sampling site. The burrows constructed by *P. serperaster* was characterized by no "foot-prints" made by fish pelvic fins around the burrow openings, while burrows of the sympatric mudskipper *Boleophthalmus boddarti* and crabs had "foot-prints" of their body parts (pelvic fins or legs). Burrow casts were made using polyester resin as described by Atkinson and Chapman (1984). The polyester resin (En Chuan Chemical industries Co., Ltd, Taiwan) was mixed with hardener (2%, v/v) in a 500 mL bottle, before it was immediately poured *in situ* into the burrow openings for casting. Mud was piled around the burrow entrance to prevent resin from spilling over the mud surface. After 24 h, the hardened casts were carefully removed from the sediment by hand, and then brought to the laboratory for analysis. In the laboratory, each burrow cast was measured for the number of openings (large and small), bulbous chambers and burrowing branches (Fig. 2.2). The casts were also measured for the maximum width and length dimensions, length of each tunnel, total burrow length, diameter of tunnel, diameter of opening and bulbous chamber, and displacement volume (Fig. 2.2). These data were used to compare the structural variation between burrows. The burrow structure was analysed based on the number of openings, interconnected chambers and burrowing branches using the method of Gonzales et al. (2008).

The relationship between fish size and burrow dimensions was tested by regression analysis between fish length and the diameter of burrow openings, bulbous chambers, tunnel diameter (i.e., the distance of tunnel cross-section), burrow depth, burrow length and width, total burrow length and burrow volume to test if a goby uses the burrow constructed by itself or just uses any existing burrows made by other fishes regardless of burrow size for hiding. Fish were caught by hand as they moved out the burrow during casting. If fish were trapped in resin or casts were broken, additional casts and fish samples were made in next fortnightly field work. Seasonal differences in burrow structure were examined between 10 burrow casts in the dry season and 14 burrow casts in the wet season to quantify the number of burrow openings and bulbous chambers, burrow depth, width and length, and total length and volume of burrows as described by Gonzales et al. (2008). These data were used to test whether the rainy season can influence the burrow structure due to the increase of mud and sand contents in flood water.

2.3.3. Laboratory experiment and burrowing behavioural observations

Although burrow casts in the field provided information on the burrows dug by fish of different sizes, the casts could not show the process of burrow building and utilisation. The burrowing activity of *P. serperaster* was thus further investigated in both laboratory and field. The laboratory experiment was conducted at Can Tho University in three replicated aquaria (50 × 40 × 60 cm) from 4 to 25 June 2013 based on the method of Dou et al. (2007) using the sediment and associated biota from the field and 10 fish (12–16 cm TL) were placed in each aquarium. The environment of the aquarium mimicked that in the field with a 15° slope muddy flat and a water level covering half of the flat (Fig. 2.3). The temperature in the aquarium was 29.5 °C, pH ≈ 7.0, and salinity of 2, which were similar to the condition in the field where fish were collected. A system with four cameras (QTC-203c, Questek Company, Taiwan) was set up to record burrowing activities of *P. serperaster* in the aquarium.

Field observations on fish burrowing behaviour were carried out twice a month and each lasted 2 days in the study area. The visual observation lasted 3 hours per day during the low tide, following the method of Bhatt et al. (2009). Inside the burrow, fish were observed using an endoscope camera (Video Borescope Inspection Camera, code: 177845, EXTECH Company) to check fish occupancy, movement, burrowing activities, and the presence of eggs or embryos inside the burrow for one hour per day during the low tide on each field trip (Ishimatsu et al. 2007). In the field and laboratory, external observations were focused on feeding, predator avoidance and reproductive uses of each burrow. In the field, observations lasted 3 hours per day during the low tide, while in the laboratory, 8 hours per day were spent to observe fish behaviour as described by Tytler and Vaughan (1983). Fish entering a burrow with head first indicates that the fish may use the burrow for predator avoidance, but not for foraging prey. On the other hand, fish entering tail first shows that fish may use burrow for foraging prey (Able et al. 1982).

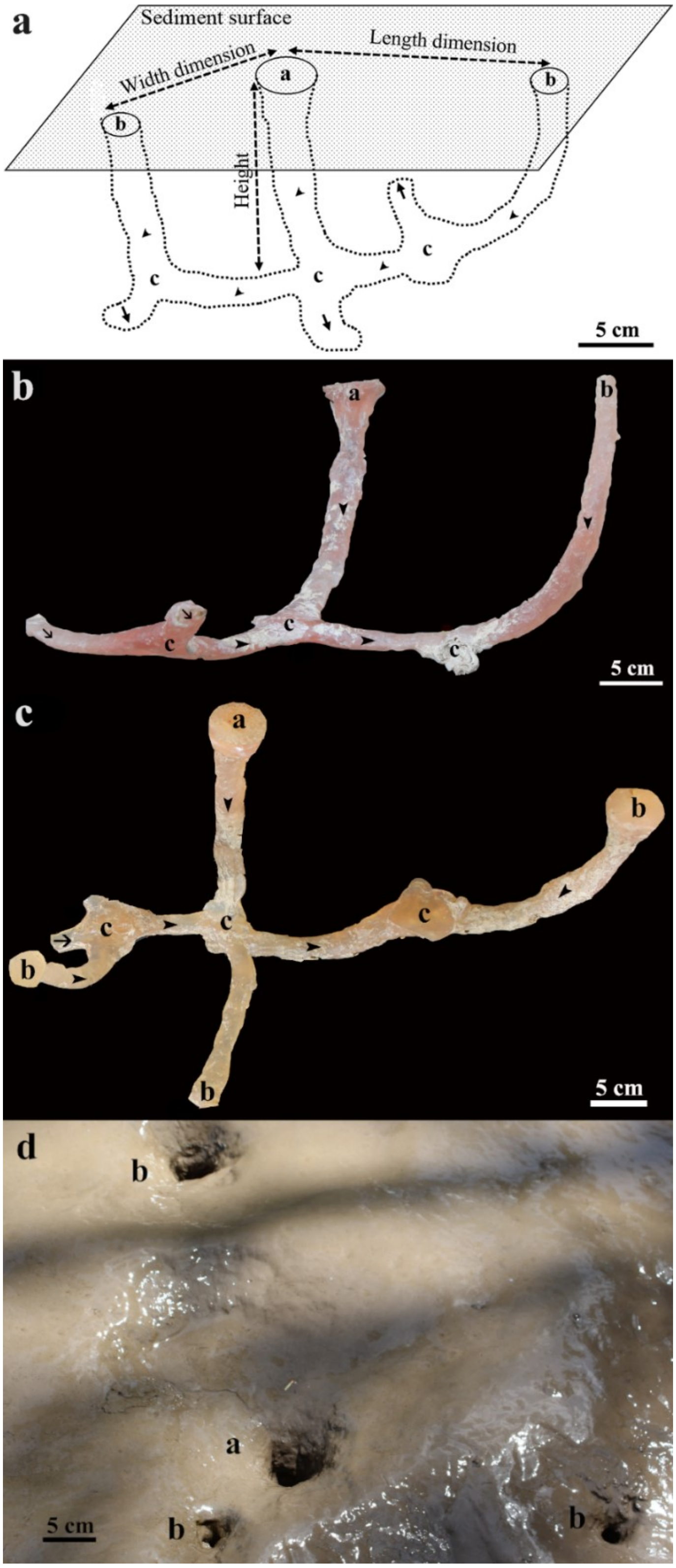

Figure 2.2 Illustration of burrow structure (a), U-shaped burrow (b), W-shaped burrow (c) and burrow openings (d). a: main opening, b: sub-opening, and c: bulbous chamber, arrow head (➤): tunnel, and arrow (→): cul-de-sac branch

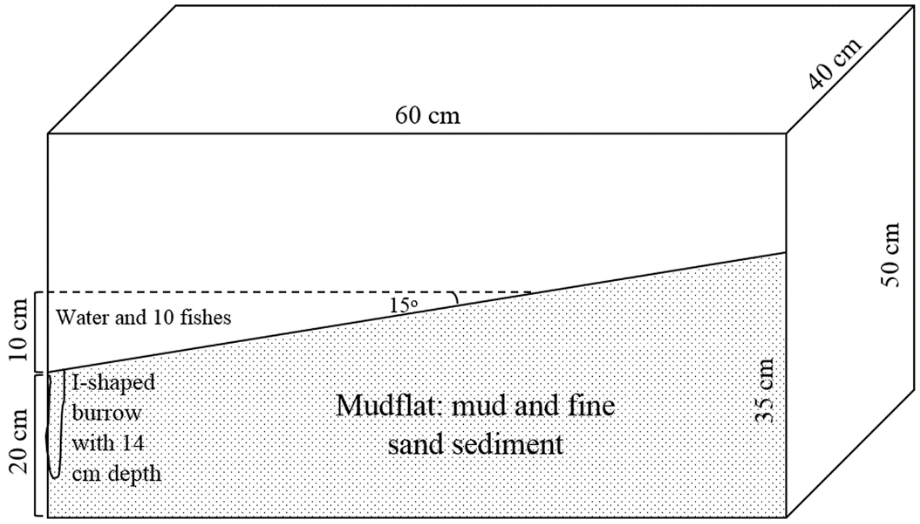

Figure 2.3 The experiment layout for observing fish burrowing behaviour in the laboratory

2.3.4. Data analysis

The 50 percentile fish body length at first maturation was considered the criterion to separate between a juvenile and an adult (Froese and Binohlan 2000). According to this criterion, the size of *P. serperaster* to reach maturation was predicted at body length 15.8 cm based on the fish collected in another study (unpublished data). In regression analysis, the independent variable was fish total length, and the dependent variables of burrow measurements included the number of openings, opening diameter, bulbous chambers, bulbous diameter, tunnel diameter, burrow depth, width and length, total burrow length and burrow volume of 24 burrow casts (two burrow casts per month). The differences of burrow depth, length and width, total burrow length and volume of burrows between dry and wet seasons were tested by MANOVA (Lawley-Hotelling test) at $p<0.05$. The Minitab package software v.16 was used for data analyses.

2.4. Results

2.4.1. Burrow structure and its relationships with fish size and season

A total of 24 goby burrows were analysed during this annual study. The burrows comprised interconnected-segments with slight slopes on the bottom with 2–5 short cul-de-sac side branches, 2–4 burrow openings, and 1–3 bulbous chambers (Fig. 2.2a). The burrow structure of *P. serperaster* was in either U-shape (Fig. 2.2b) or W-shape (Fig. 2.2c). The burrow openings were usually flat and slightly circular without a mound and “foot print” (Fig. 2.2d). The main openings were usually larger than that of sub-openings, and the former was not constricted whereas the latter was usually constricted. The tunnel walls were roughly smooth, and their cross-sections were slightly round.

Fish in the burrows were 14–18 cm in total length (TL). The fish length had a strong relationship with burrow opening diameter ($p<0.01$), bulbous chamber diameter ($p<0.01$) and tunnel diameter ($p<0.01$), resulting in significant regression coefficients (r^2, Fig. 2.4a, b, c). Similarly, fish length was correlated with burrow depth ($p<0.01$), burrow width ($p<0.01$), burrow length ($p<0.01$) and total burrow length ($p<0.01$, Fig. 2.5a, b, c, d). The volume of burrows ranged from 171 to 715 mL and had a strong relationship with fish length ($p<0.01$, Fig. 2.6).

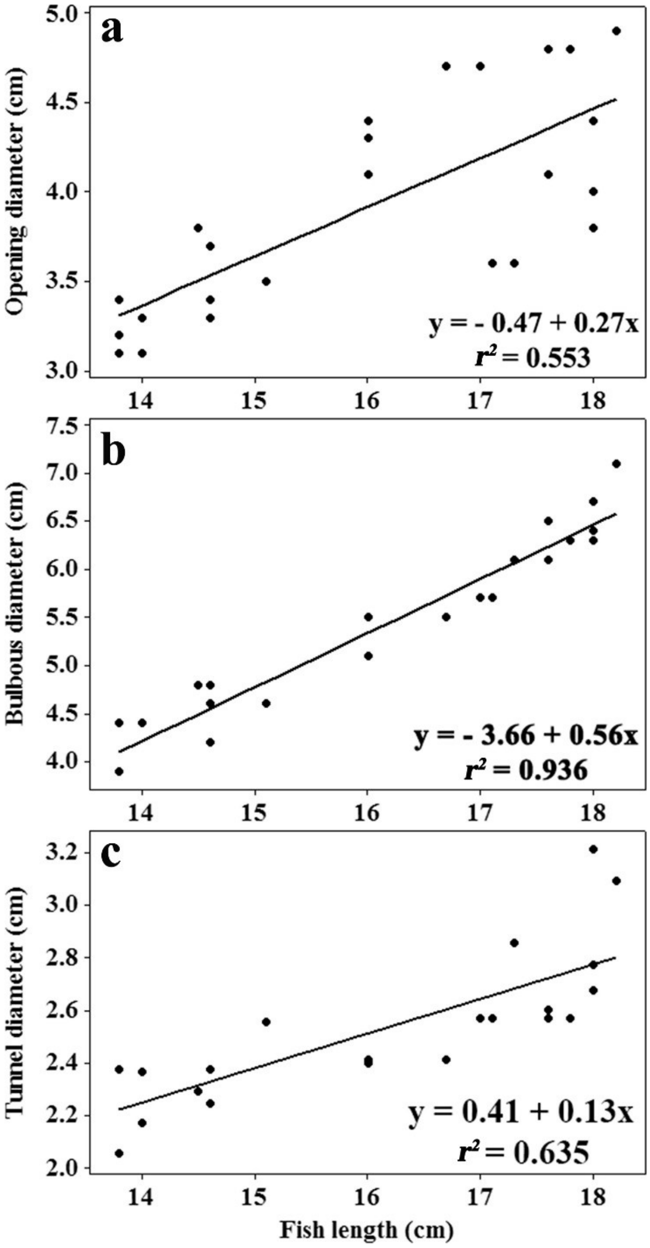

Figure 2.4 Relationships between fish body length and opening (a), bulbous chamber diameter (b) and tunnel diameter (c) (*n* = 24 in all cases)

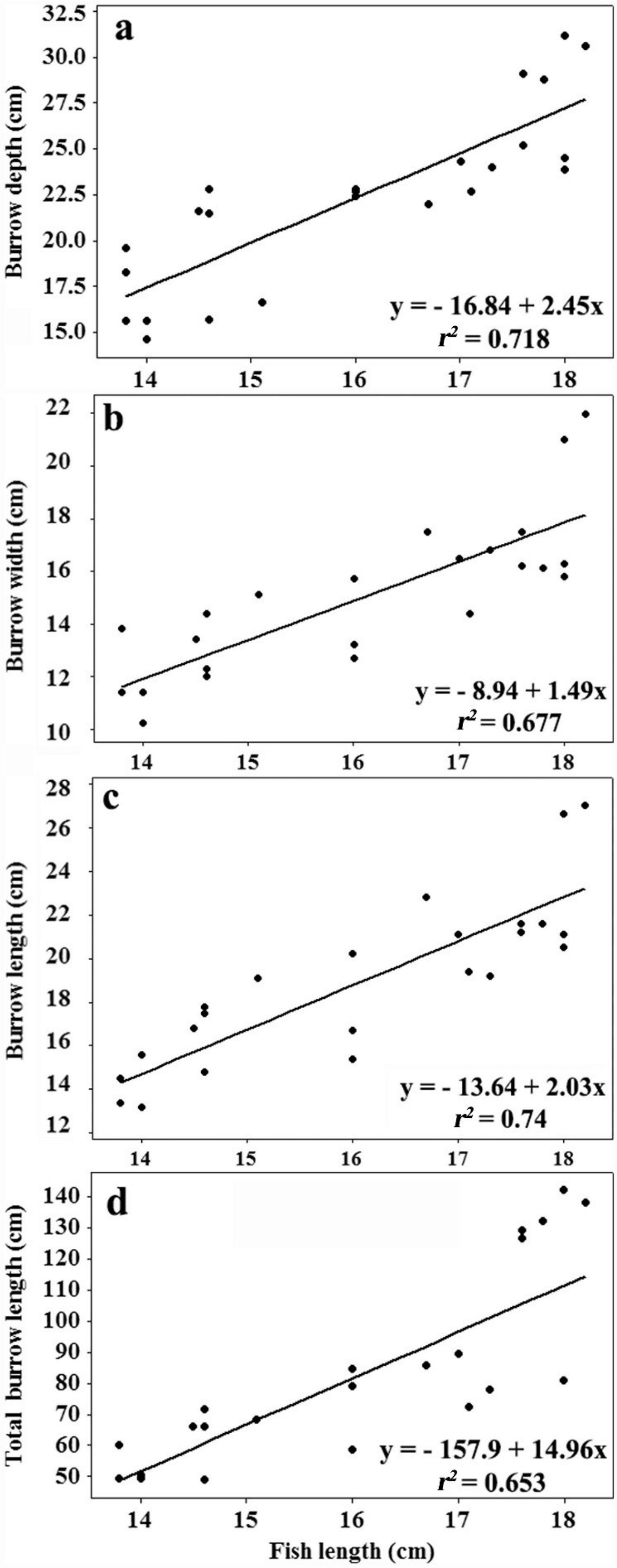

Figure 2.5 Relationships between fish length and the burrow depth (a), width (b), length (c), and total burrow length (d) (*n* = 24 in all cases)

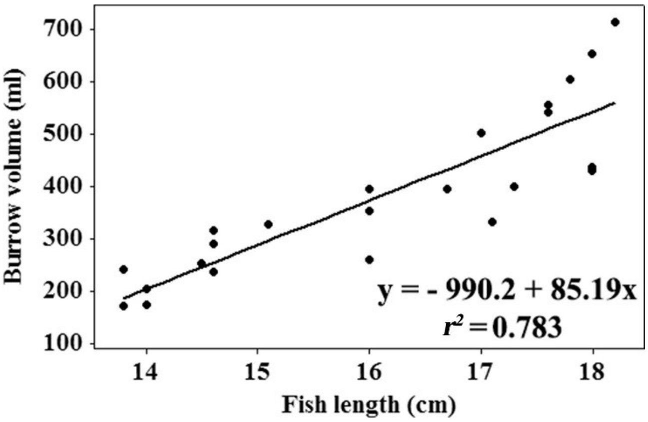

Figure 2.6 Relationships between burrow volume and fish body depth (a) and fish length (b) (*n* = 24 in all cases)

The burrow depth, width, length, and total burrow length, size of openings and volume, and the number of openings and bulbous chambers differed significantly between juvenile (<15.8 cm) and adult (15.8–18 cm) gobies (MANOVA, $F = 5.181$, $p<0.01$). These values were significantly higher for burrows occupied by adults than those by juveniles, suggesting that the burrow size, and complexity increased with fish size. The opening diameter, bulbous diameters, tunnel diameter, burrow depth, width, length, and total burrow length in the wet season were slightly greater than those in the dry season, but the difference was not significant (MANOVA, $F = 1.002$, $p = 0.445$). Similarly, the number of openings, number of bulbous chambers and volume of burrows were not significantly different between seasons (MANOVA, $F = 0.902$, $p = 0.482$, Fig. 2.7).

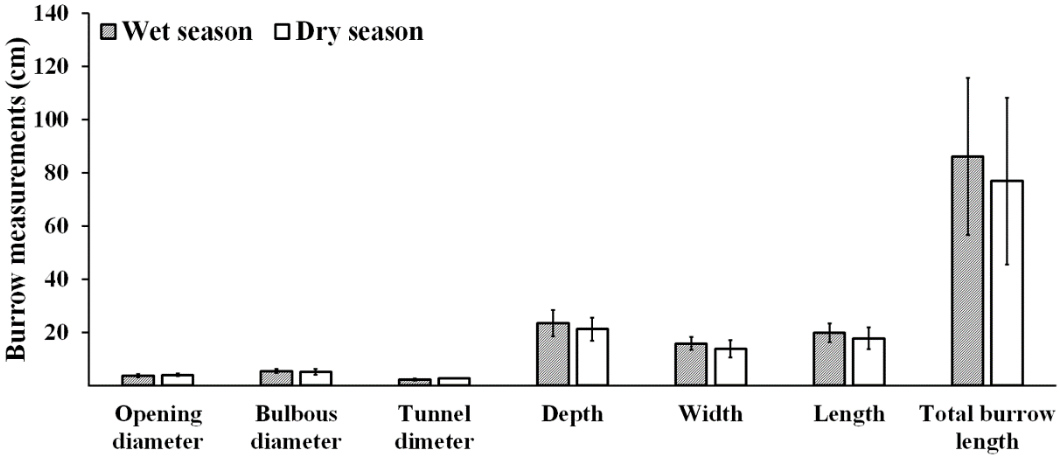

Figure 2.7 Structural features of fish burrows in the wet and dry seasons

2.4.2. Burrowing activities and utilisation

Burrowing activity of *P. serperaster* was not recorded during field observations at a spring tide because of deep and turbid water. The goby rarely moved out of its burrow at low tide until approaching to the burrow for making casts or catching fish. Yet, the goby was jumping out its burrow when the mixed polyester resin was poured into the burrow to make a cast. No other organisms were seen in the goby burrows when an endoscope camera was used to explore the burrow, or during the period of burrow casting. Neither endoscopic nor external examinations showed any indication of juvenile fish inside the burrows.

In the laboratory experiment, one of 10 fish in the aquarium excavated a burrow in the sediment at the corner of the aquarium two days after introduction into the aquarium (Fig. 2.3). The period of burrow digging activity coincided with the time of high tide in the field. The lengths of fish created burrows were 16 cm in the first and second trials and 15 cm in the third trial, and each burrow was dug through fish body movement. The fish started using the head to probe the mud, and then used its pelvic fins and body twisting movement to make the hole deeper and wider. After 4 hours, the fish stopped digging, and stayed in the hole for one day. The burrow was a simple shaft, 14 cm deep and 4.1 cm opening diameter in the first and second trials, 13.5 cm deep and 4.1 cm opening diameter in the third trial. The burrowing activity of *P. serperaster* was similar in the three trials in the laboratory.

In field observations, no evidence was found that the goby used burrows for feeding and predator avoidance as the gobies were not found moving in and out their burrows during the period of low tide observation. However, in the laboratory trials, *P. serperaster* entered burrows with head first and exited with their tail as observed in the video camera recording. No feeding activity was observed through the glass wall when the goby was hiding in the burrow.

2.5. Discussion

2.5.1. Burrowing morphology

The burrows of *P. serperaster* had a few branched with 2–4 openings, and had fewer openings and branches than those of *Odontamblyopus lacepedii* (Gonzales et al. 2008) and *Taenioides cirratus* (Itani and Uchino 2003) living in mudflats, but slightly more than those of the related species *Pseudapocryptes elongatus* inhabiting in the mudflat of Mekong Delta (Tran 2008) (Table 2.1). The burrow depth of *P. serperaster* is about half the depth of *P. elongatus* (Tran 2008) and *Odontamblyopus lacepedii* (Gonzales et al. 2008), but similar to that of *Taenioides cirratus* (Itani and

Uchino 2003) (Table 2.1). The burrow width and length of *P. serperaster* were less than that of *Odontamblyopus lacepedii* (Gonzales et al. 2008), *Taenioides cirratus* (Itani and Uchino 2003) and *P. elongatus* (Tran 2008) (Table 2.1). The total burrow length of burrows of *P. serperaster* was, however, similar to the related species *Pseudapocryptes elongatus* (Tran 2008), and much lower than that of *Odontamblyopus lacepedii* (Gonzales et al. 2008) and *Taenioides cirratus* (Itani and Uchino 2003). With regard to the burrow volume, *P. serperaster* had a smaller burrow than *Odontamblyopus lacepedii* (Gonzales et al. 2008). These comparisons for several burrow-building fish illustrate that the burrow dimensions are species specific.

Burrow openings of *P. serperaster* were usually flat and slightly circular, similar to those of *Taenioides cirratus* (Itani and Uchino 2003), *Odontamblyopus lacepedii* (Gonzales et al. 2008), and *Pseudapocryptes elongatus* (Tran 2008). The burrows of *P. serperaster* had roughly a vertical entry to the tunnels, followed by a gentle slope on the bottom with a few short cul-de-sac side branches. No "foot-prints" were observed at the burrow openings of *P. serperaster* whereas a lot of "foot-prints" surrounded the burrow openings of a neighbouring mudskipper *Boleophthalmus boddarti*. The burrows of *P. serperaster* also lacked sediment mounds on the openings, whereas there was a dead coral fragment mound on the burrow of *Valenciennea longipinnis* (Takegaki and Nakazono 2000), a sediment mound on the burrow opening in *Odontamblyopus lacepedii* (Gonzales et al. 2008), *Taenioides rubicundus* (Itani and Uchino 2003) and *Periophthalmodon schlosseri* (Ishimatsu et al. 1998). *P. serperaster* may not use a mound for storing air around the opening surface, as found in *Pseudapocryptes elongatus* (Tran 2008), whereas the mudskipper *Periophthalmodon schlosseri* use mounds to store air (Ishimatsu et al. 1998). However, it is not clear how *P. serperaster* obtain oxygen supply inside the burrows during low tide. In this study, there was no significant difference in burrow dimensions of this goby species in dry and wet seasons. It is possibly because the burrow structure of *P. serperaster* did not depend on precipitation though mud is softer in the wet season than in the dry season. In contrast, burrow dimensions of *Odontamblyopus lacepedii* in the summer and winter seasons were slightly different (Gonzales et al. 2008).

Table 2.1 Burrow morphometric measurements of *P. serperaster* and other burrowing fish species

Casts (*n*)	Openings (*n*)	Mound	Burrow depth (cm)	Burrow width (cm)	Burrow length (cm)	Total burrow length (cm)	Volume (mL)	Fish length (cm)	Species	Sites*	Sources
9	3.8±2.0	Yes	41.1±23.7	107.2±58.2	140.11±3.7	1326.1±1093.5	4971±5303	N/A	*Odontamblyopus lacepedii*	1	Gonzales et al. (2008)
2	3.5±0.7	Yes	25.0±7.1	67.5±3.54	125.0±21.21	375.0±63.6	N/A	20.0±1.4	*Taenioides cirratus*	2	Itani and Uchino (2003)
7	2.1±0.7	No	42.6±11.9	27.1±8.8	39.4±22.0	82.6±31.8	N/A	N/A	*Pseudapocryptes elongatus*	3	Tran (2008)
24	2.4±0.6	No	22.3±4.7	14.9±2.9	18.8±3.8	81.5±30.1	373±157	16.04±1.6	*Parapocryptes serperaster*	4	This study

*1: Saga Prefecture, Japan, 2: Hidaka River, Japan, 3: Bac Lieu, Vietnam, 4: Soc Trang, Vietnam

The U-shaped *P. serperaster* burrows is similar to the burrow of *Boleophthalmus boddarti* (Clayton and Wright 1989), but the W-shaped *P. serperaster* burrow is different from the *Boleophthalmus boddarti* burrow. The U- and W-shaped *P. serperaster* burrows are different from those of the related *Pseudapocryptes elongatus* (Y-shaped) (Tran 2008), *Taenioides cirratus* (Itani and Uchino 2003) and *Odontamblyopus lacepedii* (Gonzales et al. 2008). *P. serperaster* seems to effectively use the sedimentary habitat with various burrow shapes, tunnels and openings to escape from predators, as fish can use the interconnected chambers to change directions when it moves in or out of the burrow. Burrow dimensions were strongly correlated with the size of *P. serperaster* and this also occurred in the air-breathing eel goby, *Odontamblyopus lacepedii* (Gonzales et al. 2008).

2.5.2. Burrowing activities and utilisation

In the laboratory experiment, *P. serperaster* constructed burrows during the time coinciding with the occurrence of high tide in the field, whereas *Periophthalmodon schlosseri* built their shelters at low tide (Ishimatsu et al. 1998). No co-occurring macro-organism was found inside burrows of *P. serperaster*, which is similar to other burrowing fishes such as mudskipper *Boleophthalmus pectinirostris* (Chen et al. 2007), goby *Pseudapocryptes elongatus* (Tran 2008), twostripe goby *Valenciennea helsdingenii* (Clark et al. 2000), air-breathing eel goby *Odontamblyopus lacepedii* (Gonzales et al. 2008) and the goby *Taenioides cirratus* (Itani and Uchino 2003). However, the activity of *P. serperaster* is different from the goby *Cryptocentrus cryptocentrus* that work together with the pistol shrimp *Alpheus djiboutensis* (Karpulus et al. 1972). Based on the laboratory test, *P. serperaster* constructed shelters through body movement at the time coincident to high tide, as was observed on anguilliform fish (Herrel et al. 2011). However, since the configuration of burrow casts was complexity, this gobiid fish may use various combinations of body parts to dig a burrow including mouth excavation and body twisting as observed in other gobies *Periophthalmodon septemradiatus* (Bhatt et al. 2009), *Valenciennea longipinnis* (Takegaki and Nakazono 1999a), *Boleophthalmus boddarti* (Clayton and Wright 1989), and yellowhead jawfish *Opistognathus aurifrons* (Colin 1973) excavate their burrows by ejecting mud pellets or sands from mouth.

During the field observation, *P. serperaster* rarely moved out of the burrow. The field burrow of this goby species had many openings, but the burrow in the glass aquarium was a straight tunnel. Burrow casts were in various shapes and had a few side branches. However, in the laboratory observation, *P. serperaster* entered the burrow with head first, which implies that this fish may have used the burrow as a place for living and refuge to escape from predators, but may be not for foraging, which is similar to burrow utilisations in other goby species such as goby *Taenioides cirratus* (Itani and Uchino 2003) and eel goby *Odontamblyopus lacepedii* (Gonzales et al. 2008). In addition, this species could stay in the burrow for 18 hours and could adapt to the condition inside the burrow. It is likely that *P. serperaster* obtain food from outside the burrow at high tide, as we did not find any evidence that this gobiid fish feed inside the burrow.

This study provides no evidence that *P. serperaster* have used the burrow for spawning or hatching, whereas the monogamous goby *Valenciennea longipinnis* (Takegaki and Nakazono 1999b; Takegaki 2001), goby *Zosterisessor ophiocephalus* (Mazzoldi et al. 2000), *Periophthalmodon schlosseri* (Ishimatsu et al. 2009) and mudskipper *Periophthalmus modestus* (Ishimatsu et al. 2007) can use burrows for reproductive activities. However, Tran (2008) reported that another goby species *P. elongatus* can lay eggs in the burrow located offshore in Mekong Delta from June to November (wet season) with two spawning peaks in July and October in Mekong Delta. In this study, although no eggs or larvae were found in *P. serperaster* burrows, the collection of larvae and juveniles of this goby species outside the burrow may provide a hint that burrows may not be used for reproductive activities in this species in Mekong Delta.

2.6. Acknowledgments

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CHAPTER 3: GROWTH PATTERN AND CONDITION FACTOR VARIATION

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3.1. Abstract

Parapocryptes serperaster is a bottom-dwelling goby species widely distributed throughout monsoonal regions in Asia. A year-round study (2013–2014) on the length-weight relationship and body shape of this goby was conducted in the Mekong Delta, Vietnam to understand its morphometric change and growth characteristics during the dry (January–May) and wet (June–December) seasons. The sex ratio in all collected specimens was not significantly different between wet and dry seasons. The female *P. serperaster* weighed (7.2–54.5 g) more than the males (6.7–49.0 g), but female length (12.5–21.2 cm) was not different from males (12.5–23.0 cm). Length-weight relationships were similar between female and male *P. serperaster*. Male and female fish showed a similar growth pattern between wet and dry seasons and the slope of the length-weight regression was close to the standard value (b = 3) for isometric growth. The body condition factor decreased as fish grew, but the seasonal impact on the condition factor depended on gender. The condition factor of male *P. serperaster* was greater than that of females in the wet season, but not significantly different between genders in the dry season. This goby shows isometric growth and provides useful information for our understanding on gobiid morphometric variation in areas with monsoonal seasonality.

Keywords: goby, isometric growth, length-weight relationship, condition factor

3.2. Introduction

The length-weight relationship (LWR) of fishes is important to assess environmental impacts on fish growth (Froese 1998). The slope value (b) of the LWR regression can be used to predict fish growth and body condition (Froese 2006). Condition factor has been used as an indicator to compare fish wellbeing between locations, stocks (Abdoli et al. 2009a; Abdoli et al. 2009b) and to assess the effects of seasonal variation in food availability, parasitic infection and physiological fitness (Le Cren

1951). Additionally, condition factor is influenced by fish size and seasons (Froese 2006), but the annual change in condition factor in females is much more pronounced than in male fish due to different strategies of energy investment in gonadal development (Mahmood et al. 2012). The spawning time of most gobies in the tropic regions occurs in the wet season and peaks in September (Tran 2008). However, little information is available on LWR and body condition between genders in most benthic goby species, which greatly hinders our understanding on morphometric variation of small bodied fishes.

Many gobiid fish species live on mudflats (Hajisamae et al. 2006) and are an important benthic fish fauna in the Indo-Pacific region (Matics 2000; Kong and Chen 2013). *P. serperaster* (Richardson, 1846) is an elongated and round bodied goby (Khaironizam and Norma-Rashid 2000) that lives in coastal muddy areas of the Indo-Pacific and resides in burrows in estuarine mudflats (Dinh et al. 2014).

Our knowledge of *P. serperaster* is limited to its ecology (Talwar and Jhingran 1991; Dinh et al. 2014), environmental requirements (Riede 2004) and distribution (Murdy 1989; Rainboth 1996), and there is no knowledge of LWR and body shape variation of this species, especially in the Mekong Delta where dry and wet seasons are distinctive and fish growth is severely affected by heavy flood and extended periods of drought (Le et al. 2007). This study aimed to use body morphometry and condition factor as dependent variables to evaluate growth pattern under different monsoonal regimes. The results will contribute to our knowledge of growth pattern, body morphometrics and body condition in burrow dwelling fishes.

3.3. Materials and methods

3.3.1. Study site

This study was carried out in the Kinh Ba River, Cu Lao Dung district, Soc Trang Province, Mekong Delta, Vietnam (9°26'3"N, 106°13'28"E) from March 2013 to February 2014. Soc Trang Province comprises a long coastline and a large number of mudflats with semi-diurnal tide. Most of the coastline is fringed by mangroves and mudflats. There is almost no rain in the dry season (January–May) but ~400 mm monthly precipitation in the wet season (June–December). Soc Trang Province has little seasonal temperature variation (~27 °C) and represents a typical natural environment in the Mekong Delta (Soc Trang Statistical Office 2012).

3.3.2. Fish collection

Fish specimens were collected monthly with three gill nets (1.5 cm mesh in the cod end, 2.5 cm mesh in mouth and 5 m long) for a year. The nets were set behind each other with intervals of 0.5 km. Each gill net was set at the highest tide and retrieved after 2–3 h of subsequent ebb tides. After collection, specimens were immediately anesthetised using benzocaine and stored in a plastic jar with 5% formalin before transport to the laboratory. Specimens were sexually differentiated using the shape of the genital papilla, which is round in females and narrow in males (Fig. 3.1). All fish were measured to the nearest 0.1 cm in total length, and weighed to the nearest 0.01 g. Environmental parameters including surface water temperature and salinity at the study site were measured monthly using a thermometer (Model: HI98127, ±0.5ºC) and a refractometer (Model: 950.0100 PPT-ATC, ±1‰), respectively. These parameters were used to test the influence of environmental factors on sex ratios.

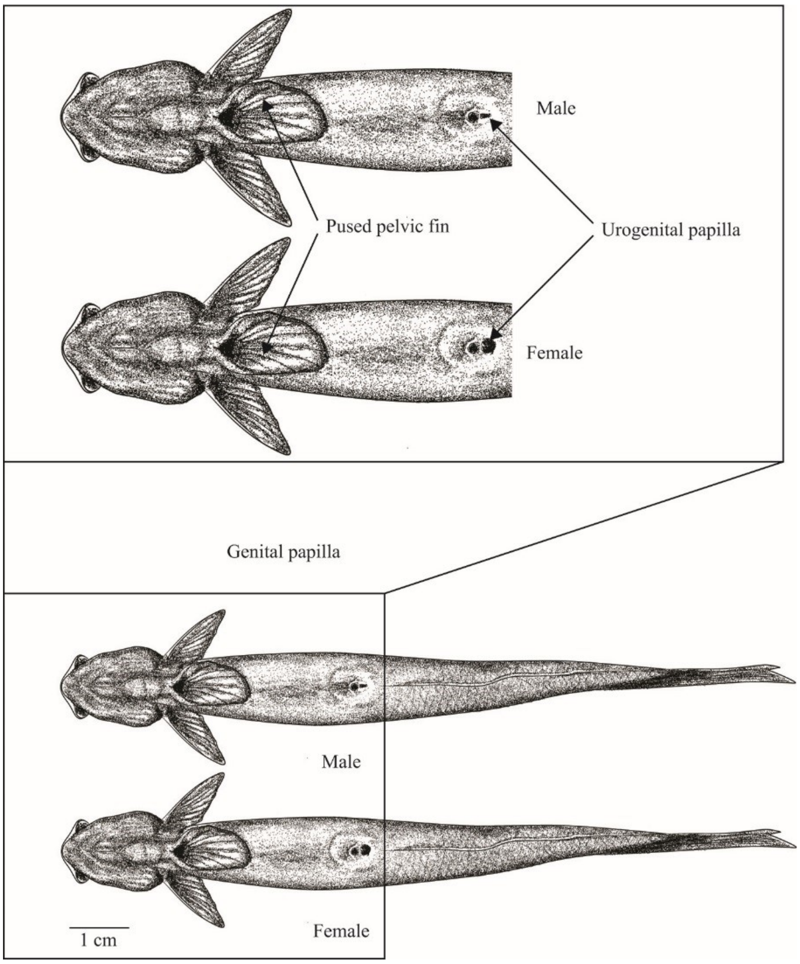

Figure 3.1 Differentiation between male and female *P. serperaster* using genital papillae

3.3.3. Data analysis

Difference in sex ratio was analysed by Chi-square test. One-way ANOVA was performed to test the differences in total length and body weight between male and female fish respectively. The LWR of male and female *P. serperaster* was quantified using the equation $W = aTL^b$ (Ricker 1973), where, W is fish weight (g); TL is total length (cm); a is the regression intercept; and b is the slope. The values of a and b were estimated using the logarithm of body weight and length, i.e., $\mathrm{Log}W = \mathrm{Log}a + b\mathrm{Log}TL$ (Froese 2006). One-way ANOVA was performed to test the difference of the regression coefficient "*b*" (e.g., regression slope) between male and female fish and between wet and dry seasons. Data associated with wet and dry seasons and genders were subject to two-way ANOVA to test possible season and gender interactions. The Student's t-test was used to test whether the *b* values obtained in the LWR equation were significantly different from the isometric value ($b = 3$) (Froese 2006).

The fish condition factor (K) was quantified using the equation $K = \frac{W}{aTL^b}$ (Le Cren 1951), where, W is fish weight (g); TL is total length (cm); a is the regression intercept and b is the slope. One-way ANOVA was performed to test for a significant difference in condition factors between male and female fish, wet and dry seasons and five size-classes (12–14, 14–16, 16–18, 18–20, and >20 cm). Fish size was divided into five classes based on the equation $\frac{\mathrm{TLmax\text{-}TLmin}}{\mathrm{Log}_{10}n}$ as described by Wand (1997), where, n is fish number; TLmax is the maximum total length; and TLmin is the minimum total length. A generalised linear model was used to test possible interactions between genders, seasons and fish sizes and the effect on fish condition factor. The Student's t-test was used to test whether the fish condition factor was equal to one. The level of significant difference for all tests was set at $p<0.05$.

3.4. Results

3.4.1. Sex ratio and morphometric measurements

A total of 409 males and 600 females were collected (Table 3.1). The male to female ratio in the total catch was 0.68:1 (0.71:1 in the dry season and 0.66:1 in the wet season), but the sex ratio was not significantly different between wet and dry seasons ($\chi^2 = 0.29$, $p>0.05$). Water temperature in the study region was not significantly different between dry (29.07±1.32 °C) and wet seasons (28.33±1.05 °C, $p>0.05$). Salinity at the study site was brackish in the dry season (8.86±3.75‰) and was significantly higher than in the wet season (2.68±2.28‰, $p<0.001$).

Table 3.1 Morphometrics and regression slopes (*b*) of fish in the study site

No.		Range of *TL* (cm) and *W* (g)				*a*		Slope (*b*)		r^2		Sampling time	
		F		M									
F	M	*TL*	*W*	*TL*	*W*	F	M	F	M	F	M	Month	Season
45	38	12.6–18.6	9.0–27.2	13.6–18.9	10.1–23.8	0.008	0.012	2.81	2.67	0.81	0.81	Mar	Dry
75	31	12.5–16.6	7.2–25.1	12.5–18.6	6.7–25.3	0.007	0.009	2.83	2.67	0.83	0.94	Apr	Dry
61	59	12.6–18.9	8.7–40.7	12.5–19.2	6.7–24.4	0.006	0.006	2.90	2.85	0.83	0.92	May	Dry
43	47	13.0–18.0	10.1–27.5	13.1–21.0	7.4–34.8	0.004	0.005	3.14	2.98	0.92	0.85	Jun	Wet
80	36	12.5–18.9	9.3–33.7	12.7–21.0	7.4–43.6	0.003	0.004	3.17	3.01	0.81	0.90	Jul	Wet
39	41	12.9–20.7	9.2–52.0	14.2–21.8	11.9–49.0	0.003	0.004	3.15	2.98	0.80	0.71	Aug	Wet
84	28	12.5–21.2	8.6–54.5	15.1–23.0	13.2–38.9	0.003	0.003	3.15	3.09	0.76	0.82	Sep	Wet
47	40	15.2–21.0	15.1–47.8	15.4–23.0	15.1–38.9	0.002	0.004	3.24	2.99	0.90	0.91	Oct	Wet
21	18	15.0–20.0	14.3–32.6	14.2–21.7	10.7–47.1	0.009	0.005	2.75	2.94	0.96	0.88	Nov	Wet
25	14	14.1–19.2	13.3–30.5	13.1–22.0	9.1–37.7	0.012	0.012	2.67	2.59	0.94	0.95	Dec	Wet
25	26	15.0–21.5	13.3–34.3	14.7–21.5	12.2–30.4	0.009	0.009	2.74	2.67	0.97	0.97	Jan	Dry
55	31	14.0–20.0	10.4–27.9	14.7–21.0	13.3–28.2	0.007	0.011	2.81	2.61	0.87	0.97	Feb	Dry

F: female, M: male

The mean length for male and female fish was 16.58±2.36 and 16.33±2.17 cm, respectively. The average body weight for male and female fish was 19.43±8.55, and 21.41±9.45 g, respectively. The TL of male and female fish was similar ($p>0.05$), but the average body weight of females was significantly higher than that of males ($p<0.01$). The TL of male and female fish was significantly different between wet and dry seasons ($p<0.001$, Fig. 3.2a), and the impact of seasonal change on fish length was dependent on gender ($p<0.01$, Fig. 3.2a). The body weight of *P. serperaster* in the wet season was significantly greater than that in the dry season ($p<0.001$, Fig. 3.2b), and females weighed more than males ($p<0.001$, Fig. 3.2b), but there was no interaction between genders and seasons with respect to fish body weight ($p>0.05$, Fig. 3.2b).

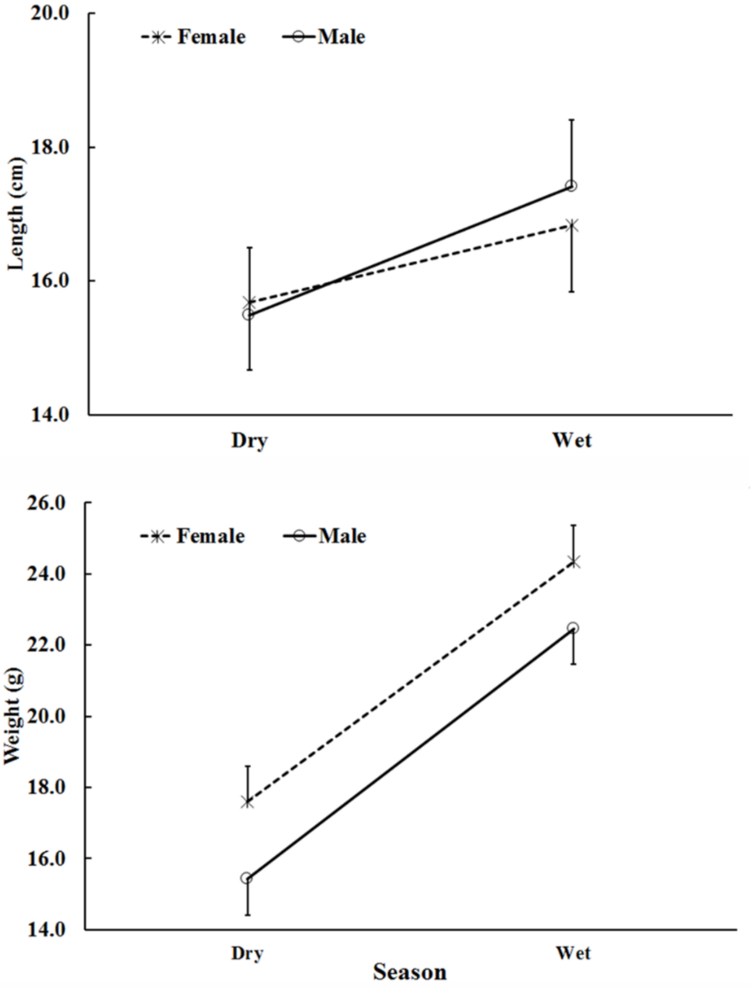

Figure 3.2 Fish total length (a) and body weight (b) of females and males in dry and wet seasons. Vertical lines represent standard error

3.4.2. Length-weight relationships

Fish weight of male and female *P. serperaster* (Table 3.1) could be predicted from fish length (Table 3.1) with high coefficients of determination (Table 3.1) in all cases ($\text{Log}W = 0.0057 + 2.874\text{Log}TL$, $n = 409$, $r^2 = 0.884$, $p<0.05$ for males, $\text{Log}W = 0.0057 + 2.921\text{Log}TL$, $n = 600$, $r^2 = 0.854$, $p<0.05$ for females). Monthly coefficient of determinations for males and females was also quantified (Table 3.1), but no significant differences were detected between months ($p>0.05$). The slope values of the linear regressions were similar in males ($b = 2.838 \pm 0.183$) and females ($b = 2.947 \pm 0.207$, $p>0.05$). Fish LWR was not significantly different between seasons ($p>0.05$), and there was no significant interaction between seasons and genders in slope values ($p>0.05$, Fig. 3.3a).

In the LWR analysis, the regression slope values (Table 3.1) for males were significantly lower than the isometric value (e.g. the cubic value of three) based on one-sample t-test ($p<0.05$ for males), whereas the slope values for females were not significantly different from the cubic value of three (*p*>0.05). Overall, the regression slope for this goby was 2.79±0.359, and was not significantly different from the isometric value (*p*>0.05). The regression slope values of LWRs showed isometric growth in *P. serperaster*, but the slope values were not significantly different between wet and dry seasons (*p*>0.05).

3.4.3. The condition factor (*K*)

Both male and female fish had a *K* value of 0.99 on average, but the condition factor was higher in males (1.02) than in females (0.96) (*p*<0.05). The male condition factor in the wet season was significantly higher than that in the dry season, whereas the female condition factor in the wet season was significantly lower than that in the dry season (*p*<0.05, Fig. 3.3b). However, the condition factor between male and female fish was not significantly different in the dry season (*p*>0.05, Fig. 3.3b). The impact of fish gender on the *K* value was not dependent on fish size (*p*>0.05).

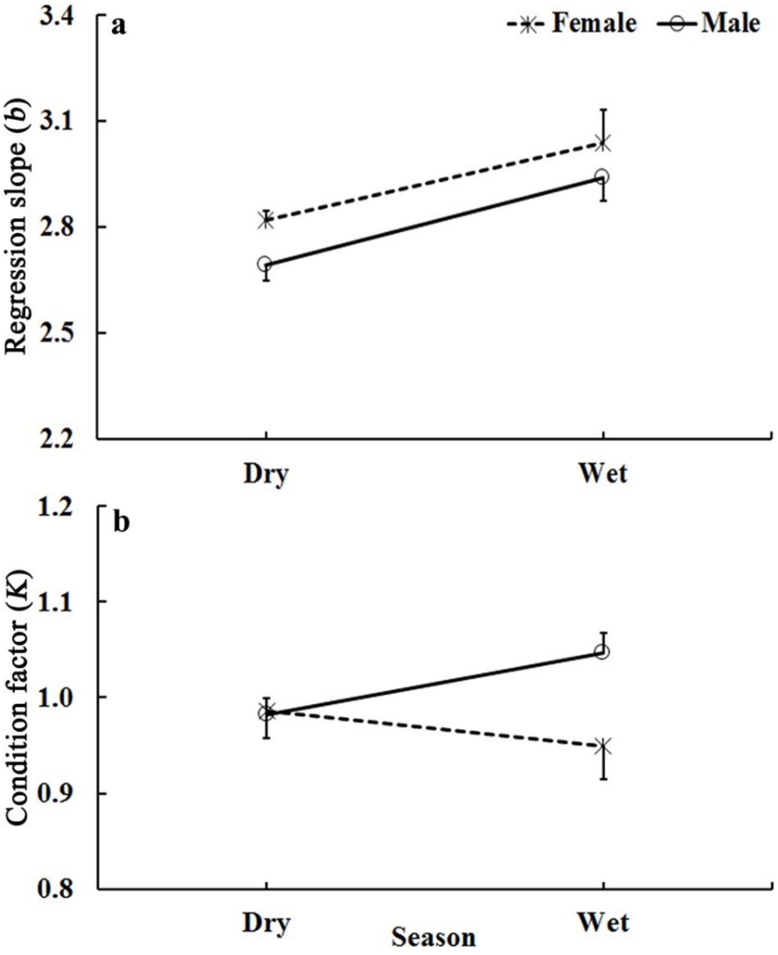

Figure 3.3 Regression slope value (a) and condition factor (b) of females and males in dry and wet seasons. Vertical lines represent standard error

The *K* value of females in size class 5 (i.e., mature fish) was significantly greater than that of males (Fig. 3.4). The condition factor of *P. serperaster* was significantly different between five size-classes (*p*<0.001), and the smallest size-class had the largest *K* value (Fig. 3.4). Among the five size classes, the condition factor of the males was significantly higher than that of females (*p*<0.05). Overall, the *K* value of *P. serperaster* was slightly lower during the main spawning period (August–September), but it was not significantly different between months (*p*>0.05, Fig. 3.5), and was close to one (*p*>0.05).

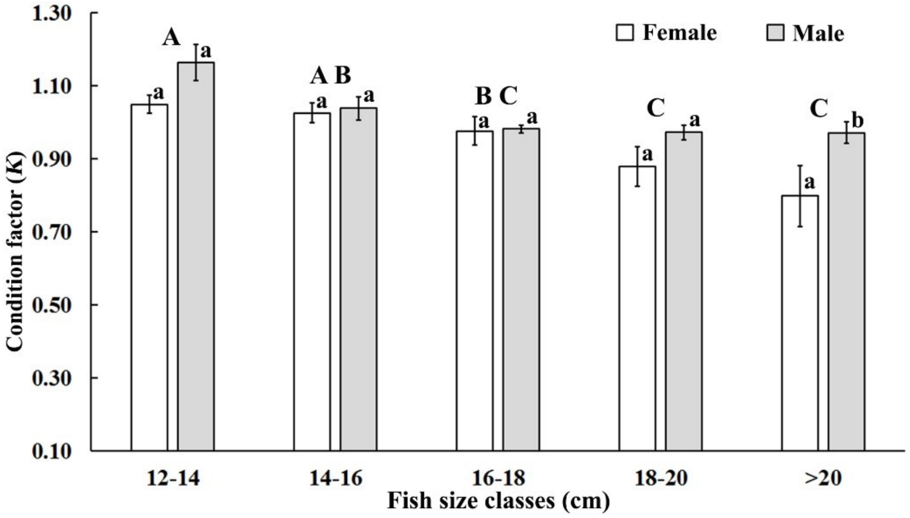

Figure 3.4 Condition factors of male and female fish in five size-classes. Different lowercase letters show significant differences between genders in each fish size, and different capital letters show significant differences between fish size-classes. Vertical lines represent standard error

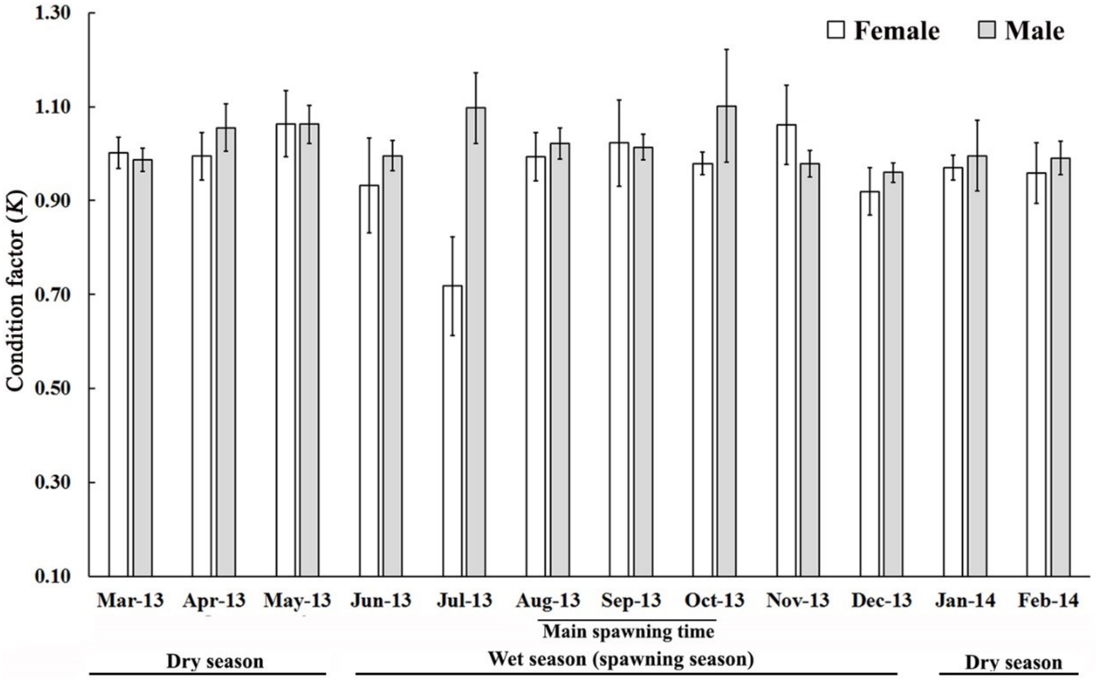

Figure 3.5 Monthly variation of condition factors across wet and dry seasons. Vertical lines represent standard error

3.5. Discussion

3.5.1. Sex ratio and morphometric traits

In some fishes, temperature affects sex determination or creates sex-specific selection pressures. For example, in Lake Thun, Switzerland, a monitoring program showed that the sex ratio of a population of grayling *Thymallus thymallus* changed from approximately 65% males before 1993 to approximately 85% males in 2011, along with a gradual temperature elevation over the last few decades (Wedekind et al. 2013). Furthermore, the sex ratio of the sand goby *Pomatoschistus minutus*, 33 *Apistogramma* species and *Oreochromis niloticus* are strongly regulated by temperature variation (Kvarnemo 1996; Abucay et al. 1999; Baroiller and D'Cotta 2001). However, the male to female sex ratio in this study is not significantly different between dry and wet seasons, suggesting that seasonal variation in temperature does not significantly regulate the relative abundance of male and female populations of this goby. Likewise, Tran (2008) collected 479 females and 461 males of another goby species *Pseudapocryptes elongatus,* from the Mekong Delta during January 2004 to June 2005 and the sex ratio was nearly 1:1. The temperature variation in the study region is minor (26.5–30 ºC) between seasons, which may explain the similar sex ratios of gobies between seasons in tropical areas.

The body lengths of males and females were not significantly different in the same age class, but females weighed more than males of a similar length. Similarly, body lengths of *Pseudapocryptes elongatus* (Tran 2008), *Periophthalmus barbarus* (Chukwu and Deekae 2011) and *Parachaeturichthys ocellatus* (Panicker et al. 2013) are also not significantly different between genders. Therefore, sexual dimorphism in gobies appears to be not significant, suggesting that male or female gobies can reach a marketable size at the same age.

3.5.2. Length-weight relationships

A positive relationship between length and weight was found in juveniles and mature, male and female gobies, indicating that fish weight can be estimated from fish length regardless of fish developmental stage. Positive relationships between length and weight were also found in the goby *P. elongatus* (Tran 2008), the Atlantic mudskipper *Periophthalmus barbarus* (Chukwu and Deekae 2011) and the goby *Parachaeturichthys ocellatus* (Panicker et al. 2013). However, in this study, the

LWR coefficient value (*b*) was influenced by fish age as mature male and female fish showed significantly higher *b* than that of juvenile fish, suggesting that gonadal development can lead to a high value of *b* in mature fish.

The slope value of the length-weight regression in females was slightly higher than that of males, implying that the size of the gonads in females during the spawning season may affect the LWR. Kalaycı et al. (2007) also found that gonadal development leads to a difference in the *b* value in both genders of *Gobius niger* in Turkey, but not in the mudskippers *Periophthalmus barbarus* (King and Udo 1998; Chukwu and Deekae 2011) and *Parachaeturichthys ocellatus* (Panicker et al. 2013). In the present study, the regression coefficients of *P. serperaster* were not significantly different between seasons. This could be due to similar food availability as environmental variables such as temperature between wet and dry seasons in this region are alike (Tran 2008). Similarly, the growth pattern of *Ilisha melastoma* in Pakistan is also not affected by season, including pre-monsoon (January–April), monsoon (May–September) and post-monsoon (November–December) (Mahmood et al. 2012). These authors reported that precipitation in the monsoon season was higher than in other seasons, which is similar to the wet season with heavy rain in the Mekong Delta region in Vietnam. In the present study, the impacts of season and gender on the change of the regression coefficient of *P. serperaster* were independent of each other. Furthermore, the *b* value of *Periophthalmus barbarus* caught in Nigeria, where the tropical monsoon is a feature of the local climate, is also not affected by the interaction of season and gender (King and Udo 1998). Clearly, both male and female gobies have similar growth trajectories over time in wet and dry seasons, suggesting that *P. serperaster* can successfully adapt to climate change in this area.

The growth pattern of *P. serperaster* was isometric because the *b* value in the regression equation is not significantly different from the cubic value of 3, indicating that the growth pattern of *P. serperaster* falls into the "well-being" category as defined by Froese and Binohlan (2000). Isometric growth usually occurs in fish when environmental conditions and food supply meet the fish requirements. These conditions can be simulated in captivity to achieve fast fish growth. Our results suggest that the environmental conditions that *P. serperaster* inhabit are suitable for fish feeding and growth. In favourable conditions, isometric growth has been found in *Sardinella sindensis*, *Liza carinata*, *Alepes kleinii* and *Alepes melanoptera* collected from the Manora Channel and backwaters in the northern Arabian Sea (Khatoon et al. 2013), *Barbatula barbatula* in the Iberian Peninsula (Oscoz et al. 2005), and mudskipper *Periophthalmus barbarus* in Nigeria (King and Udo 1998). Mahmood et al. (2012) reported that *Ilisha melastoma* showed negative allometric growth ($b < 3$), whereas *Periophthalmus argentilineatus* and *Periophthalmus spilotus* showed positive allometric growth ($b > 3$) (Khaironizam and Norma-Rashid 2002), resulting in a slim body shape in *I. melastoma* and fat body shape in *P. argentilineatus* and *P. spilotus*. The growth pattern is species-specific, but environmental conditions can also regulate growth outcomes. For example, the *b* value of *Gobius niger* varies in different habitats from 2.81 in Black Sea, 2.89 in Egypt, to 3.85 in Mediterranean (Kalaycı et al. 2007). In addition, the *b* value of *Gobio gobio* is also different from the *b* value among Iberian Peninsula (3.33), Thames River (3.05), and Rokytna River (3.55) (Oscoz et al. 2005).

3.5.3. Condition factor (*K*)

Condition factor can be affected by fish developmental stage and it is usually high in females towards the end of gonadal maturation. In this study, the condition factor was slightly lower in females than in males during the wet season (spawning season), which is similar to the report on *Ilisha melastoma* (Mahmood et al. 2012), but not the mudskipper *Periophthalmus barbarus* (King and Udo 1998; Chukwu and Deekae 2011). The low *K* value may be due to the release of mature eggs after spawning in the wet season. The variation in the condition factor of *P. serperaster* was significantly dependent on fish size-classes, and smaller fish had a higher *K* value than larger fish before the age of maturation or after spawning. It seems that the energy spent on gonadal development of larger fish contributes to the low *K* compared to juveniles.

The *K* value of *P. serperaster* in this study was close to one, which is above the average condition of other fishes in the same habitat such as *Pseudapocryptes elongatus* (Tran 2008). Mahmood et al. (2012) also reported that *Ilisha melastoma* lived in a favourable environmental condition as the condition factor of this fish is close to the wellbeing threshold value of one. The change in *K* value of *P. serperaster* was not significantly different between wet and dry seasons, suggesting that the nutritional condition in this study is favourable for the growth of this species. Like *P. serperaster*, the *K* values of *Ilisha melastoma* (Mahmood et al. 2012) and

Periophthalmus barbarus (King and Udo 1998; Chukwu and Deekae 2011) are also not significantly different between seasons. However, the *K* value variations in *Ilisha melastoma* (Mahmood et al. 2012) and *Periophthalmus barbarus* (King and Udo 1998; Chukwu and Deekae 2011) are associated with seasonal change. The change of condition factor between male and female *P. serperaster* depended on the season, whereas the impact of gender on the condition factor of *Ilisha melastoma* (Mahmood et al. 2012) and *Periophthalmus barbarus* (King and Udo 1998; Chukwu and Deekae 2011) does not. The interactive effect of gender and season on the variation of *K* values of *P. serperaster* indicates that *K* values of male and female *P. serperaster* changed significantly in spawning season (wet season), but these values were similar during the dry season. The impact of season on the change of *K* value may be attributed to the difference in resource availability between seasons.

In conclusion, the sex ratio was not significantly different between dry and wet seasons, but the length and weight of this goby varied between genders and seasons. The value of the regression slope of *P. serperaster* was near 3, suggesting isometric growth in this species. The value of the slope was significantly different between male and female fish, but not between wet and dry seasons. This goby lives in a condition suitable for growth as the *K* value was close to one, falling into the wellbeing category. The *K* value of this goby was different between genders and fish sizes, but not between seasons, suggesting that this goby has adapted to the variation of precipitation between dry and wet seasons.

3.6. Acknowledgments

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CHAPTER 4: FOOD AND FEEDING HABIT

This chapter has been submitted to Ichthyological Research as:
Dinh QM, Qin JG, Dittmann S, Tran DD (reconsider acceptance after revision) Seasonal variation of food and feeding in burrowing goby *Parapocryptes serperaster* (Gobiidae) at different body size (manuscript number: ICRE-D-15-00136).

4.1. Abstract

Diet composition and feeding habits of the burrowing fish *Parapocryptes serperaster* were investigated on different fish sizes across dry and wet seasons in the Mekong Delta, Vietnam. The gut length was positively related to fish length and the relative gut length to total length was 1.57±0.30, which is in the range for omnivore (1–3), consuming detritus, algae and copepods. The diet composition showed seasonal and intraspecific variations in all fish size groups. The diet breadth varied with fish size group and the dry-wet season pattern, and smaller fish had a wider diet breadth than larger fish. The index of relative diet importance and Costello graphic analysis indicate that this goby is a generalist feeder and feeds mainly on detritus, followed by diatoms. The goby could feed on the bottom and at the water column. The feeding intensity of *P. serperaster* was higher in the wet season than in the dry season, but was not significantly affected by fish size. *P. serperaster* fed on *Navicula* spp. in the wet season, but on *Nitzschia* spp. in the dry season. The understanding of food and feeding habits of *P. serperaster* contributes to our knowledge of feeding adaptation of small-bodied bottom dwelling gobies to the mudflat habitats in tropical monsoonal regions.

Keywords: trophic guild, dietary composition, feeding habit, omnivore

4.2. Introduction

Knowledge of food and feeding is fundamentally important to understand fish biology and trophic interactions between species in a fish community (Brodeur and Pearcy 1992; Wootton 1996; Blaber 2000). Fish stomach contents vary with the time of day, e.g., the round goby *Neogobius melanostomus* mainly feed on chironomid and hydropsychid larvae during the day, but on thironomid pupae and heptageniid nymphs at night (Carman et al. 2006), and the Japanese seabass *Lateolabrax japonicus*, a visual feeder, mainly ingests copepods during daytime, but ceases feeding at night (Hibino et al. 2006). Additionally, seasonal change can influence fish stomach contents, e.g., *Schilbe intermedius* feed on insects and *Oreochromis mossambicusthe* feed on algae in a wet season, whereas in a dry season *S. intermedius* predominantly feed on fish and *O. mossambicus* feed on detritus (Teferra et al. 2003), and the food composition of *Pseudorhombus jenynsii* is strongly influenced by season as this species mainly ingest crabs and polychaetes in the autumn but mysids in the summer. Moreover, fish stomach contents also vary with fish size, e.g., the juvenile *Sillago bassensis* feed on calanoid copepods and cladocerans whereas the adults feed on polychaetes and carid shrimp, *Sillago vittata* juveniles feed calanoid copepods but adults feed on small fish (Schafer et al. 2002), and Chironomid larvae are found in the stomach of small *Economidichthys pygmaeus*, while the larger fish mainly feed on copepods (Gkenas et al. 2012). The discrepancy of food composition is attributable to the foraging behaviour of fish and the prey availability in the environment. Thus, study of variation of food types of fish at different season and age is critically important to improve our understanding of fish adaptation to the changing environment and habitat.

Stomach contents reflect habitat separation in fish as the analysis of stomach content can reveal the habitat where fish feed (Gumus et al. 2002). Although diet composition of fish is species-specific, it varies with food availability in the environment. For instance, *Aphia minuta* feed on copepods, cirripede and mysid larvae in the north-eastern Atlantic and the Mediterranean, but mainly feed on copepods in the Black Sea and the north-western Adriatic Sea (Tirelli et al. 2014). When the habitat changed from an artificially made habitat to the natural habitat in the western basin of Lake Erie, the diet of the round goby *Neogobius melanostomus* switched from grain particles to copepods (Thompson and Simon 2014). In the shoreline of Hron River (Slovakia), the monkey goby *Neogobius fluviatilis* mainly feed on chironomids, but the diet is mainly composed of crustacean *Corophium curvispinum* in the shoreline of the Danube River (Slovakia). Likewise, the round goby *N. melanostomaus* mainly feed on molluscs and crustaceans in the Danube River (Yugoslavia), whereas the fish ingest a wide range of benthic macroinvertebrates in the St. Clair River (Michigan, USA) (Adámek et al. 2007).

The guild of fish feeding habit is generally classified into herbivore, omnivore and carnivore, and the morphology and function of the alimentary tract vary between fish species of different feeding habits (Geevarghese 1983). However, the feeding habit can be altered by the change of food availability and habitat structure. Most gobiids are considered omnivorous and feed on benthic algae and detritus. For example, *Oxyurichthys tentacularis*, *O. microlepis*, *Stenogobius gymnopomus* and *Oligolepis acutipennis* mainly feed on benthic diatoms and detritus (Geevarghese 1983), but both *Psuedapocryptes elongatus* (Tran 2008) and *Boleophthalmus boddarti* (Ravi 2013) mainly ingest diatoms. The coastal areas in tropical Southeast Asia are important nursery grounds for numerous fishes including gobies (Blaber 2000; Hajisamae and Chou 2003; Tue et al. 2012). Onxudercine gobies are typically found on mudflats in creeks, estuaries and coastal waters at low tide (Murdy 1989; Takita et al. 1999; Hajisamae et al. 2006), including the Mekong Delta (Tran et al. 2013). The change of feeding habit and food partitioning among fish species can significantly affect the abundance and community structure of food organisms (Garrison and Link 2000). However, food and feeding habits of gobiid species are poorly understood due to their small size, cryptic behaviour and low economic value.

P. serperaster (Richardson, 1846; Gobiidae) is widely distributed in the Indo-Pacific region including southern China, India, Malaysia, Myanmar, Singapore, Thailand and Indonesia (Murdy 1989; Talwar and Jhingran 1991; Kottelat et al. 1993; Khaironizam and Norma-Rashid 2000), and is common in the estuarine region of the Mekong Delta, Vietnam (Tran et al. 2013). This species is an elongated and round bodied fish (Murdy 1989; Rainboth 1996), and builds burrows by body twisting movement in the coastal and muddy areas to escape from predation (Dinh et al. 2014). In the riverine areas of Sungai Sembilang, Jeram, Malaysia, where the substrates are very soft and muddy, *P. serperaster* mainly feed on diatoms (Khaironizam and Norma-Rashid (2000). However, it is not clear if the change of food and feeding habit would depend on season and fish size in the field. An understanding of the diet breadth and feeding intensity is important for the management and conservation of aquatic ecosystems. This study aims to understand the variation of food and feeding habit of *P. serperaster* with season and fish size as measured by diet breadth and feeding intensity. The results of this study will provide an insight into diet flexibility and adaptation of small-bodied fish under the change of environmental conditions.

4.3. Materials and methods

4.3.1. Study site

This study was conducted in the Kinh Ba River, Soc Trang Province, Mekong Delta, Vietnam (9°26'3"N, 106°13'28"E) from April 2014 to March 2015. The distance from the riverbank to the riverbed of the mudflat was nearly 3 m at the lowest tide. Tides are semi-diurnal with a spring tidal range of ~0.7 m. Soc Trang Province comprises a long coastline connected to mangroves and mudflat habitats. The monthly precipitation in the wet season (June–December) is roughly 400 mm, but there is almost no rain in the dry season (January–May). The average annual temperature is ~27 °C, and Soc Trang is a typical region for the natural environment in the Mekong Delta (Soc Trang Statistical Office 2012).

4.3.2. Fish collection

Fish specimens were collected monthly over a year using three sets of gill net with a distance of 0.5 km apart. The gill net had 1.5 cm mesh in the cod end, 2.5 cm mesh in the mouth and were 5.0 m long. These gill nets were set at the highest tide and retrieved after 2–3 h during ebb tide. After separating fish sexes using the feature of the genital papilla, specimens were immediately anesthetised using benzocaine and preserved in 5% formalin before transport to the laboratory.

4.3.3. Feeding habit and intensity determination

After measurement of total length (nearest 0.1 cm) and body weight (nearest 0.01 g), fish specimens were dissected to measure the length of the gastrointestinal tract (nearest 0.1 cm) to calculate the relative gut length (RGL) as the ratio of the gut length to the total length. This ratio was used to determine fish feeding habit, e.g., herbivores ($RGL > 3$), carnivores ($RGL < 1$) or omnivores ($RGL = 1–3$) (Al-Hussaini 1947; Nikolsky 1963; Geevarghese 1983).

Alimentary tracts were weighed to the nearest 0.01 mg to determine the gut fullness index (FI), which is calculated as $FI = W_i \times 10000/BW$, where, W_i is the weight of stomach and BW is fish body weight, as an index for fish feeding intensity (Watanabe et al. 2004). This index was used to test if fish feeding intensity changes between seasons and fish size classes based on the method of Bakhoum and Fatas (2003).

4.3.4. Diet composition and index of relative importance

Stomach contents of each fish were diluted in 1 L distilled water, and three sub-samples (1 mL/each) were examined in a Sedgewick-Rafter chamber on an inverted microscope connected with a digital camera (Motitc Digital Microscope, Model: DM1802) to count food items. Moreover, the food items in the Sedgewick-Rafter chamber were also photographed for counting and measurement of each food item using Image Pro Plus software v.2. Diet composition was quantified by the prey occurrence in fish stomach ($\%O_i = \frac{O_i}{N} \times 100$), and the prey number ($\%N_i = \frac{N_i}{N_{total}} \times 100$), where, O_i is the number of fish consuming prey *i*; *N* is the total number of fish examined, excluding individuals with an empty stomach; N_i is the number of prey *i*; and N_{total} is the total number of prey individuals (Hynes 1950; Hyslop 1980; Baeck et al. 2013). For quantification of meal size, the biovolumetric percentage ($\%V_i = \frac{V_i}{V_{total}} \times 100$) was used to determine the diet volume in the fish stomach, where, V_i is the biovolume of prey *i*; and V_{total} is total biovolume of prey individuals. The biovolume of prey *i* (V_i) was obtained by multiplying the standard biovolume of prey *i* with the number of prey *i* in each stomach. The 30 random photos per sub-sample (90 photos per stomach content) were used to measure the area (e.g., biovolume) of each prey (e.g., prey *i*) using Image Pro Plus before obtaining the standard biovolume of prey *i*. The smallest prey item was ascribed one point and was used to calculate the points for the larger food item. This method was modified from the volumetric method of Hynes (1950) and Hyslop (1980) and the biovolumetric method of Vitule et al. (2008) and Alcaraz et al. (2015).

The index of relative importance (*IRI*) of a diet was used to determine the most important food item in the stomach. The percentage of the index of relative importance was calculated as $\%IRI = \frac{(\%N_i + \%\mathrm{V}_i) \times \%O_i}{\sum_i^n (\%N_i + \%\mathrm{V}_i) \times \%O_i} \times 100$, where, the index of relative importance (*IRI*) of *i* prey type is calculated based on the percentage of numerical abundance (N_i), frequency of occurrence (O_i) and biovolume (V_i) of *i* prey relative to total prey items from prey *i* to *n* in the stomach (Baeck et al. 2013).

Values greater than 250 were used to indicate significant food items that were important in the diet (Pinkas 1971; Gumus et al. 2002).

4.3.5. Diet breadth and feeding strategy

The dietary breadth of this goby was estimated using the Shannon-Wiener index as $H' = -\sum_{i=1}^{n} p_i \mathrm{Log} p_i$, where p_i is the percentage of i prey item among the total number of prey (Vitule et al. 2013). The Costello (1990) graphical method modified by Amundsen et al. (1996) was used to plot the percentage of number, biovolume vs. frequency occurrence of food items to determine diet specialisation (e.g., feeding strategy) and prey importance. In this graph, the most important prey items are closer to the top right corner and the prey items with low occurrence but important by number are closer to the top left corner of the graph (Adámek et al. 2007).

To obtain relative abundance and food items available in the field, samples were taken from the river. For the analyses of phytoplankton, zooplankton and detritus, 100 L water was concentrated through a ~50 μm mesh to obtain 1 L water sample. Phytoplankton, zooplankton and detritus in the water was collected at the same site and the same period of fish collection from October 2014 to March 2015 to determine food availability and the overlap of food prey in the fish stomach and the environment (Sabatés and Saiz 2000; Pratchett 2005). After prey identification, the biovolume of food items in the environment was estimated using the same method as in the fish stomach. Thereafter, the biovolume of food items was used to estimate the diet electivity (E) using the equation $E = (r_i - p_i)/(r_i + p_i)$, where, r_i and p_i are the proportion of each food category in the fish stomach and environment respectively, and E ranges from –1 (e.g., strong negative election) through 0 (e.g., random election) to 1 (e.g., strong positive election) (Strauss 1979; Lückstädt and Reiti 2002; Gkenas et al. 2012). When the value of E index exceeds 0.6 for a diet item, this index is considered biologically significant for food selection (Wallace 1981; Gkenas et al. 2012).

4.3.6. Data analysis

The influences of two seasons (dry and wet) and three fish size classes (TL <16, 16–18, and >18 cm) on the variations of the index of relative importance, diet breadth and feeding intensity were analysed using two-way ANOVA (Alcaraz et al.

2015). If there was no interaction between season and fish size, one-way ANOVA was used to test the fish size effect, and t-test was used to test the season effect. One-way ANOVA was also used to test the effect of fish size on the relative gut length and monthly variation of feeding intensity. The χ^2 test was used to test if the proportion of empty stomachs differs between wet and dry seasons (SPSS v.21). If the ANOVA assumptions of normality and homogeneity of variance were not met, PERMANOVA was used to examine the influences of season and fish size on the variations of diet breadth and feeding intensity. The biovolume of all food items was analysed using PRIMER (v.6.1.11) (Clarke and Gorley 2006) with PERMANOVA+ v.1.0.1 add-on package (Anderson et al. 2008) to compare food composition between season and fish size (Baeck et al. 2013). If the food composition was significantly different between season or fish size, Mann-Whitney U-tests or Kruskal-Wallis tests were used to examine which prey contributed to the seasonal difference or intraspecific change. The significance level in all tests was set at $p<0.05$.

4.4. Results

4.4.1. Relative gut length and gut fullness

The analysis of feeding habit was based on a total of 240 fish (116 female TL = 9–21 cm and 124 male TL = 8–24 cm, Table 4.1). The relative gut length (*RGL*) of *P. serperaster* was 1.57±0.30 (mean±SD, $n = 240$). The proportion of fish with empty stomach was not significantly different between dry and wet seasons (χ^2, $p>0.05$, Table 4.1). Similarly, the number of full and moderately full stomachs in the dry season was not significantly different from that in the wet season (χ^2, $p>0.05$, Table 4.1). Although the *RGLs* varied significantly with fish size (ANOVA, $p<0.05$, Fig. 4.1), the *RGLs* of *P. serperaster* generally fell into the omnivorous feeding category, i.e., *RGL* = 1–3. The length of gut was positively related to the total body length ($r = 0.644$, $p<0.001$).

The gut fullness index (*FI*) varied significantly between months (ANOVA, $p<0.001$), and steadily increased from dry season to the pre-spawning period in July before falling to the lowest point during the main spawning period in September (Dinh et al. 2015) (Fig. 4.2), coinciding with a high percentage of empty stomachs (55%) in September (Table 4.1). After post-spawning recovery in October, the *FI* was slightly decreased from the late wet season to the dry season (Fig. 4.2). The *FI* in the wet season was significantly higher than in the dry season (t-test, $p<0.05$, Fig. 4.3a), whereas this index did not differ significantly between fish sizes (ANOVA, $p>0.05$, Fig. 4.3b). The seasonal change of *FI* of *P. serperaster* did not depend on fish size either (ANOVA, $p>0.05$).

Table 4.1 Gut fullness of *P. serperaster* in different months and seasons

Sampling time	Fish (No.)		Gut fullness (%)		
	Female	Male	Full	Moderately full	Empty
Apr-14	11	9	10	65	25
May-14	8	12	25	50	25
Jun-14	12	8	25	60	15
Jul-14	9	11	75	25	0
Aug-14	8	12	35	55	10
Sep-14	11	9	15	30	55
Oct-14	11	9	100	0	0
Nov-14	7	13	75	25	0
Dec-14	9	11	90	10	0
Jan-15	9	11	75	15	10
Feb-15	12	8	35	35	30
Mar-15	9	11	70	10	20
Wet season	67	73	59	29	12
Dry season	49	51	43	35	22

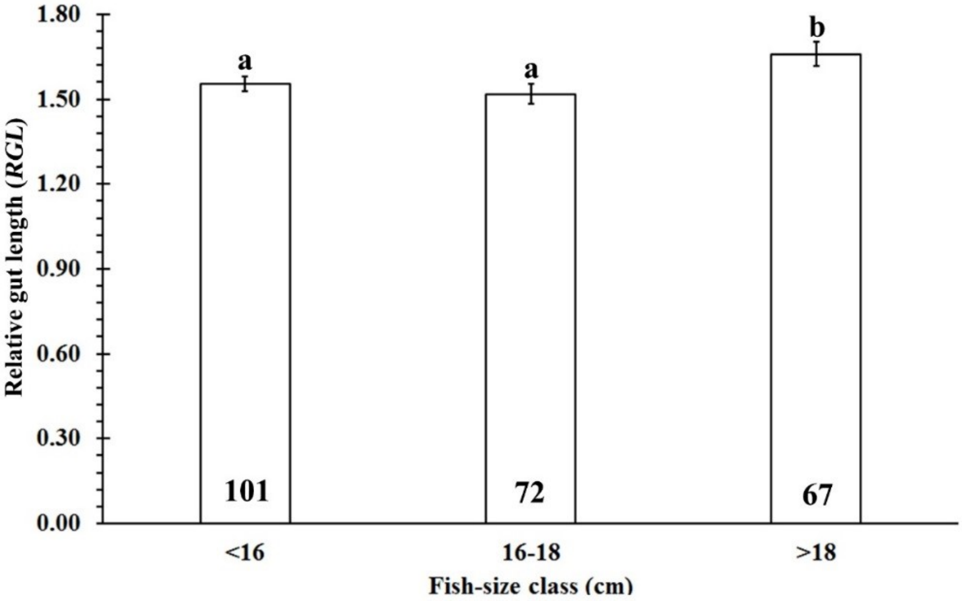

Figure 4.1 The variation of the relative gut length between three fish size groups (group 1: TL <16, group 2: TL = 16–18 and group 3: TL >20 cm). Different letters represent significant differences in *RGL* (mean±SE, $p<0.05$). The number of fish in each size group is given in each column

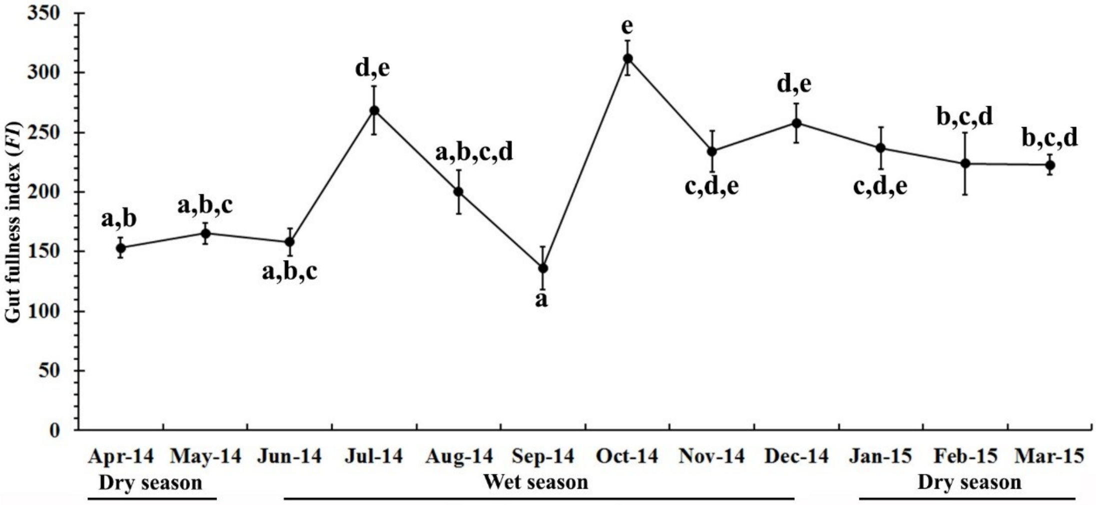

Figure 4.2 The monthly variation of the gut fullness index (*FI*) of *P. serperaster*. Different letters show the significant differences between months (n = 202, p<0.05)

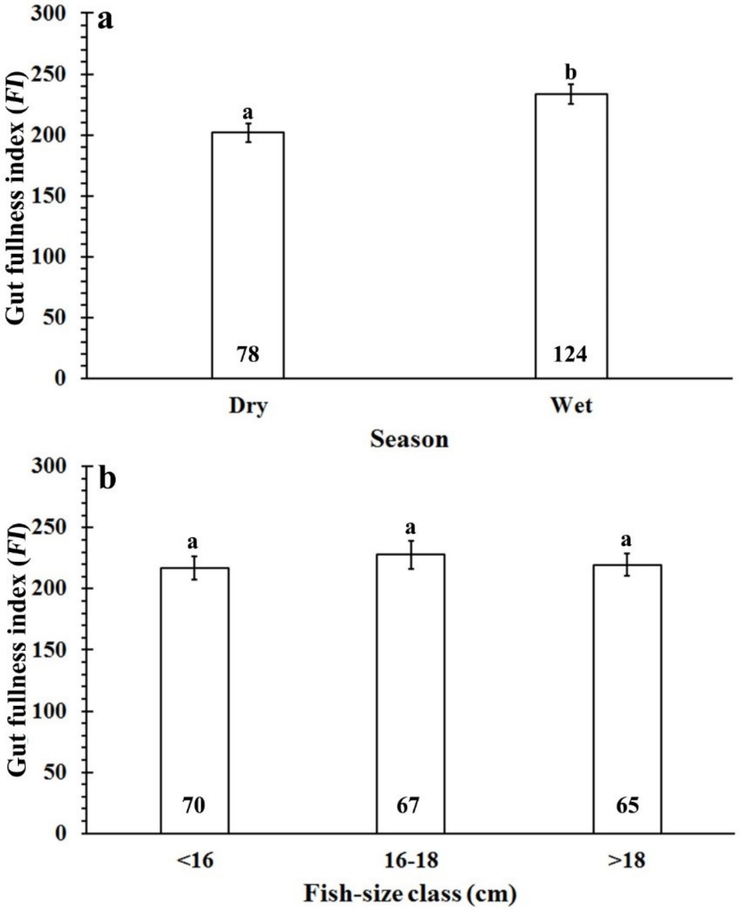

Figure 4.3 The variation of the gut fullness index (*FI*) of *P. serperaster* between seasons (a) and fish size groups (b). Different letters indicate significant differences (mean±SE, p<0.05). The number of fish used for analysis is given in each column

4.4.2. General diet composition

A total of 202 individuals (102 females with 12.8–21.0 cm in TL and 100 males with 9.7–23.5 cm in TL) were used to examine diet composition and fish with an empty stomach were excluded (Table 4.1). Fourteen food items were detected from fish stomachs (Table 4.2). The analysis on prey number showed that *P. serperaster* mainly fed on detritus due to the highest percentage of this item in fish stomachs, followed by Bacillariophyta, whereas the biovolume analysis showed that both detritus and Bacillariophyta contributed similarly to the fish stomach content. This fish also fed on Cyanophyta and copepods (Table 4.2). The changes of the index of relative importance (*IRI*) of 14 food items were recorded in Table 4.2. Detritus (*IRI* ≈ 64%) was the most important food, *Coscinodiscus* spp., *Thalassiosira* sp. and *Pleurosigma* sp. (Bacillariophyta), *Oscillatoria* sp., *Spirulina* sp. and *Anabaena* spp. (Cyanophyta), *Acartia* sp., *Calanus* sp. and *Paracalanus* sp. (copepods) contributed little to the diet of this goby (value of *IRI* <250 or percentage of *IRI* <2%). Based on *IRI* analysis, detritus was the primarily consumed item, and *Nitzschia* spp., *Navicula* spp. and *Gyrosigma* sp. (Bacillariophyta) were secondarily important, whereas the other food items were rarely consumed.

4.4.3. Seasonal and intraspecific variations in diet composition

The food composition of *P. serperaster* was significantly different between seasons (PERMANOVA, $p<0.001$) and fish size ($p<0.001$). The difference of diet composition between the dry and the wet seasons was found in most prey items (Mann-Whitney U, $p<0.05$), except for detritus, *Thalassiosira* sp., *Biddulphia* spp., *Pleurosigma* sp., *Oscillatoria* sp., *Acartia* sp. and *Calanus* sp. (Mann-Whitney U, $p>0.05$, Table 4.2). The food items contributing most to the difference of diet composition between fish sizes were detritus, *Nitzschia* spp., *Oscillatoria* sp., *Spirulina* sp., *Anabaena* spp. and *Canalus* sp. (Kruskal-Wallis, $p<0.05$, Table 4.3). The PERMANOVA analysis of four major food categories showed that the diet composition differed significantly between the dry and wet seasons ($p = 0.004$) and between the three fish size groups ($p<0.001$). Cyanophyta species contributed most to the difference between seasons (Mann-Whitney U, $p<0.05$), while both detritus and Cyanophyta contributed to the diet differences between three fish sizes (Kruskal-Wallis, $p<0.05$, Table 4.4). The amount of detritus in the wet season (51%) was not different from that in the dry season (50%, Mann-Whitney U, $p>0.05$, Table 4.4). The diet of larger *P. serperaster* was predominantly composed of detritus compared to the smallest fish (Kruskal-Wallis, $p<0.05$, Table 4.4), whereas the reverse was the case for Cyanophyta ($p<0.05$, Table 4.4) based on the biovolume data.

Table 4.2 Percentage of the frequency of occurrence, number, biovolume and relative important index (*IRI*) of 14 food items of *P. serperaster* in the dry and wet seasons

Food	Occurrence		Number (Mean±SE)		Biovolume (Mean±SE)		*IRI* (Mean±SE)	
	Dry	Wet	Dry	Wet	Dry	Wet	Dry	Wet
Detritus	100	100	72.74±1.14^a	74.25±0.59^a	50.25±1.51^a	51.23±0.69^a	62.58±1.31^a	63.92±0.63^a
Bacillariophyta								
Coscinodiscus spp.	87.18	91.13	0.87±0.14^a	0.34±0.03^b	1.02±0.14^a	0.42±0.04^b	0.87±0.13^a	0.35±0.03^b
Thalassiosira sp.	71.79	85.48	0.52±0.07^a	0.53±0.05^a	0.66±0.08^a	0.70±0.07^a	0.49±0.06^a	0.50±0.05^a
Navicula spp.	100	100	7.95±0.58^a	10.61±0.42^b	5.49±0.41^a	7.27±0.29^b	6.83±0.50^a	9.11±0.36^b
Gyrosigma sp.	98.72	100	8.37±0.76^a	2.70±0.19^b	13.83±1.18^a	4.87±0.32^b	11.27±0.98^a	3.84±0.26^b
Biddulphia spp.	97.44	98.39	2.34±0.33^a	1.71±0.11^a	5.20±0.60^a	4.20±0.27^a	3.78±0.47^a	2.95±0.19^a
Pleurosigma sp.	80.77	97.58	1.36±0.17^a	1.27±0.11^a	2.65±0.31^a	2.67±0.21^a	1.86±0.22^a	1.83±0.15^a
Nitzschia spp.	100	100	4.95±0.34^a	7.64±0.31^b	12.07±0.79^a	18.91±0.69^b	8.65±0.56^a	13.51±0.50^b
Cyanophyta								
Oscillatoria sp.	64.1	95.97	0.30±0.04^a	0.33±0.03^a	2.07±0.24^a	2.49±0.19^a	1.01±0.12^a	1.21±0.09^a
Spirulina sp.	5.13	24.19	0.01±0.01^a	0.02±0.01^b	0.05±0.03^a	0.16±0.03^b	0.01±0.01^a	0.02±0.01^b
Anabaena spp.	71.79	97.58	0.20±0.03^a	0.23±0.02^b	1.40±0.17^a	1.80±0.12^b	0.71±0.09^a	0.91±0.06^b
Copepods								
Acartia sp.	44.87	59.68	0.10±0.02^a	0.11±0.01^a	1.34±0.23^a	1.53±0.16^a	0.40±0.07^a	0.46±0.05^a
Calanus sp.	66.67	84.68	0.25±0.03^a	0.21±0.02^a	2.96±0.39^a	2.55±0.22^a	1.28±0.17^a	1.09±0.10^a
Paracalanus sp.	35.9	54.84	0.06±0.01^a	0.08±0.01^b	0.96±0.18^a	1.19±0.13^b	0.25±0.05^a	0.31±0.03^b

Different letters in each category show significant differences ($p<0.05$) between seasons

Table 4.3. Percentage of the frequency of occurrence, number, biovolume and relative important index (*IRI*) of 14 food items of three *P. serperaster* size groups (1: TL <16, 2: 16–18, 3: >18 cm)

	Occurrence			Number (Mean±SE)			Biovolume (Mean±SE)			*IRI* (Mean±SE)		
Food	Group			Group			Group			Group		
	1	2	3	1	2	3	1	2	3	1	2	3
Detritus	100	100	100	71.88±0.39^{a}	74.98±0.87^{b}	74.22±0.80^{a,b}	47.75±1.48^{a}	53.09±1.02^{b}	51.88±1.07^{b}	61.12±1.32^{a}	65.14±0.94^{a}	64.07±0.75^{a}
Bacillariophyta												
Coscinodiscus spp.	88.57	86.57	93.85	0.73±0.15^{a}	0.55±0.08^{a,b}	0.34±0.04^{b}	0.81±0.13^{a}	0.71±0.10^{a}	0.43±0.06^{a}	0.71±0.13^{a}	0.57±0.08^{a,b}	0.35±0.04^{b}
Thalassiosira sp.	78.57	76.12	86.15	0.71±0.10^{a}	0.41±0.05^{a}	0.45±0.04^{a}	0.88±0.12^{a}	0.56±0.07^{a}	0.61±0.06^{a}	0.65±0.09^{a}	0.40±0.05^{a}	0.43±0.04^{a}
Navicula spp.	100	100	100	8.92±0.69^{a}	9.92±0.60^{a}	9.94±0.53^{a}	5.87±0.45^{a}	7.01±0.42^{a}	6.92±0.39^{a}	7.56±0.58^{a}	8.61±0.51^{a}	8.56±0.46^{a}
Gyrosigma sp.	100	98.51	100	5.99±0.72^{a}	4.72±0.65^{a}	3.87±0.52^{a}	9.48±1.02^{a}	8.51±1.09^{a}	6.96±0.90^{a}	7.88±0.88^{a}	6.69±0.88^{a,b}	5.48±0.71^{b}
Biddulphia spp.	100	94.03	100	2.42±0.35^{a}	1.75±0.20^{a}	1.65±0.14^{a}	5.31±0.62^{a}	4.32±1.09^{a}	4.07±0.34^{a}	3.89±0.49^{a}	3.03±0.33^{a}	2.85±0.24^{a}
Pleurosigma sp.	87.14	89.55	96.92	1.76±0.20^{a}	0.95±0.10^{a}	1.18±0.15^{a}	3.42±0.38^{a}	2.07±0.21^{a}	2.44±0.28^{a}	2.42±0.27^{a}	3.03±0.33^{b}	2.85±0.24^{a,b}
Nitzschia spp.	100	100	100	6.33±0.39^{a}	5.88±0.42^{a}	7.63±0.46^{b}	14.94±0.89^{a}	14.98±0.99^{a}	19.02±1.02^{b}	10.85±0.64^{a,b}	10.61±0.72^{a}	13.53±0.75^{b}
Cyanophyta												
Oscillatoria sp.	84.29	77.61	89.23	0.43±0.04^{a}	0.29±0.04^{a,b}	0.23±0.02^{b}	3.01±0.28^{a}	2.2±0.27^{b}	1.73±0.17^{b}	1.47±0.14^{a}	1.06±0.13^{b}	0.83±0.08^{b}
Spirulina sp.	10	11.94	29.23	0.01±0.01^{a}	0.01±0.01^{b}	0.02±0.01^{b}	0.09±0.04^{a}	0.08±0.03^{a}	0.20±0.04^{b}	0.01±0.01^{a}	0.01±0.01^{a}	0.02±0.01^{b}
Anabaena spp.	90	83.58	89.23	0.31±0.03^{a}	0.18±0.02^{b}	0.15±0.01^{b}	2.23±0.22^{a}	1.46±0.14^{b}	1.22±0.11^{b}	1.14±0.11^{a}	0.73±0.07^{b}	0.61±0.06^{b}
Copepods												
Acartia sp.	55.71	52.24	53.85	0.13±0.02^{a}	0.09±0.02^{a}	0.08±0.01^{a}	1.79±0.24^{a}	1.36±0.25^{a}	1.22±0.18^{a}	0.53±0.07^{a}	0.40±0.07^{a}	0.36±0.05^{a}
Calanus sp.	80	76.12	76.92	0.30±0.04^{a}	0.18±0.02^{a,b}	0.18±0.03^{b}	3.47±0.40^{a}	2.42±0.30^{a,b}	2.19±0.32^{b}	1.51±0.18^{a}	1.03±0.13^{b}	0.94±0.14^{b}
Paracalanus sp.	38.57	50.75	53.85	0.64±0.01^{a}	0.08±0.01^{a}	0.07±0.02^{a}	0.98±0.18^{a}	1.23±0.19^{a}	1.10±0.18^{a}	0.26±0.05^{a}	0.32±0.05^{a}	0.28±0.05^{a}

Different letters in each category show significant differences each prey between fish size groups ($p<0.05$)

Detritus was predominantly ingested by fish in three size groups in the dry and the wet seasons (*IRI* ≈ 65%, Tables 4.2 and 4.3). Among seven food items of Bacillariophyta, *Gyrosigma* sp. was mostly consumed during the dry season followed by *Nitzschia* spp. and *Navicula* spp., while *Nitzschia* spp. contributed predominantly to the food of *P. serperaster* during the wet season, followed by *Navicula* spp. and *Gyrosigma* sp. (Table 4.2). Although *Nitzschia* spp. and *Navicula* spp. were the secondarily important prey ingested by three fish groups, *Nitzschia* spp. were significantly consumed by large fish compared to small ones (Kruskal-Wallis, $p<0.05$, Table 4.3).

Table 4.4. Percentage of frequency of occurrence, number, biovolume of four major categories of food items between three fish size groups (group 1: TL <16 cm, group 2: TL 16–18 and group 3: TL >18 cm) during the dry and wet seasons

Food			Detritus	Bacillariophyta	Cyanophyta	Copepods
Occurrence	Fish size group	1	100	100	92.86	82.86
		2	100	100	88.06	88.06
		3	100	100	93.85	83.08
	Season	Dry	100	100	98.39	87.9
		Wet	100	100	80.77	79.49
Number (Mean±SE)	Fish size group	1	71.88 ± 1.20^{a}	26.87 ± 1.17^{a}	0.75 ± 0.06^{a}	0.50 ± 0.05^{a}
		2	74.98 ± 0.87^{a}	24.19 ± 0.87^{a}	0.47 ± 0.04^{b}	0.35 ± 0.03^{a}
		3	74.22 ± 0.80^{a}	25.04 ± 0.79^{a}	0.40 ± 0.03^{b}	0.33 ± 0.04^{a}
	Season	Dry	72.74 ± 1.14^{a}	26.35 ± 1.11^{a}	0.50 ± 0.05^{a}	0.41 ± 0.05^{a}
		Wet	74.25 ± 0.59^{a}	24.79 ± 0.59^{a}	0.57 ± 0.03^{b}	0.39 ± 0.03^{a}
Biovolume (Mean±SE)	Fish size group	1	47.75 ± 1.48^{a}	40.70 ± 1.39^{a}	5.32 ± 0.38^{a}	6.23 ± 0.59^{a}
		2	53.09 ± 1.02^{b}	38.16 ± 1.13^{a}	3.74 ± 0.34^{b}	5.00 ± 0.44^{a}
		3	51.88 ± 1.07^{b}	40.46 ± 1.11^{a}	3.14 ± 0.24^{b}	4.51 ± 0.49^{a}
	Season	Dry	50.25 ± 1.51^{a}	40.97 ± 1.42^{a}	3.52 ± 0.35^{a}	5.27 ± 0.55^{a}
		Wet	51.23 ± 0.69^{a}	39.03 ± 0.73^{a}	4.46 ± 0.24^{b}	5.28 ± 0.35^{a}

Different letters in each category (fish size groups and seasons) show significant differences ($p<0.05$) between fish size groups and seasons

Table 4.5. Diet electivity (*E*) of *P. serperaster* of three fish size groups (group 1: TL <16 cm, group 2: TL 16–18 and group 3: TL >18 cm) during dry and wet seasons

Food items	Diet electivity (*E*)				
	Wet	Dry	Group 1	Group 2	Group 3
Detritus	0.21	0.33	0.43	0.45	0.45
Bacillariophyta					
Rhizosolenia sp.	–1	–1	–1	–1	–1
Navicula spp.	0.69	0.54	0.67	0.72	0.73
Nitzschia spp.	0.60	0.76	0.39	0.22	0.45
Thalassiosira sp.	–0.58	–0.78	–0.72	–0.8	–0.79
Pleurosigma sp.	–0.27	–0.34	–0.49	–0.65	–0.62
Biddulphia spp.	0.32	0.34	0.04	–0.13	–0.16
Coscinodiscus spp.	–0.15	–0.55	–0.28	–0.45	–0.58
Ditylum spp.	–1	–1	–1	–1	–1
Gyrosigma sp.	0.62	0.26	0.36	0.28	0.02
Cyanophyta					
Oscillatoria sp.	–0.24	–0.35	–0.81	–0.85	–0.89
Spirulina sp.	–0.85	–0.75	–0.97	–0.99	–0.97
Anabaena spp.	–0.31	–0.88	–0.96	–0.97	–0.98
Chlorophyta					
Cosmarium spp.	–1	–1	–1	–1	–1
Euglenozoa					
Phacus sp.	–1	–1	–1	–1	–1
Copepods					
Acartia sp.	–0.73	–0.75	–0.97	–0.98	–0.98
Calanus sp.	–0.44	–0.53	–0.92	–0.95	–0.95
Paracalanus sp.	–0.79	–0.63	–0.98	–0.98	–0.98
Larva	–1	–1	–1	–1	–1

4.4.4. Diet breadth, feeding strategy and prey election

This goby showed a wide diet breadth as the value of *H’* index was high ($n = 202$, $H' = 1.37 \pm 0.02$ SE). The food item diversity of *P. serperaster* was not significantly different between dry and wet seasons (PERMANOVA, $p = 0.30$, Fig. 4.4a), whereas the smallest fish size group had a wider diet breadth compared to groups 2 and 3 ($p = 0.04$, Fig. 4.4b). The variation in the diet breadth of this goby species was not significantly different for the interaction of seasons and fish size groups ($p = 0.88$).

The Costello graphic analysis on the relationship between diet number (Fig. 4.5a), biovolume (Fig. 4.5b) and occurrence of four major food categories showed that *P. serperaster* was a generalist feeder and fed mainly on detritus and Bacillariophyta. Similarly, the graphic analyses on the frequency of occurrence and number (Fig. 4.6a) and biovolume (Fig. 4.6b) for 14 food items also revealed that the feeding strategy of this goby species was a generalist feeder as no food items fell into the scenario of low occurrence but high in prey number or biovolume (Fig. 4.6). Likewise, the Costello graphic analysis for three fish size groups in both dry and wet seasons showed that *P. serperaster* was also the generalist feeder and fed mainly on detritus and Bacillariophyta.

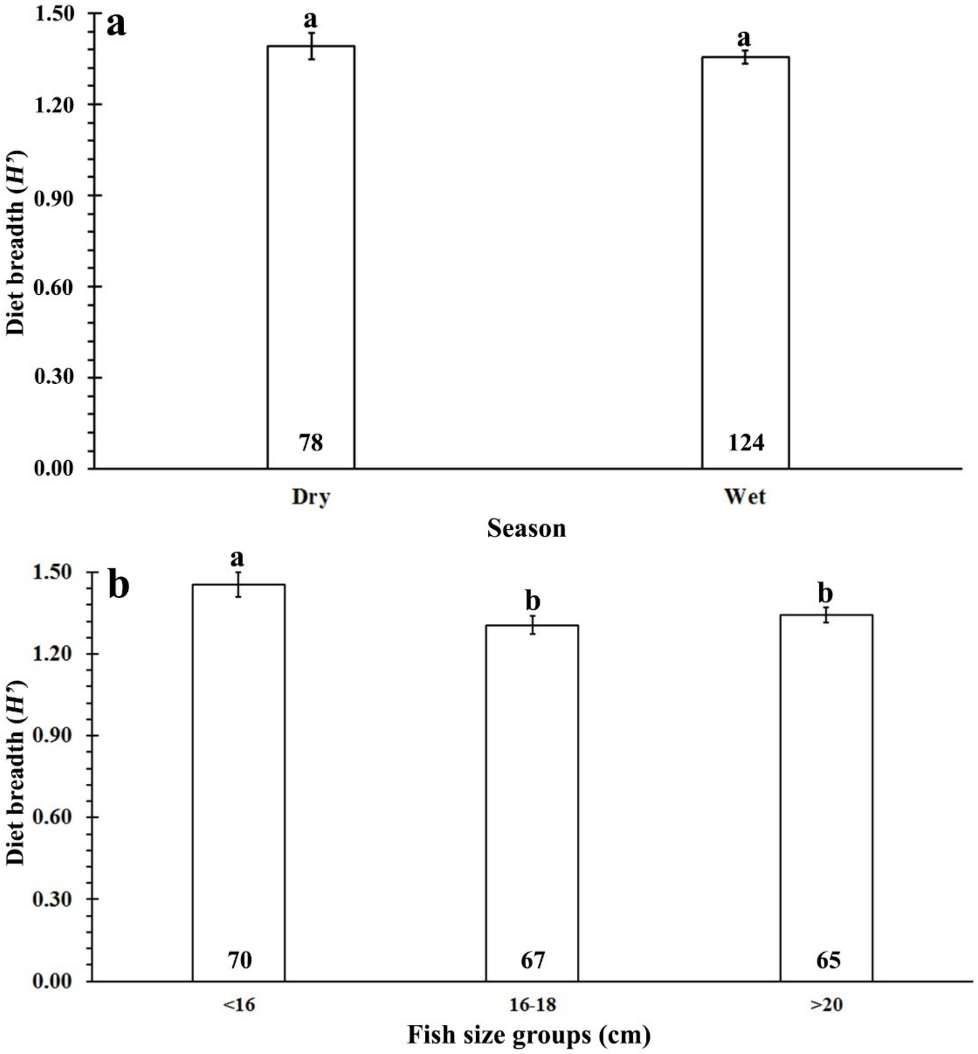

Figure 4.4 Variations of diet breadth (*H'*) of *P. serperaster* between seasons (a) and fish size groups (group 1: TL <16 cm, group 2: TL 16–18 and group 3: TL >18 cm, b). Different letters show significant differences (mean±SE, *p*<0.05). The number of fish used for analysis is given in each column

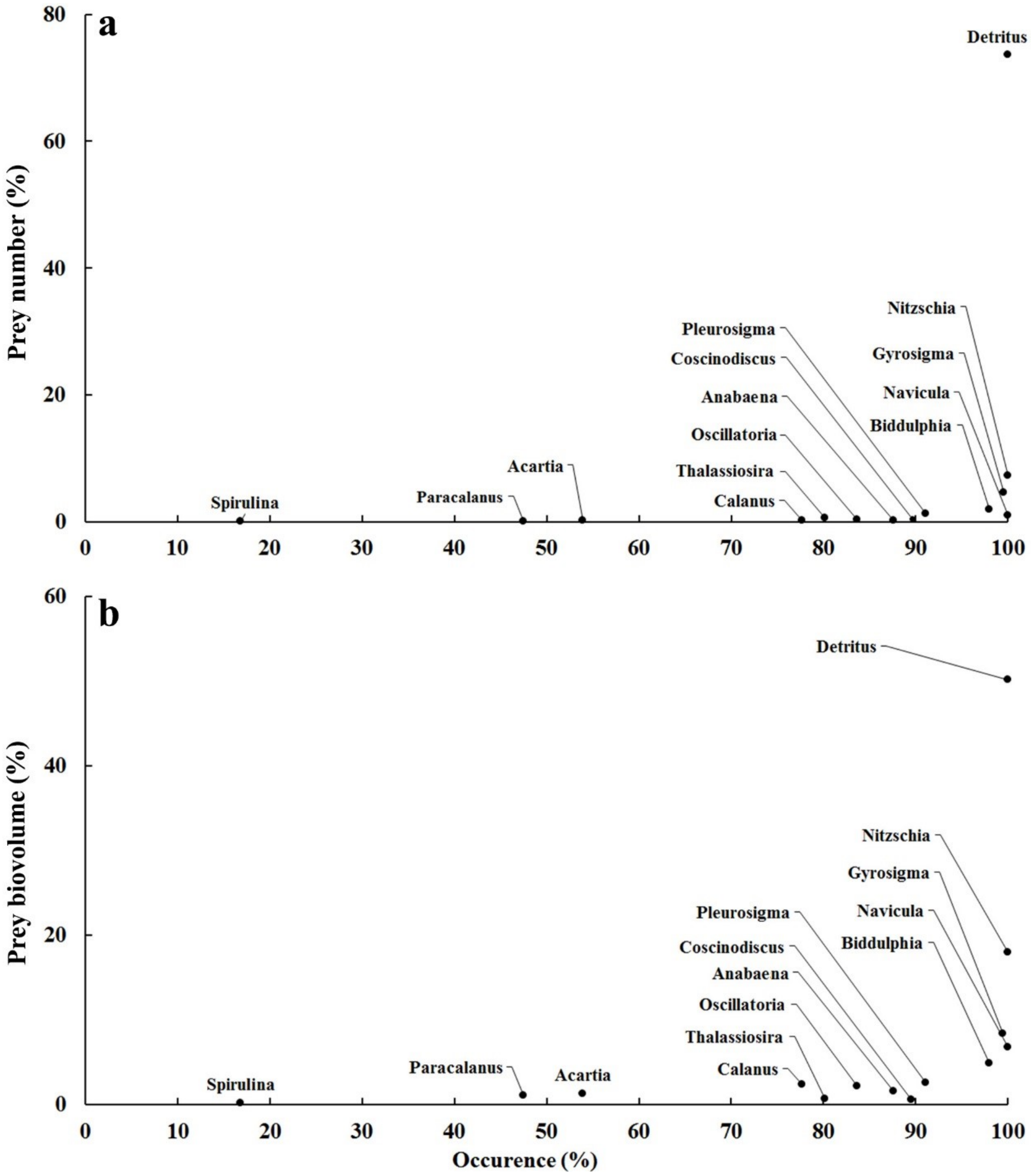

Figure 4.5 The modified Costello graphics represents feeding strategy based on plotting the relationship between percentage of number (a) and biovolume (b) and frequency of occurrence of four main food items for *P. serperaster*

P. serperaster fed on *Navicula* spp. (E = 0.69) in the wet season, but on *Nitzschia* spp. (E = 0.76) in the dry season (Table 4.5). *Navicula* spp. were mostly fed on by the goby in all size groups with an increasing tendency as fish size increased. In the wet season, *Spirulina* sp. was rarely ingested, followed by *Paracalanus* sp. and *Acartia* sp., but in the dry season, *Anabaena* spp. were rarely consumed followed by *Spirulina* sp., *Paracalanus* sp. and *Acartia* sp. (Table 4.5). Cyanophyta and copepods were rarely fed by the fish at all sizes, followed by *Thalassiosira* sp. and *Pleurosigma* sp. (Table 4.5), suggesting the possibility of feeding in the water column as pelagic copepods were found in fish stomach.

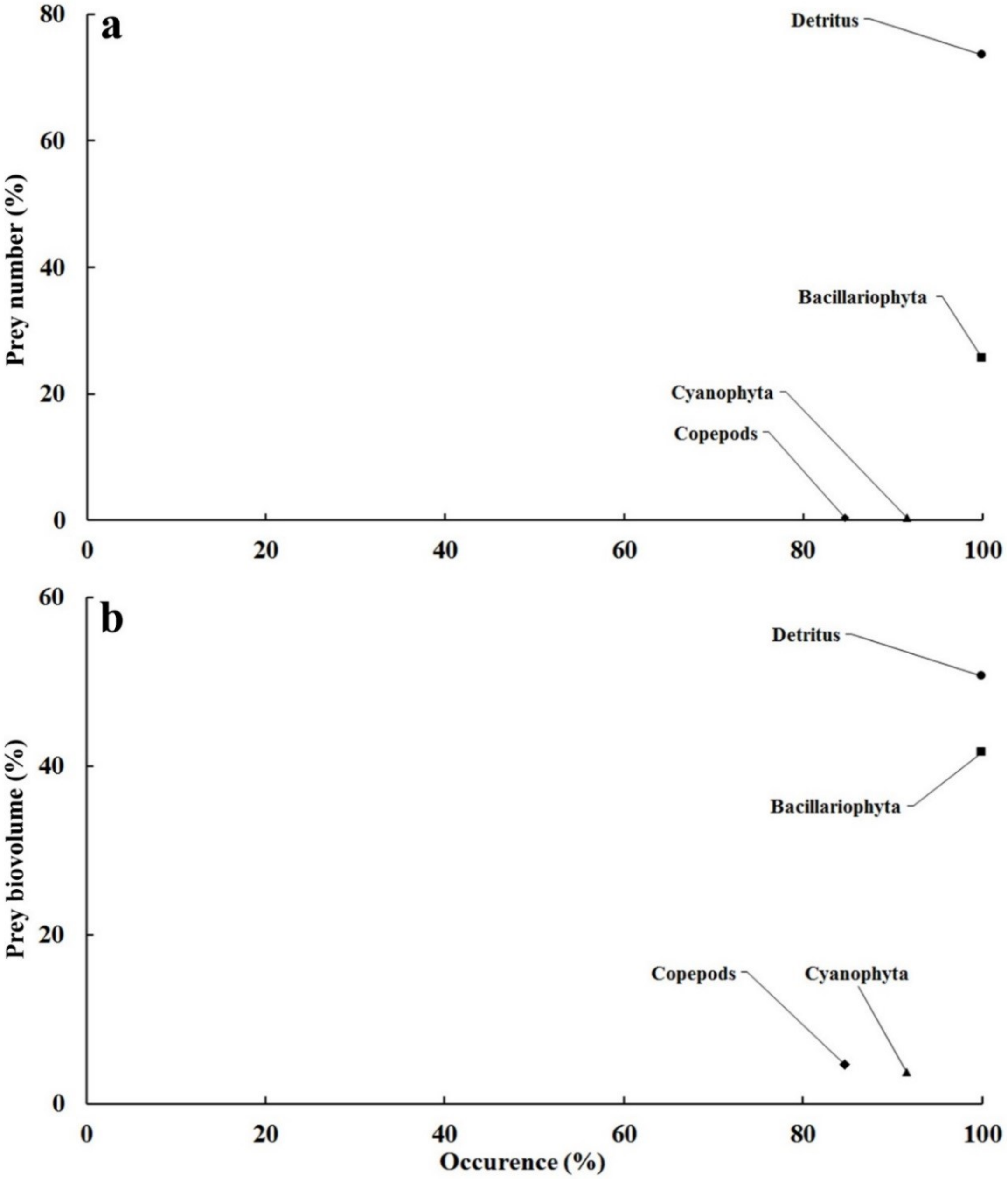

Figure 4.6 The modified Costello graphics represents feeding strategy based on plotting the relationship between the percentage of number (a) and biovolume (b) and frequency of occurrence of 14 prey items for *P. serperaster*

4.5. Discussion

As gut length is proportional to fish body length, the relative gut length index (*RGI*) has been used as an indicator to classify feeding habits (Xie et al. 2001; Pouilly et al. 2003; Hernaman et al. 2009; Berumen et al. 2011). In the present study, the *RGL* showed that *P. serperaster* is omnivorous according to the scale described by Geevarghese (1983), coinciding with a previous study on this goby in Malaysian waters (Khaironizam and Norma-Rashid 2000). The omnivorous category is found in the goby *Pseudapocryptes elongatus* (Tran 2008), while other gobiid fish such as *P.*

modestus and *P. magnuspinnatus* in Korea are carnivorous (Baeck et al. 2013). Although the *RGL* of *P. serperaster* varied with fish size, the feeding habits of this goby species did not change as fish grew up and the values of *RGLs* were 1–3, an indicator of omnivorous feeding. Similarly, black prickleback *Xiphister atropurpureus* is a carnivore feeding on harpacticoid copepods in early life, but switches to gammarid amphipods as fish grow. In contrast, the monkeyface prickleback *Cebidichthys violaceus* change food from small invertebrates (carnivores) to algae (herbivores) as fish grow (German and Horn 2006). The mudskipper *Periophthalmus koelreuteri* change from herbivorous feeding mainly on aquatic macrophytes and algal filaments to carnivorous mostly on crustaceans, aquatic and terrestrial insects and polychaetes as fish grow (Bob-Manuel 2011). The *RGL* has been successfully used to classify the trophic guild in most fish species (Geevarghese 1983; Elliott and Bellwood 2003; Karachle and Stergiou 2010) though it did not reliably reflect the trophic level of some gobiid species in a tropical Indo-Pacific seagrass bed (Pogoreutz and Ahnelt 2014). In the present study, the *RGL* was successfully applied for *P. serperaster* as supported by the proportion of relative values between fish total length and gut length. The analysis of stomach contents also revealed omnivorous feeding of this fish as its stomach mainly contained detritus, phytoplankton and zooplankton.

Feeding intensity of *P. serperaster* varied with month as demonstrated by monthly variation in the gut fullness index (*FI*). This goby species actively fed during the pre- and post-spawning periods to gain the energy needed for gonad development and compensate energy loss due to spawning (Dinh et al. 2015). In comparison to the dry season, this goby species showed intensive feeding in the wet season but low feeding in the dry season possibly due to seasonal variation in food availability (Nedeco 1993). A seasonal change in feeding intensity is also found in the Bayad fish *Bagrus bajad* in Egypt (Bakhoum and Fatas 2003) and the naked goby *Gobiosoma bosc* (D'Aguillo et al. 2014) in western Atlantic estuaries. However, feeding intensity did not vary with fish size in the present study, suggesting that food competition is not severe between small and large fish.

P. serperaster primarily ingested detritus, coinciding with a high proportion of this item in the environment. This goby also fed on Bacillariophyta, which is similar to the mudskipper *Boleophthalmus pectinirostris* that feeds mainly on diatoms (Yang et al. 2003). A small percentage of Cyanophyta and copepods was found in the stomach of *P. serperaster*, suggesting that these prey items were randomly fed, which is supported by the low occurrence of these items in the environment. This finding is similar to a related goby *P. elongatus* that also feeds on detritus and Bacillariophyta (Tran 2008). In contrast, Khaironizam and Norma-Rashid (2000) reported that *P. serperaster* mainly ingested Bacillariophyta and suggested that the food availability in the environment is the main factor involved in food selection, which is similar to the round goby *Neogobius melanostomus*, a non-random or nonopportunistic feeder that can adapt to different environments (Thompson and Simon 2014). The common goby *Pomatoschistus microps* is an opportunistic carnivore feeding on prey according to its availability as the fish stomach comprises Mysidacea in the upper Tagus estuary (Salgado et al. 2004) but not in fish found in the Mondego estuary due to low prey abundance in the environment (Leitão et al. 2006).

The high rainfall in the wet season leads to the change of nutrient input (Nedeco 1993), resulting in the variation of food composition of *P. serperaster* between the dry and the wet seasons. The seasonal change in diet composition may also be caused by the large size of gonad that occupied the major space in the body cavity prior to spawning. Seasonal variation was also found in the diet of the sand goby *Pomatoschistus minutus* feeding mainly on foraminifers in the autumn and mysids in the summer, and the common goby *Pomatoschistus microps* mostly ingesting copepods in the winter and polychaetes in spring and summer in the upper Tagus estuary, Portugal (Salgado et al. 2004). The diet of the round goby *Neogobius melanostomus* also varied with season, feeding mainly on fish eggs in the spring and ostracods in the autumn in the Kingston Basin, Lake Ontario (Brush et al. 2012). The differences in diet composition of *P. serperaster* between wet and dry seasons suggest that the diet composition is reflected by prey availability in the environment. The increasing body size or increasing feeding activity as fish grow may result in the difference in diet composition of *P. seperaster* in three size groups. A variation of diet with fish size is also found in the sand goby *P. minutus* feeding mainly on copepods in small fish but on oligochaetes, fish, bivalve and shrimp in large fish, whereas the common goby *P. microps* shows no clear trend of diet preference between fish sizes (Salgado et al. 2004).

The *IRI* is used as a tool to determine fish feeding habit and used as a biological indicator to classify the type of food items (Gumus et al. 2002), but it is difficult to apply for omnivorous, detritivorous, zooplanktivorous and phytoplanktivorous fish due to small prey size. To overcome the difficulty of small prey size, the percentage of food weight or volume was replaced by food biovolume (e.g., area of prey) to estimate the *IRI* of each prey. Based on the estimate of food biovolume, *P. serperaster* mainly fed on detritus and some Bacillariophyta (*Navicula* spp., *Nitzschia* spp. and *Gyrosigma* sp.), but this fish rarely consumed Cyanophyta and copepods. The possible reason is that the Cyanophyta such as *Oscillatoria* sp. and *Spirulina* sp. are not easy to digest compared to other algae (Vu and Duong 2013), and *Acartia* sp., *Calanus* sp. and *Paracalanus* sp. (copepods) rarely appeared in the environment. The biovolume method is consistent with the result of other methods for food item determination and can be used to determine the seasonal and intraspecific variations in small fish that feed on small-sized prey. The contribution of four major food categories to *P. serperaster* diet varied with food analysis methods, especially for the diet occurrence frequency, suggesting that prey size eventually influenced its determination for the trophic guild. This situation was found in round goby *N. melanostomus* (Thompson and Simon 2014) and in the mudskipper *B. pectinirostris* (Yang et al. 2003), suggesting that the biovolumetric method is useful to study dietary composition of small fishes such as gobiids.

Like most other goby species (Thiel et al. 1996; D'Aguillo et al. 2014), *P. serperaster* is considered a generalist feeder as various food types (14 type of preys) were found in its stomach, enabling *P. serperaster* to adapt to a variety of environmental conditions. The wide diet breadth was also found in other gobiids such as *P. modestus* and *P. magnuspinnatus* (Baeck et al. 2013), *Achondrostoma arcasii, Pseudochondrostoma duriense*, *Salmo trutta* and *Gasterosteus gymnurus* (Sánchez-Hernández et al. 2011), *P. minutus* and *P. microps* (Salgado et al. 2004; Leitão et al. 2006). By contrast, the goby *Economidichthys pygmaeus* has a narrow diet breadth and is a specialist feeder, feeding mainly on copepods and chironomids (Gkenas et al. 2012). Although only 19 food items belonging to six main categories such as detritus, Bacillariophyta, Cyanophyta, Chlorophyta, Euglenozoa and copepods were found in the environment, more microalgae might have been found if more samples had been collected. In the wet season, *Navicula* spp. were mostly ingested whereas *Spirulina* sp. was rarely fed. In the dry season, *Nitzschia* spp. were mostly fed, while *Anabaena* spp. were rarely consumed. *Navicula* spp. were mainly ingested, whereas Cyanophyta and copepods were rarely fed by fish at all size. The diet overlap of food preys between the fish stomach and the water column suggests that *P. serperaster* may feed in the water column. Likewise, the co-occurring *P. elongatus* at the same site also feed in the water column (Tran 2008).

In conclusion, *P. serperaster* was an omnivorous fish, consumed mainly detritus and some Bacillariophyta, and its feeding habits varied with the availability of food items in the environment. The food composition of this goby varied with fish size and season, and this fish showed a wide diet breadth and was a generalist feeder. Its feeding intensity peaked before and after spawning and varied with season but did not vary with fish size. The present study provides a basis for further research on the feeding biology of this goby species, and the biovolume method is useful to determine dietary composition and feeding intensity of gobiids that feed on small-size food items.

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CHAPTER 5: REPRODUCTIVE BIOLOGY

This chapter has been published in in Ichthyological Research as:
Dinh QM, Qin JG, Dittmann S, Tran DD (2015) Reproductive biology of the burrow dwelling goby *Parapocryptes serperaster* in the Mekong Delta. Ichthyol Res, 1-9. doi: 10.1007/s10228-015-0502-7.

5.1. Abstract

Parapocryptes serperaster is a bottom dwelling fish widely distributed in monsoonal regions in Asia, but its reproductive biology is poorly understood. This study assessed important variables associated with *P. serperaster* reproduction through monthly field collection and histological analysis in the Mekong Delta, Vietnam over a year. This goby species is a multiple spawner as the ovaries contained oocytes at different developmental stages during the spawning season, coinciding with the spawning period in the wet season. Peak spawning occurred in September when gonadosomatic index was at its highest. This goby shows sexual dimorphism in body size, and the average size at first sex maturation was 15.8 cm total length in the female and 16.3 cm in the male. Fish reproduction was characterised by high variation of fecundity and heterogeneous size of eggs in the ovary, indicating a possible adaptation to the monsoonal region. This study provides new understanding on the spawning pattern and reproductive biology of *P. serperaster* living in the mud flat habitat in tropical monsoon climate.

Keywords: gonad, ovary, testis, reproduction, fecundity, Mekong Delta, Vietnam.

5.2. Introduction

Fishes have evolved unique reproductive strategies to adapt to the environmental and biological changes in nature (Rocha et al. 2008). Fish reproductive behaviour and spawning modes are species-specific but are generally classified as semelparity, i.e., individuals spawn once in the whole life and die, and iteroparity including synchronous and asynchronous ovarian organization, i.e., individuals have several sexual maturation cycles in their life (Wootton 1990). The fish with synchronous iteroparity contain a uniform pattern of ovarian development from oogonia to egg ovulation (Yamasaki et al. 2011). In contrast, fish with asynchronous iteroparity spawn several times a year and release multiple batches of eggs at discrete intervals (Plaza et al. 2007; Teletchea et al. 2009).

Semelparity rarely occurs in gobies but has been found in the ice goby *Leucopsarion petersi* in Miomote and Hayagawa rivers in the northern part of Niigata Prefecture (Tamura and Honma 1969; La Mesa 2011), the transparent goby *Aphia minuta* in the coast of Ortona (Caputo et al. 2001) and the crystal goby *Crystallogobius linearis* in the coast of the Adriatic Sea (Caputo et al. 2003). Iteroparity is the dominant mode of reproduction in most gobiids that repeatedly spawn and lay submerged eggs across a breeding season (Miller 1984). For example, the gobids *Sicyopterus japonicus*, *Awaous melanocephalus*, and *Stenogobius* sp. lay eggs from May to November in the nest under rocks (Yamasaki et al. 2011), while *Pomatoschistus minutus* deposit eggs from June to July into the nest excavated by the male under mussel shells (Lindström et al. 2006). After laying eggs, the female usually leaves the spawning site and the male guards the fertilised eggs during the period of embryonic development such as in *Neogobius melanostomus* (Meunier et al. 2009), and *P. minutus* (Lindström et al. 2006; Lissåker and Kvarnemo 2006), but female *Awaous guamensis* also attend eggs (Ha and Kinzie 1996). Although parental care enhances development and survival of progeny (Lissåker and Kvarnemo 2006), this is not the only strategy used by gobiids to increase their evolutionary fitness in nature. Therefore, it is necessary to study the reproductive biology of gobiids to understand the recruitment strategy of these small but numerous benthic estuarine fishes.

Many oxudercine gobies are found in coastal and estuary areas (Murdy 1989; Takita et al. 1999) and form an important benthic fauna in the Indo-Pacific region (Matics 2000). The estuaries are an important area for fish feeding, reproduction and larval development (Blaber 2000). For example, amphidromous gobies including *Lentipes* spp., *Sicyopterus* spp. and *Stenogobius* spp. grow and spawn in rivers but complete their pelagic life in the sea (Keith 2003). In contrast, the goby *Pseudapcryptes elongatus* lays eggs in the wet season in the sea, and the larvae move back to the estuarine and river regions to grow and complete the life cycle (Tran 2008). On the other hand, the goby *Periophthalmodon schlosseri* deposit eggs *in situ* inside burrows on the mud flat in mangrove forests and the eggs are hatched in the burrows (Ishimatsu et al. 2009). This reproductive pattern is also reported for *P. modestus* (Ishimatsu and Graham 2011) and *P. minutus* (Takeda et al. 2012), but not on *P. serperaster* (Dinh et al. 2014).

P. serperaster is widely distributed in the Indo-Pacific region including China, India, Taiwan, Malaysia, Myanmar, Singapore, Thailand and Indonesia (Murdy 1989; Talwar and Jhingran 1991; Kottelat et al. 1993; Khaironizam and Norma-Rashid 2000) and is commonly found in Mekong Delta (Rainboth 1996; Tran et al. 2013). Like other estuarine fish, *P. serperaster* live in bays and brackish water estuaries from Sri Lanka to China, and in the freshwater tidal zone of the Mekong Delta (Rainboth 1996). Despite its wide distribution in the monsoonal region, our current understanding on this goby is limited to its morphological description (Murdy 1989), food and feeding habit (Khaironizam and Norma-Rashid 2000), living habitat in the Mekong Delta (Dinh et al. 2014, Chapter 2) and growth patterns between genders and among seasons (Dinh et al. 2015, Chapter 3). The knowledge of its reproductive biology including reproductive pattern, spawning season, sex maturity, fecundity, egg size and gonadal development has been fragmental. Therefore, this study aims to investigate the basic reproductive biology of *P. serperaster* in an attempt to better understand its life history, which will help interpretation of the pattern of adaptation in benthic gobiids to a changing environment.

5.3. Materials and methods

5.3.1. Study site description and specimen collection

This study was carried out in Kinh Ba River, Cu Lao Dung district, Soc Trang Province, Mekong Delta, Vietnam (9°26'3"N, 106°13'28"E) for one year. Soc Trang is a typical province representing the natural environment in the Mekong Delta. Dry (January–May) and wet (June–December) seasons are distinctive with an average annual temperature of ~27 ºC, and ~400 mm monthly precipitation in the wet season. Soc Trang Province comprises a long coastline connected to mangroves and muddy flat habitats with semi-diurnal tides and the difference between low and high tides was 0.7–1 m. The distance from the riverbank to the riverbed was about 3 m and mudflats were exposed to the air at the lowest tide (Soc Trang Statistical Office 2012).

Fish were collected monthly using three gill nets (1.5 cm mesh in the cod end, 2.5 cm mesh in the mouth and 5 m long) for a year-round cycle. Each gill net was set at the highest tide and retrieved after 2–3 h during the ebb tide. Three gill nets were set next to each other with a distance of 0.5 km apart. Four hundred and fifty four fish (11.9–23.5 cm TL) were collected and analysed over 12 months from

March 2013 to February 2014. Fish specimens were sexually differentiated using the shape of the genital papilla (e.g., the female with an oval genital papilla and the male with a narrow papilla). Fish samples were immediately anesthetised using benzocaine and preserved in 5% formalin before being transported to the laboratory for analysis. The water temperature and salinity at the study site were measured monthly using a thermometer (Model: HI98127) and a refractometer (Model: 950.0100 PPT-ATC), respectively.

5.3.2. Classification of gonad stage and gamete development

Fish total length (TL) and body weight (BW) were measured to the nearest 0.1 cm and 0.01 g, respectively. The male and female gonads were removed and first visually classified into six maturation stages according to the criteria for the black goby *Gobius niger* (Vesey and Langford 1985). The gonads were then examined histologically as an indicator for oocyte development patterns using the staining method described by Carleton et al. (1980). Firstly, gonads were fixed in Bouin solution for 24 h before being washed and stored in 70% alcohol for dehydration. After being embedded in paraffin wax, gonads were sectioned into 6-µm thick slices using a microtome. Finally, the thin sections were mounted on a glass slide for staining with Mayer's haematoxylin and Eosin solution. The diameter of 30 oocytes at each oocyte stage was measured using the Motic Image Pro Plus software version 2.0 to estimate the average size of oocytes at each gonadal stage. The terminology used for staging the oocytes and spermatocytes were based on Yamamoto (1956), Yamazaki (1965) and Palmer et al. (1995).

5.3.3. Gonadosomatic index, body length at sexual maturity and fecundity

Gonads were weighed to calculate the gonadosomatic index (GSI) using the equation $GSI = 100 \times GW/BW$, where GW is gonad weight (0.1 mg), and BW is body weight (0.01 g) (Lloret and Rätz 2000). This index was used to evaluate the development of gonad and spawning pattern of *P. serperaster* over time based the method of Alonso-Fernández et al. (2011).

Fish spawned at the beginning of the breeding season are expected to be larger than those spawned later. The length at first maturity (L_m) is defined as the length at which 50% of the population reaches sexually maturity (King 2007). The proportion of spawning population was estimated using the equation $P = 1/(1+exp[-r\times(TL - L_m)])$, where P is the proportion of mature individuals in a length class (*TL*); L_m is the length with 50% of fish reaching sexual maturity; and r is a model parameter (Zar 1999).

Batch fecundity (F) refers to the number of viable eggs released by a serial spawner during a pulse of spawning, which was estimated from the number of oocytes in 30 mature (stage IV) and ripe (stage V) ovaries. According to Bagenal and Braum (1978) and Tran (2008), the mature and ripe ovaries were placed in the Gilson fluid for 7 days to release the oocytes from the ovarian tissue before being washed and stored in 50% isopropyl alcohol. All hydrated oocytes of each ovary were counted in a petri dish to estimate the batch fecundity.

5.3.4. Data analyses

The relationships among batch fecundity, fish size and fish weight were determined using logarithmic regression, and the determination coefficient (r^2) was used to confirm the degree of association between variables (Metin et al. 2011). The sex ratios of *P. serperaster* within a month or a season, and between wet and dry seasons were tested using χ^2 test. Student t-test was used to examine the difference of GSIs between wet and dry seasons, and between the male and female (Hajji et al. 2012). As Imai and Tanaka (1994) suggested that a normal distribution usually occurs in egg size distribution in iteroparous fish, Kolmogorov-Smirnov test was used to examine the normal distribution of oocyte diameters in each ovary stage to confirm fish iteroparity. One-way ANOVA was used to test the differences in oocyte diameters between ovarian stages. SPSS software v.21 was used for data analysis, and the level of significant difference for all tests was set at $p<0.05$.

5.4. Results

5.4.1. Environmental variables and sex ratio

Four hundred and fifty four fish (219 females and 235 males) were collected for reproductive analysis. Fish total length ranged from 11.9 to 21.5 cm for females and 12.0 to 23.5 cm for males. The female *P. serperaster* to male ratio was not significantly different between months or between seasons based on χ^2 tests, and the sex ratio was nearly 1:1. Water temperature in the study region was not significantly different between dry (29.07±1.32°C) and wet seasons (28.33±1.05 °C, $p>0.05$). The salinity of the study site in the dry season (8.86±3.75‰) was significantly higher than that in the wet season (2.68±2.28‰, $p<0.001$).

5.4.2. Development of ovary and testis

Ovaries: Stage I ovaries were connected by connective tissue and were not easy to differentiate from testes. Stage I ovaries contained germ cells, oogonia and primary oocytes (Fig. 5.1a). Stage II ovaries were yellowish, 1 mm wide and contained prominent blood vessels. The two ovaries at stage II were separated at the halfway to the posterior end. The surface of ovaries was smooth and uniform in texture. Stage II ovaries contained primary oocytes, germ cells and oogonia. Vitellogenesis occurred in the oocytes as indicated by yolk granules in the cytoplasm (Fig. 5.1b). In stage III, the paired ovaries were separated and became transparent with visible orange-yellow eggs. The ovary surface was covered with prominent blood vessels, but the left ovary was slightly larger than the right one. Mature oocytes showed migratory nucleus towards the centre and contained more yolk accumulation (Fig. 5.1c). The stage IV ovaries contained small whitish eggs at the posterior end, and the blood vessels on the ovary surface were prominent. The stage IV ovaries mainly consisted of post vitellogenic oocytes with the nucleoli in the centre of the nucleus (Fig. 5.1d). At stage V, the ovaries became opaque and were covered by a smooth and firm membrane, containing hydrated oocytes. The post vitellogenic oocytes were absent, but oogonia were scattered between primary and secondary vitellogenic oocytes. Hydrated mature oocytes were present (Fig. 5.1e). The completely spent ovary (stage VI) was not found in this study. The egg size frequency at each ovarian stage showed a normal distribution (Kolmogorov-Smirnov, $p>0.05$), suggesting that this goby was iteroparous as the ovary contained a range of different sized eggs. Oogonia and primary oocytes were found in all stages of ovaries. The oocyte diameter increased significantly during vetellogenesis from primary oocytes, primary vitellogenic oocytes, post vitellogenic oocytes and secondary vitellogenic oocytes to hydrated mature oocytes (Table 5.1).

Testes: Stage I testes were very thin (<1 mm), elongated, and paired but had a smooth surface in pale pink. Many spermatogonia appeared in stage I testes (Fig. 5.1f). Testes increased to >1 mm wide at stage II and were pale pinkish, smooth and uniform in texture. At stage II, the number of primary and secondary spermatocytes increased, and a few spermatogonia appeared (Fig. 5.1g). The stage III testes continued to increase in size and became translucent and smooth on the surface. The stage III testes mainly consisted of primary and secondary spermatocytes, and a few spermatids and spermatozoa in the sperm ducts (Fig. 5.1h). When the testes reached a maximum size in stage IV, the gonad became milky in colour, with noticeable blood vessels on the surface. The stage IV testes comprised mature spermatozoa in the testicular cavity and sperm ducts (Fig. 5.1i). Stage V and VI testes, however, were not found in this study.

From July to October, the ovaries developed from stage I, II to V. Most females with stage V ovaries were found in late September (Fig. 5.2a). Immature (stage I) and maturing (stage II) ovaries were found throughout the study, while nearly matured ovaries (stage III) appeared from the late dry to the end of wet season (April to December, Fig. 5.2a). The mature (stage IV) and ripe (stage V) ovaries appeared mostly in the wet season, whereas ripe ovaries were mainly found in the wet season (August–October, Fig. 5.2a). This pattern suggests that *P. serperaster* was a multi-spawner releasing eggs from July to October, but mainly deposited eggs in September. The development of testes shared a similar pattern with ovaries (Fig. 5.2b), but the stage V testis was not found in this study. Specifically, the immature and maturing testes were found during the study period, while early matured testes appeared from April to December and the mature testes were dominant during August–October (Fig. 5.2b).

5.4.3. Gonadosomatic index, length at first maturation and fecundity

The gonadosomatic index (GSI) fluctuated during the study period (Fig. 5.3), especially in the wet season (i.e., the spawning season). The GSI values of male and female *P. serperaster* increased from April (i.e., late dry season) to September (wet season) before decreasing from the late wet season to the dry season (January–May). The GSI values during the wet season were significantly higher than those during the dry season ($p<0.001$). Furthermore, the GSI values were significantly higher in females than males ($p<0.001$). The results indicated that the breeding season occurred from July to October with a spawning peak in September.

The length at sexual maturity (L_m) was estimated from the maturity curve, and 50% females and males reached sexual maturity at 15.8 cm and 16.3 cm, respectively (Fig. 5.4a, b). A large fish may release more eggs throughout the breeding season as the fecundity had a positive relationship with fish length ($p<0.001$, Fig. 5.5a) and body weight ($p<0.001$, Fig. 5.5b).

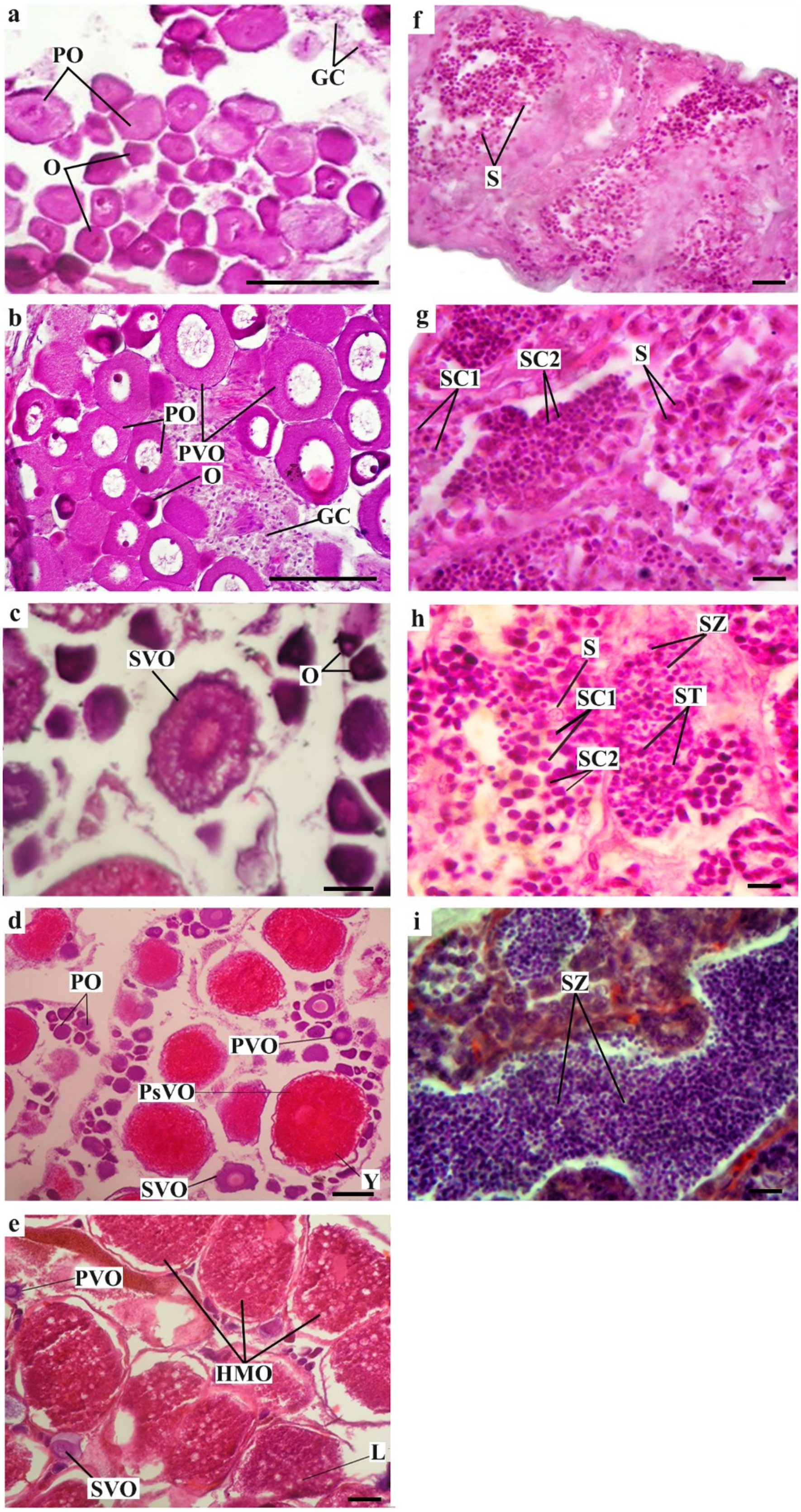

Figure 5.1 Gonad cross-sections of *P. serperaster* (a, b, c, d, and e represent ovary stages I, II, III, IV and V; f, g, h and i represent for testis stages I, II, III and IV, respectively). Ovary sections show germ cells (GC), oogonia (O), primary oocyte (PO), primary vitellogenic oocytes (PVO), secondary vitellogenic oocytes (SVO), post vitellogenic oocytes (PsVO), hydrated mature oocytes (HMO), lipid (L), yolk (Y). Testis sections show spermatogonia (S), primary spermatocytes (SC1), secondary spermatocytes (SC2), spermatid (ST) and spermatozoa (SZ). Scale bar: 200 µm.

Table 5.1 The oocyte diameter at different stages of *P. serperaster* ovary

Maturity stage	Oocyte diameter mean±SD (μm)
Stage I	59.18±16.01^{a}
Stage II	94.79±13.42^{b}
Stage III	352.03±83.96^{c}
Stage IV	406.16±72.21^{d}
Stage V	638.37±55.75^{e}

Different letters (a, b, c, d and e) show significant difference in oocyte diameter between ovarian stages ($p<0.05$).

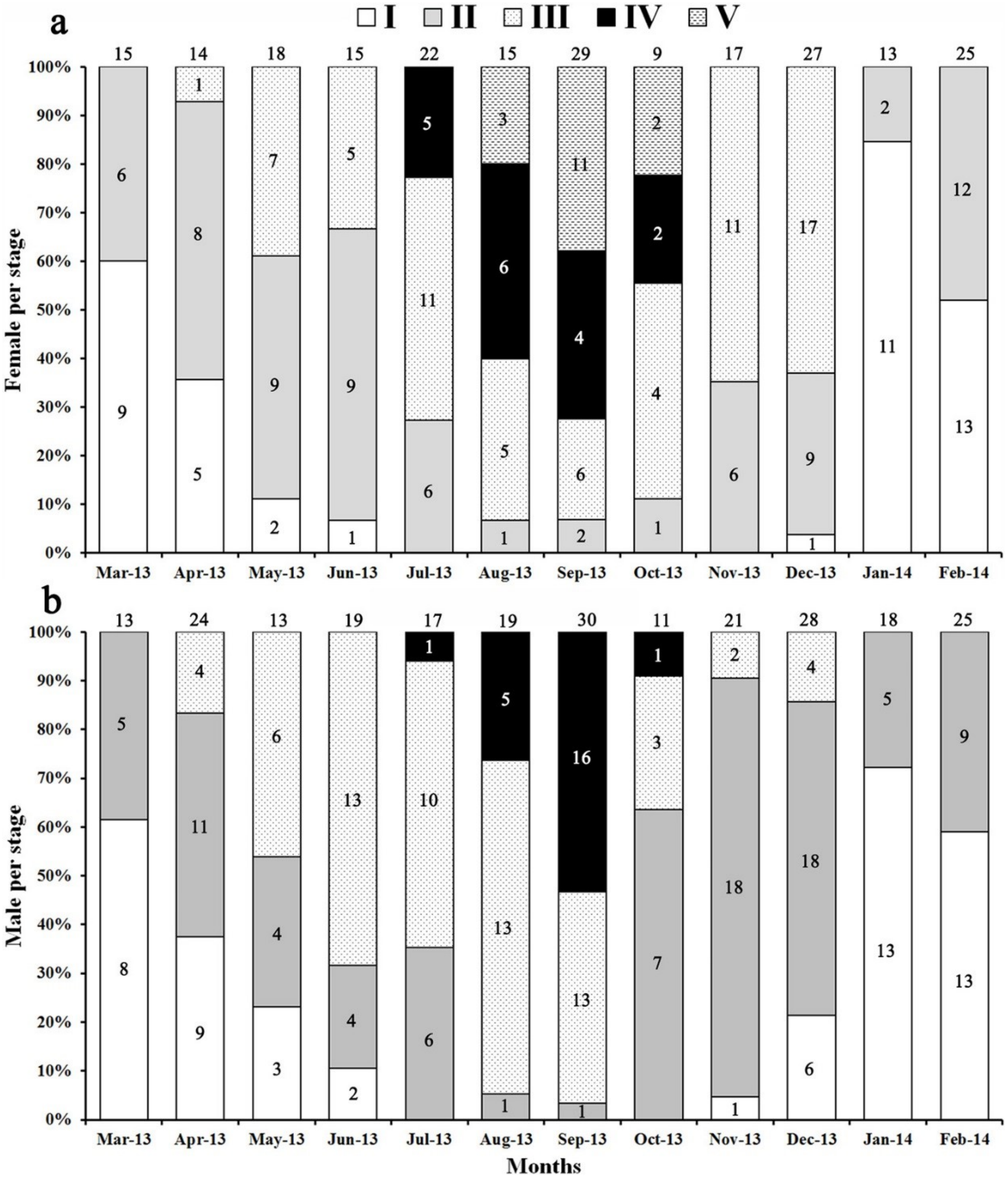

Figure 5.2 Seasonal changes in gonad stage compositions of females (a) and males (b). Top number: total fish collected; Number in a column: fish at each gonad development stage

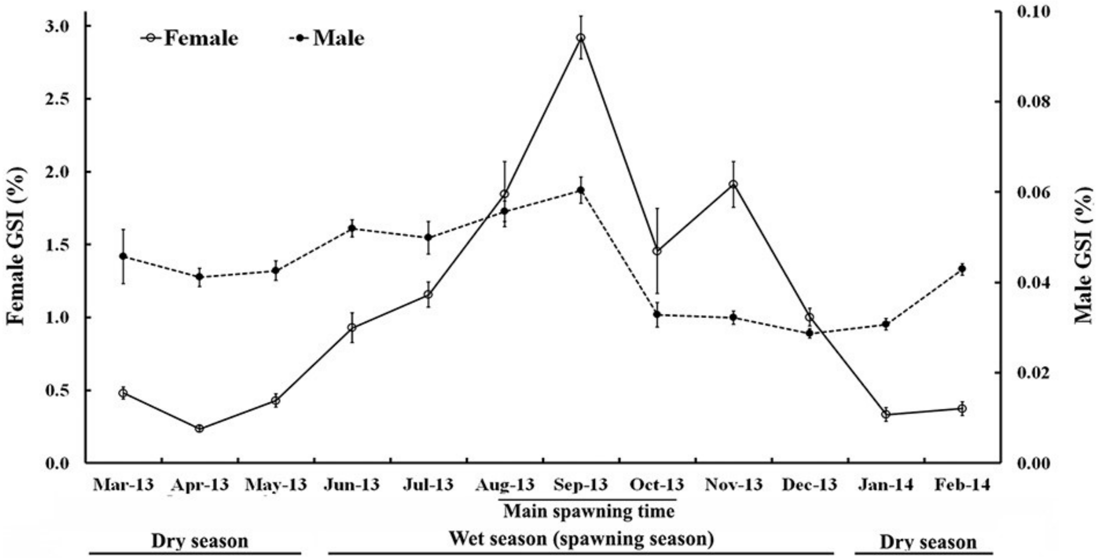

Figure 5.3 Seasonal changes in GSI (mean±SE) of female and male fish

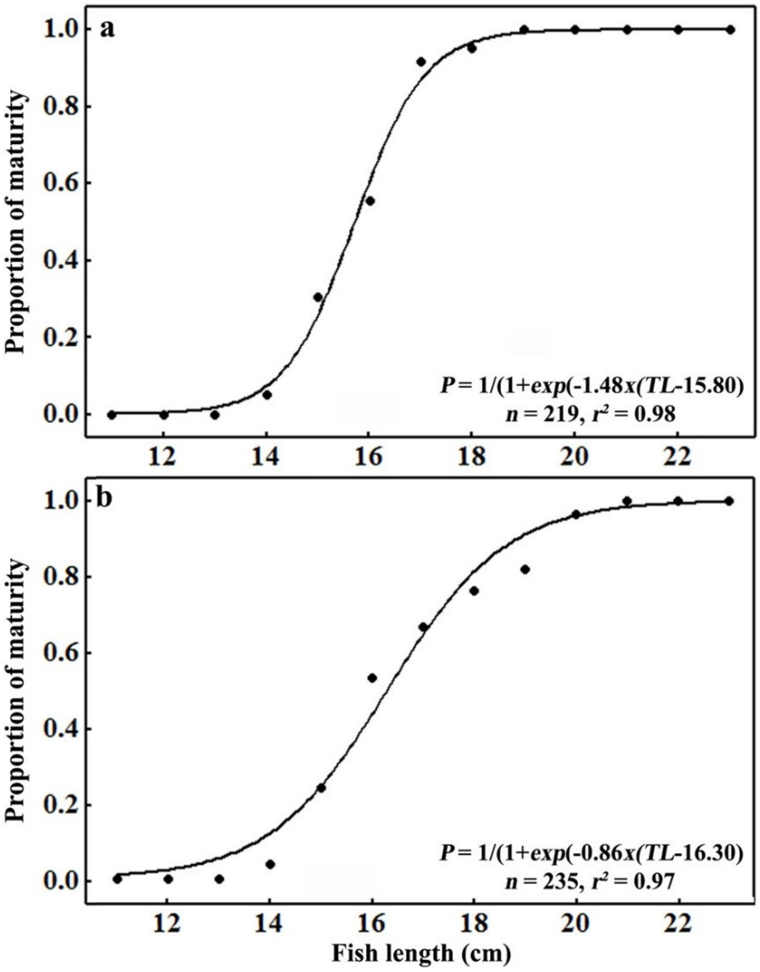

Figure 5.4 Length at first mature of female (a) and male (b)

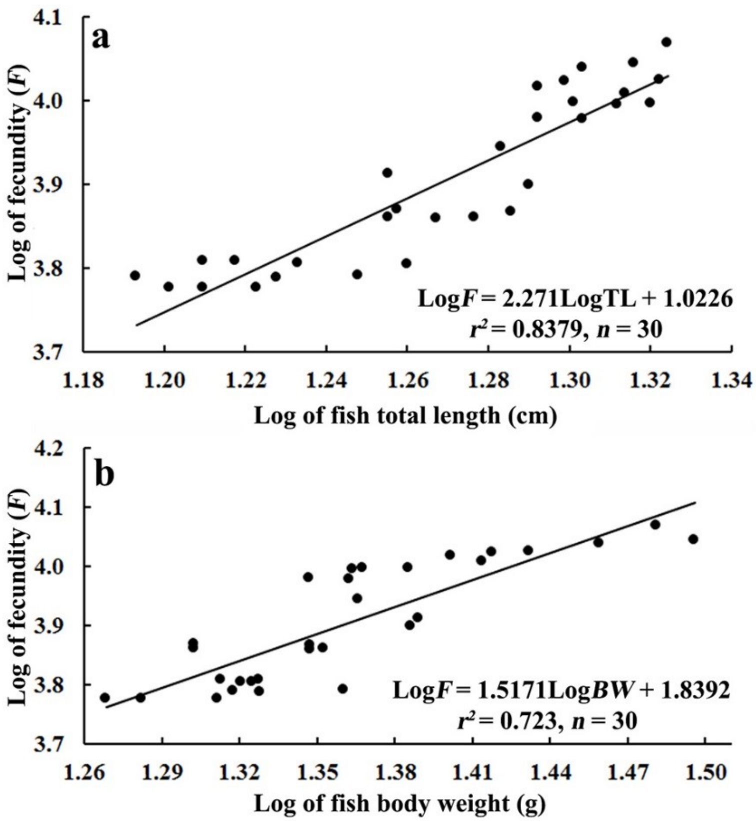

Figure 5.5 Relationship between fecundity (Log*F*) and (a) total length (LogTL) and (b) body weight (Log*BW*)

5.5. Discussion

Most gobies are serial spawners shedding eggs more than once through a spawning season rather than giving a single spurt of egg release (Longhurst and Pauly 1987). The goby in the present study was a multiple spawner, as supported by the coexistence of five oocyte types (oogonia, primary oocytes, secondary oocytes, and vitellogenic and hydrated mature oocytes) in the stage V ovaries. Interestingly, no true stage VI ovary was found in this study, suggesting that there is no completely spent ovary in this multiple spawning species. Besides, the stage IV testes comprised spermatocyes at different developmental stages including primary spermatocyte, secondary spermatocyte, spermatid and spermatozoa, suggesting that male goby can release sperm several times during the reproductive season.

The high runoff after precipitation may increase food availability in larval fish nursery due to incoming nutrients in the water running into the coastal area (Nedeco 1993; Nguyen et al. 2000; Tran 2008). The spawning season of a related goby species *Pseudapocryptes elongatus* is also from June to November with two spawning peaks in July and October (Tran 2008), which overlaps with the spawning period of *P. serperaster*. *P. serperaster* seems to be a semi-estuarine resident and oviparous fish since a few *P. serperaster* juveniles were collected along the coastline using a larval fish net during the study period (Dinh et al. 2014).

Estuarine ecosystems provide a habitat for various fish species, especially gobiid fish, and are used as spawning and nursery grounds for many fish species (Whitfield 1990; Blaber 2000; Elliott et al. 2007). A great volume of sediment is carried towards the coastal area of the Mekong Delta during the wet season and it brings in a large quantity of nutrients (Nedeco 1993). The spawning season of *P. serperaster* from July to October coincides with the main period of precipitation in the Mekong Delta. During the spawning period, the male *Gobius vittatus* tend eggs and embryos, resulting in more females than males in fish catch (Kovačić 2007), and a similar case occurs with *Periophthalmus barbarus* in Imo estuary, Nigeria (Etim et al. 2002). There was no male *P. serperaster* with stage V testis caught during the breeding season in the present study, suggesting that matured males might tend eggs in the mud substrate. The egg caring activity by a male during the spawning season was also found in other gobiid fishes such as *Periophthalmus magnuspinnatus* (Baeck et al. 2008), *Periophthalmus modestus* (Ishimatsu et al. 2007; Ishimatsu and Graham 2011), and *Periophthalmus minutus* (Takeda et al. 2012). In the present study, the monthly change of gonadosomatic index (GSI) was similar between males and females. The GSI values of *P. serperaster* were high prior to the spawning period due to the relative increase of the ovary mass to body weight. Tran et al. (2007) reported that the spawning season of *Pseudapocryptes elongatus* ranges from June to October, and Macinnis and Corkum (2000) predicted that the goby *Neogobius melanostomus* in Canada spawn from May to July based on the variation of monthly GSI. In the present study, the GSI was low in April, which coincides with the early stages of gonad development, and reached the highest value in September, matching the histological development of the stage V ovary. With the development of ovary maturity, the increase of yolk content and hydratation of oocytes, the value of GSI reached a maximum in the spawning season. After egg release, the GSI declined from September onward due to the degeneration of follicular layers.

The size and age at first maturity in fish primarily depend on species and environmental factors (Wootton 1990). The fish size at first sexual maturation is regulated by the biotic factors such as parental care and predation (Abrams and Rowe 1996) and fecundity (Pampoulie et al. 2000). For example, the marbled goby

Pomatoschistus marmoratus show that parental care leads to a larger size of males at first maturation than females (Mazzoldi et al. 2002). Besides, abiotic factors also affect the size at first sexual maturation in fish. For instance, *P. marmoratus* reach the first sexual maturation at 2.4 cm in Suez Canal (Fouda et al. 1993) but 2.7 cm in Mauguio Lagoonare (Bouchereau et al. 1993). The high abundance of *P. marnoratus* at the bottom in Suez Canal may contribute to its smaller size at first mature comprared in Mauguio Lagoonare. In the present study, the size at first mature of the male *P. seperaster* was similar to females, suggesting no sexual dimorphism in growth for both sexes of this gobiid species.

Most gobies are repetitive spawners, and the fecundity and egg size depend on fish size (Miller 1984; Kovačić 2007). Similarly, the present study reveals that *P. serperaster* is a serial spawner, and its fecundity is positively related to fish size. In this study, the total fecundity of *P. serperaster* (6,000–11,700 eggs, 11.9–21.5 cm TL) is lower than some gobiid species such as *Valenciennea strigata* (60,000–160,000 eggs, 9.3–12.9 cm SL) (Reavis 1997), *Amblygobius phalaena* (37,000–38,000 eggs, 12.1–12.4 cm TL) (Takegaki 2000), and *Periophthalmus barbarus* (900–24,000 eggs, 3.4–13.6 cm TL) in mangrove swamps of Imo estuary, Nigeria (Udo 2002). However, the fecundity of *P. serperaster* is in a similar range to *Periophthalmus papilio* (e.g., *P. barbarus*, 500–16,000 eggs, 9.1–18.0 cm TL) in mangrove swamps of Lagos Lagoon, Nigeria (Lawson 2011), *Periophthalmus barbarus* (860– 14,000 eggs, 4.1–14.2 cm TL) in new Calabar River, Nigeria (Chukwu et al. 2010) and *Pseudapocryptes elongatus* in the Mekong Delta (2,600–29,400 eggs, 12.4–22.4 cm TL) (Tran 2008), but higher than other gobiid species such as *Boleophthalmus dentatus* in the northern Arabian sea (970–4100 eggs, 7.5–11.0 cm TL) (Hoda and Akhtar 1985), *Boleophthalmus dussumeri* in Karachi coast (980–4900 eggs, 1.7–11.0 cm TL) (Hoda 1986), *Neogobius melanostomus* (80–600 eggs, 4.2–9.2 cm SL) (Macinnis and Corkum 2000), *Pomatoschistus marmoratus* in Venetial lagoon (410 – 2,900 eggs, 3.1–5.7 cm TL) (Mazzoldi et al. 2002), *Crystallogobius linearis* (200–700 eggs, 1.8–3.3 cm TL) (Caputo et al. 2003), *Rhinogobius kurodai* (1,000–2,700 eggs, 3.65–4.32 cm TL) and *Rhinogobius* sp. (150–1,100 eggs, 2.67–3.99 cm TL) in Lake Biwa, Japan (Takahashi 2013). It seems that the fecundity of gobiids is species-specific and is also related to the size of fish.

In summary, *P. serperaster* was a serial spawner shedding eggs over four months during the wet season. The present study provides a basis to further understand the reproductive biology of benthic gobies in tropical regions. As a wet-season spawning fish, its spawning success could be influenced by climate change and the variation of rainfall patterns. The effect of climate change on this goby species may spin off on other benthic fishes in this region, which may further influence the ecosystem function via the impact on their predators through food web interaction.

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CHAPTER 6: POPULATION AND AGE STRUCTURE

This chapter has been published in Turkish Journal of Fisheries and Aquatic Sciences as:
Dinh QM, Qin JG, Dittmann S, Tran DD (2015) Population and age structure of the goby *Parapocryptes serperaster* (Richardson, 1846; Gobiidae: Oxudercinae) in the Mekong Delta. Turk J Fish Aquat Sci 15: 345–357.

6.1. Abstract

Parapocryptes serperaster lives on the mudflats in estuaries and has been increasingly exploited as food fish in Mekong Delta, Vietnam, but little is known of its population, age structure and growth. This study aims to understand some basic parameters of its population biology and age used for fishery management and size-selective fishing. Based on monthly samples over a year, a total of 3002 fish were used for population structure analysis. Fish length distribution and otoliths were used for fish aging. The sex ratio of *P. serperaster* was near 1:1 and fish size at first entry to fishery was 14.6 cm. The longevity of this goby was 4.05 yr, but fishing mortality (1.57 yr^{-1}) and natural mortality (1.51 yr^{-1}) accounted for 51% and 49% of the total mortality (3.07 yr^{-1}), respectively. Relative yield-per-recruit and biomass-per-recruit analyses revealed the levels of maximum exploitation yield (E_{max} = 0.83), maximum economic yield ($E_{0.1}$ = 0.71) and the yield at 50% reduction of exploitation ($E_{0.5}$ = 0.37). Readable otoliths from female (n = 164) and male (n = 196) gobies with proper otolith morphometry were used for age determination. The mean age estimated by reading otolith annual rings matched the age estimate from length frequency distribution. The otolith morphometry is a reliable method to age this goby and possibly also applicable for other tropical gobies. The fishery analysis indicates that this goby stock is not overexploited and shows high population recruitment in the Mekong Delta.

Keywords: *Parapocryptes serperaster*, mortality, growth, exploitation, otolith, Vietnam

6.2. Introduction

Growth and mortality rates are important population parameters to understand the population dynamics and recruitment pattern for a fish cohort (Al-Husaini et al. 2001). Moreover, fish age determination using length-frequency distribution and otolith analysis (Devries and Frie 1996; Pilling et al. 2003; Cardinale and Arrhenius

2004; Tran 2008) is a fundamental step for fishery management and stock assessment as fish age is an important estimate for fish growth, recruitment, sexual maturation and population structure (Beatriz 1992; Cardinale et al. 2000).

Most fish species in the family Gobiidae live on mudflats and contribute significantly to fishery catch in Indo-Pacific regions (Matics 2000; Hajisamae et al. 2006). *Parapocryptes* is one of 10 genera of Gobiidae and consists of two species such as *P. rictuosus* (Valenciennes, 1837) and *P. serperaster* (Richardson, 1846) living in soft-bottomed tidal areas and estuaries (Murdy 1989; Murdy 2011). *P. serperaster* has an elongated and round body (Khaironizam and Norma-Rashid 2000), and is distributed in the Indo–West Pacific region including the Mekong Delta, Vietnam (Froese and Pauly 2015). *P. serperaster* is a species living in coastal muddy areas and dwelling in burrows situated in estuarine mudflats (Dinh et al. 2014), and is a commercially important food species in Taiwan, Vietnam and other Asian countries (Ip et al. 1990). The use of versatile fishing gears including hook, gill net, deep net, even hand catching has significantly reduced the goby resource and may affect future sustainable exploitation (Dinh et al. 2014). Understanding the effects of heavy floods on the population and age structure of *P. serperaster* in the Mekong Delta is limited (Le et al. 2007). Therefore, this study aims to understand the population and age structure of this goby using length-frequency distribution and test if otolith morphometry is related to fish growth and aging. The result will contribute to our knowledge of population and age structure for this goby species and other burrow dwelling tropical fishes.

6.3. Materials and methods

6.3.1. Study site

This study was carried out in the Kinh Ba River, Cu Lao Dung district, Soc Trang Province, Mekong Delta, Vietnam (9°26'3"N, 106°13'28"E), from March 2013 to February 2014. Soc Trang is a typical region in the Mekong Delta with a long coastline connected with mangroves and large areas of mudflats with semi-diurnal tides and a spring tidal range from 0.7 to 1 m. This region has a dry (January to May) and wet (June to December) seasonal cycle. The average annual temperature is ~27 ºC and the monthly precipitation in the wet season is about 400 mm, which represents a typical natural condition in the Mekong Delta (Soc Trang Statistical Office 2012).

6.3.2. Fish collection

Fish were collected monthly from a commercial fishery site using three gill nets with 1.5 cm mesh size in the cod end, 2.5 cm mesh size in mouth and 5 m in length for a year-round cycle. Each gill net was set at the highest tide (stagnant) and retrieved after 2–3 hours during the ebb tide. Three gill nets were set behind each other with 0.5 km apart. In the field, after sex determination using external morphology of genital papilla shape (oval shape for female and roughly triangle sharp for male), all fish were measured to the nearest 0.1 cm in total length and weighed to the nearest 0.01 g. Among 3002 individuals, 476 individuals were chosen over a period 12 months, from March 2013 to February 2014, and were immediately anesthetised using benzocaine and were stored in a plastic jar in 5% formalin before transport to the laboratory or fish age and population analysis (Table 6.1). The rest of fish specimens were released to the river. Thermometer (Model: HI98127, ±0.5ºC) and refractometer (Model: #950.0100 PPT-ATC, ±1‰) were used to measure monthly the environmental parameters including surface water temperature and salinity at the study site, respectively. These parameters were used to test the influence of environmental factors on the sex ratio of *P. serperaster*.

In the laboratory, the otoliths from each side of all specimens were extracted by cutting the head along the mid-sagittal plane and removing the otoliths from the brain cavity. The otoliths were subsequently cleaned in water to remove any attached tissues, dipped in 75% ethanol and stored dry on plastic culture plates (David and John 1992). Otoliths were measured in maximum length and width (Fig. 6.1a) using the Motic Image Pro Plus software v.2 and weighed to the nearest 0.1 mg.

6.3.3. Population structure

Monthly data of length measurements of *P. serperaster* were used to estimate population parameters using the FiSAT II software (Gayanilo et al. 2005). The difference in sex ratio was analysed by Chi-square test. After the Powell-Wetherall procedure was used to obtain the initial asymptotic length (L_∞) (Powell 1979; Pauly 1986; Wetherall 1986), the Beverton and Holt length-based Z-equation was obtained and expressed as a linear regression equation: $\bar{L} - L' = a + bL'$, where, L' is the cut off length (e.g., the length threshold larger than the smallest fish length in the capture samples); $\bar{L}$ is the mean length of all fish (≥*L'*) and was calculated as $\bar{L} = \frac{L_\infty + L'}{1 + Z/K}$.

From the linear regression, the asymptotic length L_∞ was calculated as a/b and Z/K was defined as $-(1+b)/b$, where, a is the regression intercept and b is the regression slope. The initial L_∞ was used in the ELEFAN I procedure (Pauly and David 1981; Pauly 1982; Pauly 1987) to optimise the asymptotic length (L_∞) and the growth parameter (K). The L_∞ and K were used to obtain the t_0 from the equation $\text{Log}(-t_0) = -0.392 - 0.275 \times \text{Log}L_\infty - 1.038 \times \text{Log}K$, where, the initial L_∞ was set at 22.9 cm and Z/K was 2.757 (Pauly 1979). The initial L_∞ was then used to determine the optimised growth parameters, i.e., $L_\infty = 25.2$ cm, $K = 0.74$ yr^{-1}. The value of t_0 was estimated as -0.22 yr^{-1} from the above equation.

Mortality rate (Z) was computed from the length-converted capture curve (Beverton and Holt 1957; Ricker 1975). The natural mortality rate (M) was estimated by the following empirical model $\text{Log}M = -0.0066 - 0.279\text{Log}L_\infty + 0.6543\text{Log}K + 0.463\text{Log}T$, where, L_∞ and K were estimated from the ELEFAN I procedure and T was the average monthly water temperature (°C) in the study area (Pauly 1980). The attainment of reasonable estimates of Z and M was used to estimate fishing mortality (F) based on the equation $F = Z - M$. The exploitation ratio (E) was then defined as $E = F/Z$ (Ricker 1975). The probability of capture for each size class was analysed using the length-converted catch curve (Pauly 1987). The seasonal recruitment pattern of *P. serperaster* was reconstructed using the whole length-frequency dataset. By plotting the cumulative probability of capture against the class mid-length, a resultant curve was obtained to determine the fish length at first capture (L_c), which was then taken as the cumulative probability of capture at 50% (Pauly 1987).

The yield-per-recruit model of Beverton and Holt (1957) was used to describe the stock and yield of fish when all fish are vulnerable to catch after recruitment (Sparre and Venema 1992). The yield-per-recruit (Y'/R) of *P. serperaster* was calculated using the equation of Beverton and Holt (1966):

$Y'/R = EU^{M/K}\left(1 - \frac{3U}{1+m} - \frac{3U^2}{1+2m} - \frac{U^3}{1+3m}\right)$, where, $U = 1 - (L_c/L_\infty)$ is the fraction of growth to be completed by this species after entering the exploitation phase:

$m = \frac{1-E}{M/K} = \frac{K}{Z}$. The biomass-per-recruit relation ($^{B'}\!/_{R}$) of this species was computed from the equation $^{B'}\!/_{R} = \frac{^{Y'}\!/_{R}}{F}$.

The exploitation rate (E) of *P. serperaster*, obtained by dividing fishing (F) and total mortality (Z) rates, was compared to the anticipated values of E_{max} (the maximum yield exploitation rate), $E_{0.1}$ (the maximum economic exploitation rate at which the marginal increase of $^{Y'}\!/_{R}$ is 10% of its value at $E = 0$), and $E_{0.5}$ (the exploitation rate with the reduction of stock to 50% of its unexploited biomass). The ratio of natural mortality (M) to growth parameter (K), and the ratio of the length at first capture (L_c) to the asymptotic length (L_∞) were used to estimate the values of E_{max}, $E_{0.1}$ and $E_{0.5}$ based on knife-edge selection described by Beverton and Holt (1966). The effect on yield derived by the change of exploitation rate and the fraction of length at first catch and asymptotic length (L_c/L_∞) was estimated by the yield isopleth diagram. This diagram was used to determine the fishing status of *P. serperaster* based on four quadrants of the yield contour described by Pauly and Soriano (1986).

For comparison of the von Bertalanffy growth parameters of *P. serperaster* and other goby fishes dwelling in the same habitat, the index of overall growth performance (Φ') was calculated from the following equation (Pauly and Munro 1984): $\Phi' = \mathrm{Log}K + 2\mathrm{Log}L_\infty$, where, K and L_∞ were parameters obtained from the von Bertalanffy curve. The longevity ($t_{\max}$) of *P. serperaster* was calculated from the following equation $t_{\max} = \frac{3}{K}$, where, K was the growth parameter and t_0 was the age when the egg was fertilised (Taylor 1958; Pauly 1980).

6.3.4. Fish age determination using otolith

The otolith burning method for fish age determination was first described by Møller Christensen (1964), and has been used by many researchers for fish aging. For example, Stransky et al. (2005) aged *Sebastes marinus* and *S. mentella* by using this method, which was also used by Campana et al. (2015) when they age *Sebastes mentella* and *S. fasciatus*) caught from the eastern coast of Canada. Each otolith was randomly selected and cut through the core of the otolith and perpendicular with the sulcus by a pair of scissors before placing the otolith on the flame of an alcohol burner. The broken otolith was removed from the flame when its colour became ash grey. The otolith fragments were then fixed on a microscope slide using crystal-ball glue. The fractured otolith surface was facing upward under the digital microscope equipped with a camera (Motic model: 143-FBGG-C). The otolith surface was gently brushed with vegetable oil before capturing the image under reflected light against a dark background using this microscope. With this method, the hyaline rings appeared as brown on a yellow background. The otolith photo was also used to determine the fish age because it was easier than reading on a digital microscope.

For fish otolith without obvious annuli, the fish age was determined based by the number of daily rings on the otolith. The otoliths were embedded in clear polyester resin mixed with hardener (ratio: 49 resins and 1 hardener). The mounted otoliths were grinded by using the 600-grit and 1000-grit silicon carbide papers until the core of the otolith appeared. The otoliths were polished lightly on a polishing disc with diamond compound. During grinding, the otoliths were regularly checked for the ring structure on a microscope. If some rings appeared, the polishing was stopped and then the otoliths were washed with soap water (David and John 1992). Otolith aging was undertaken with a transmitted light microscope, and the number of daily rings on the otolith was counted from the centre to the edge.

Each otolith was read at three independent times with a 3-week break between readings, and the result would be used only if the two of three readings agreed (Steven 1992). The percent error between three readings on annual rings was quantified using the index of average percent errors (*IAPE*):

$IAPE = \frac{1}{N}\sum_{j=1}^{N}\left[\frac{1}{R}\sum_{i=1}^{R}\frac{\left|X_{ij} - X_j\right|}{X_j}\right]$, where, *N* was the number of fish aged; *Xj* was the *i*th age calculated for the *j*th fish; *Xij* was the *i*th age determination of the *j*th fish (Beamish and Fournier 1981). The result of age determination was accepted when the value of *IAPE* between three readings was less than 5%.

6.3.5. Fish age determination using length-frequency distribution

The age of this *P. serperaster* was estimated using the following equation (Pauly 1987): $L_t = L_\infty\left(1 - e^{-K(t-t_0)}\right)$, where, L_t is the length of fish at *t* age; L_∞ is asymptotic length; *K* is the growth parameter; and t_0 is the age at which egg is fertilised. This method was successfully used by Tran (2008) to determine the fish age of

Pseudapocryptes elongatus, and by Mazlan and Rohaya (2008) to determine the age of the mudskipper *Periophthalmodon schlosseri*. The fish age estimated from otolith and that from length-frequency distribution was compared by *t*-test. Differences in percentage of male and female *P. serperaster* within months and seasons, and between dry and wet seasons were tested using the χ^2 test. All data were analysed using SPSS software v.21 and the level of significant differences for all tests was set at $p<0.05$.

6.4. Results

6.4.1. Sex ratio and environmental factors

A total of 3002 fish (8–24 cm TL) including 496 juveniles, 1279 females and 1227 males were used for population analysis. The proportion of female and male *P. serperaster* was not significantly different within the month and the season or between dry and wet seasons based on χ^2 tests, and the sex ratio of *P. serperaster* was near 1:1 (Table 6.1). Among 3002 individuals, 476 individuals (246 male and 230 female) were randomly selected for otolith analysis on age determination, and the sex ratio was also close to 1:1 (Table 6.1).

Table 6.1 Monthly sex ratio of *P. serperaster* from March 2013 to February 2014 in the study area tested by χ^2 analysis ($p<0.05$)

Month	Population analysis					Aging analysis			
	Juveniles	Female	Male	Sex ratio	*P*-value	Female	Male	Sex ratio	*P*-value
Mar-13	65	85	89	1 : 0.96	0.762	15	13	1 : 1.15	0.705
Apr-13	65	76	84	1 : 0.90	0.527	19	24	1 : 0.79	0.446
May-13	106	162	161	1 : 1.01	0.956	18	13	1 : 1.38	0.369
Jun-13	32	108	101	1 : 1.07	0.628	21	19	1 : 1.11	0.752
Jul-13	8	75	79	1 : 0.95	0.747	22	17	1 : 1.29	0.423
Aug-13	8	109	101	1 : 1.08	0.581	15	19	1 : 0.79	0.493
Sep-13	3	135	121	1 : 1.12	0.382	25	20	1 : 1.25	0.456
Oct-13		96	77	1 : 1.25	0.149	19	16	1 : 1.19	0.612
Nov-13		109	127	1 : 0.86	0.241	17	21	1 : 0.81	0.516
Dec-13	6	96	82	1 : 1.17	0.294	27	28	1 : 0.96	0.893
Jan-14	77	121	105	1 : 1.15	0.287	23	18	1 : 1.27	0.435
Feb-14	126	107	100	1 : 1.07	0.627	25	22	1 : 1.24	0.662
Dry	439	551	539	1 : 1.02	0.761	100	90	1 : 1.11	0.468
Wet	57	728	688	1 : 1.06	0.288	146	140	1 : 1.04	0.723
Total	496	1279	1227	1 : 1.04	0.299	246	230	1 : 1.07	0.463

In the study region, water temperature (29.07±1.32°C) in the dry season was not significantly different from that in the wet season (28.33±1.05°C, t-test, $t = 1.78$, $p > 0.05$). However, the water in the study site was brackish in the dry season (8.86±3.75‰) and significantly higher than in the wet season (2.68±2.28‰, t-test, $t = 16.67$, $p < 0.001$).

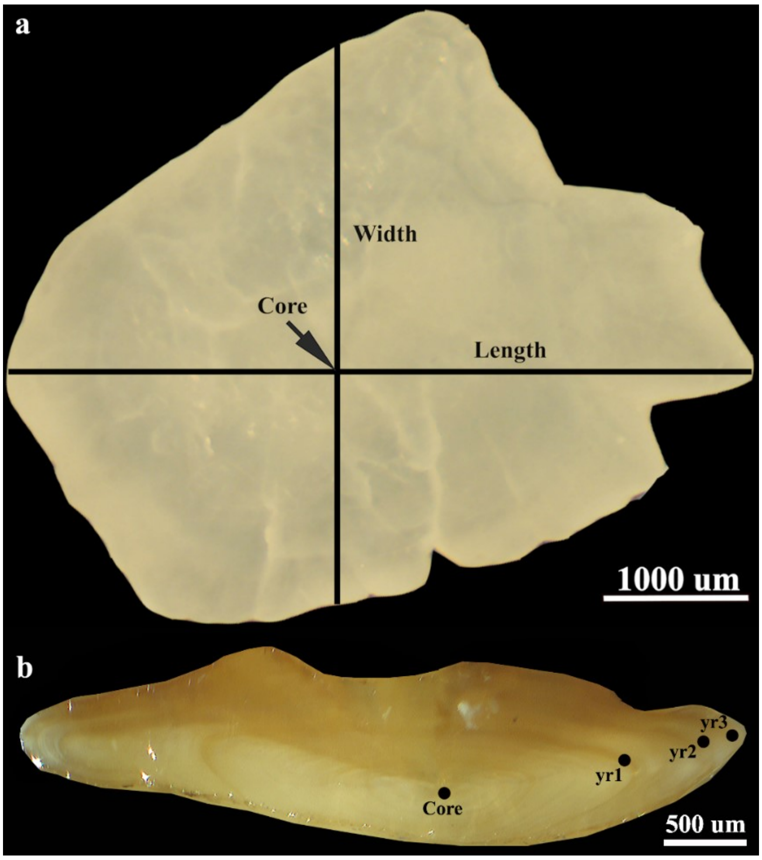

Figure 6.1 The otolith of a three-year old *P. serperaster*. (a) left external morphology, (b) annuli at core, yr 1, yr 2 and yr 3

6.4.2. Population structure

The length frequency analysis of 3002 fish were used to estimate the population parameters such as asymptotic length, growth rate, longevity, mortality, recruitment, exploitation indices and yield-per-recruit. The growth curves of *P. serperaster* from the length-frequency data showed that most of fish size in this study was greater than the length at first capture (L_{50}) in the spawning season from August to October (Fig. 6.2). Moreover, there were four fish size groups, i.e., four growth curves represented by four dark lines (Fig. 6.2) in the population of *P. serperaster* in this study area, and the slight slope in the bigger fish compared to smaller fish leading to large fish grew more slowly than small ones.

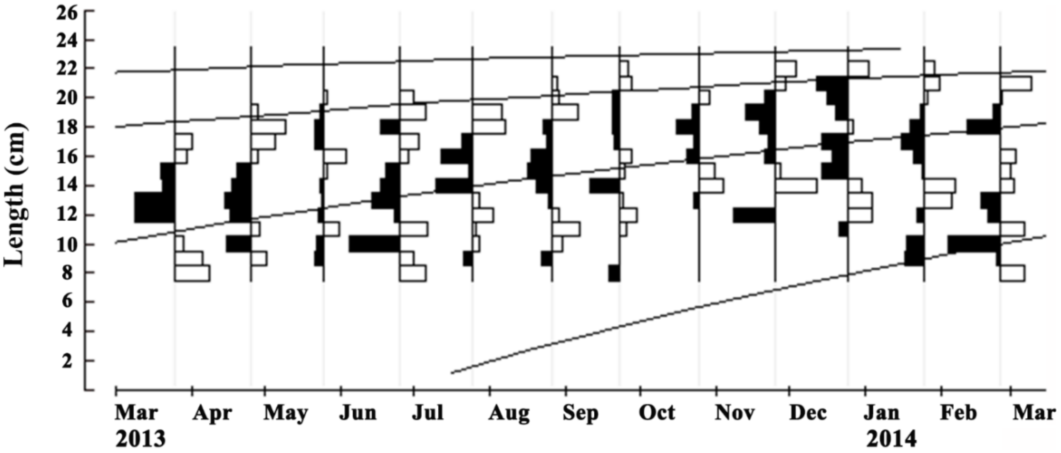

Figure 6.2 The von Bertalanffy fish growth curve. Black and white bars represent positive and negative deviation from the weighted average of the length classes, and curves represent fish length over time

The total mortality (*Z*) of *P. serperaster* was 3.07 yr^{-1} (intercept: $a = 11.372$, slope: $b = -2.306$, $r = 0.967$, $n = 9$, confidence interval: 2.4–3.8 yr^{-1}), which was estimated from the length-converted catch curve (Fig. 6.3a). The natural mortality (*M*), fishing mortality (*F*) and exploitation (*E*) rates of *P. serperaster* were 1.57 yr^{-1}, 1.51 yr^{-1}, and 0.49, respectively (Fig. 6.3a). Recruitment pattern showed that the variation in fishery recruitment over time, and the two recruitment peaks occurred in March and September with different magnitudes. The means of the two peaks were separated by a time interval of 6 months (Fig. 6.3b). The analysis of capture probability indicated that the fish length at first capture (L_c or L_{50}) was 14.6 cm (Fig. 6.3c).

The yield-per-recruit and biomass-per-recruit of this *P. serperaster* were analysed using the knife-edge selection. The maximum sustainable yield (E_{max}), the optimum yield ($E_{0.1}$) and the yield at stock reduction of 50% ($E_{0.5}$) were 0.83, 0.71 and 0.368, respectively (Fig. 6.4a). The yield isopleths were used to predict the response of relative yield-per-recruit of the fish to the changes in L_c and *E*. The stock of *P. serperaster* was under-fishing as the yield isopleths with L_c/L_∞ being 0.57 and *E* being 0.49 (Fig. 6.4b) failed to fit into quadrant A, one of four quadrants of the yield contour. The relative index of growth rate and the asymptotic length, e.g., growth performance (Φ') was calculated with the equation $\Phi' = \text{Log}K + 2\text{Log}L_\infty$, whereas the longevity (t_{max}) was estimated from the equation $t_{\max} = \frac{3}{K}$. The growth performance value of *P. serperaster* was 2.67, and longevity was 4.05 yr.

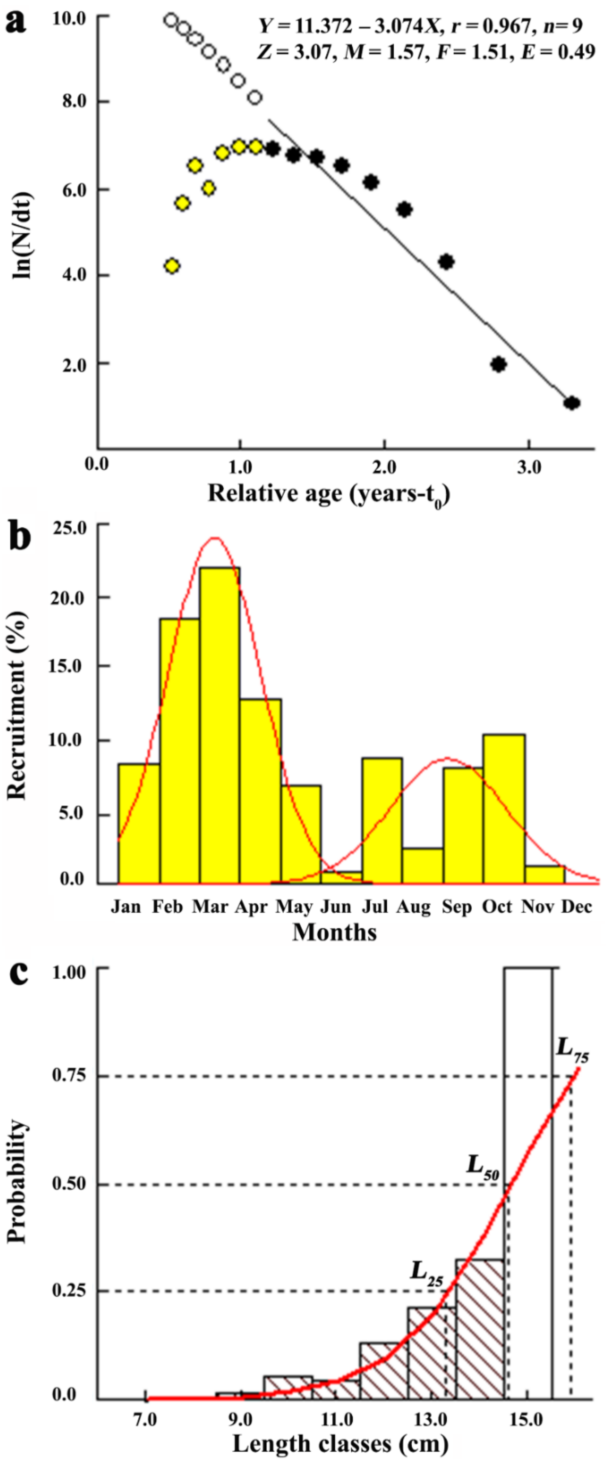

Figure 6.3 (a) The fish length converted catch curve (dark points: data for calculating least square linear regression; yellow points: data excluded from regression analysis; and open points: data for fish age expectation); (b) Recruitment pattern estimated from length frequency data; and (c) The probability of capture of each length class (L_{25} = 13.3, L_{50} = 14.6 and L_{75} = 15.9 cm, estimated from the logistic transform curve)

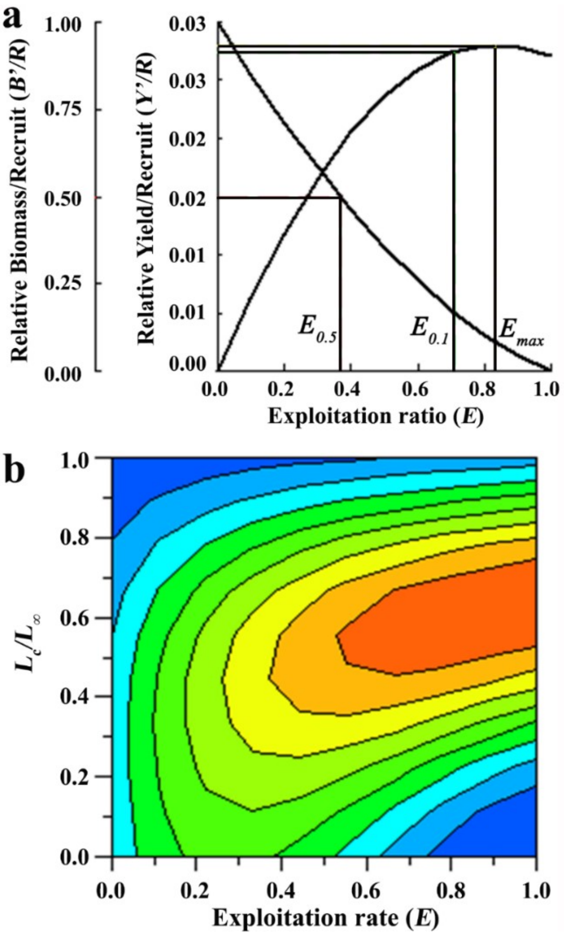

Figure 6.4 (a) The relative yield-per-recruit and relative biomass-per-recruit using the knife-edge procedure (E_{max} = 0.83, $E_{0.1}$ = 0.71 and $E_{0.5}$ = 0.37); and (b) the yield isopleths

6.4.3. Otolith morphometric analysis

Among 476 individuals, only 360 individuals were successfully aged including 164 females and 196 males as otolith was broken down after removing from fish specimens. Fish length was 10.7–23 cm for the female and 12.1–23.1 cm for the male (Fig. 6.5). The left otolith morphometric measurements (e.g., length, width and weight) of male and female fish were used to examine the potential use of otolith to age this goby by regression analysis. The result showed that the otolith was able to be used for fish age determination as the fish length (TL) of two genders had positive relationships with the otolith length ($OL_{female} = 309.5 + 222.02TL$, $n = 164$, $r^2 = 0.683$, $F = 283.56$, $p<0.001$; $OL_{male} = 313.02 + 218.72TL$, $n = 196$, $F = 500.16$, $r^2 = 0.721$, $p<0.001$, Fig. 6.6a); $OWidth_{female} = -890.47 + 256.75TL$, $n = 164$, $F = 356.07$, $r^2 = 0.838$, $p<0.001$; $OWidth_{male} = -515.21 + 232.96TL$, $n = 196$, $r^2 = 0.791$, $F = 451.94$, $p<0.001$, Fig. 6.6b); and weight ($LogOW_{female} = 0.3426 + 0.0441TL$, $n = 164$, $r^2 = 0.780$, $F = 575.15$, $p<0.001$; $LogOW_{male} = 0.1727 + 0.0536TL$, $n = 196$, $r^2 = 0.862$, $F = 1214.39$, $p<0.001$, Fig. 6.6c).

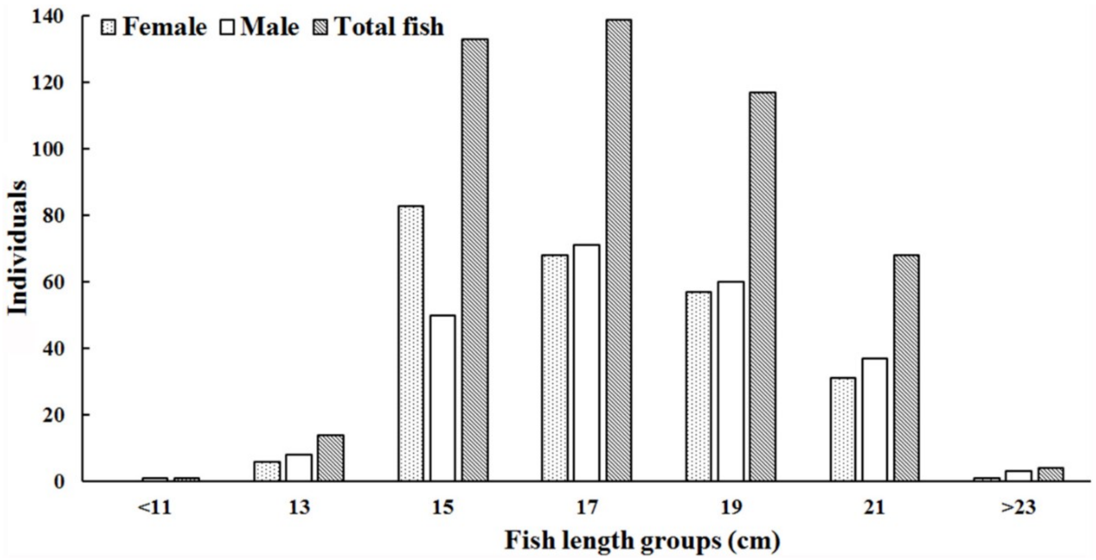

Figure 6.5 Length distribution of male, female and total number of fish used for aging ($n = 476$)

6.4.4. Age determination using fish-length distribution and otoliths

The length frequency analysis showed that L_∞ was 25.2 cm, $K = 0.74$ yr^{-1} and $t_0 = –0.22$ yr^{-1}; and fish length could reach 15 cm at age 0+, 20.4 cm at age 1+, 22.9 cm at age 2+, and 23.1 cm at age 3+ (Fig. 6.7a). The analysis of otolith annual rings (Fig. 6.1b) showed that fish could reach 15.2 cm at age 0+, 20 cm at age 1+, 21.7 cm at age 2+, and 23.1 cm at age 3+ (Fig. 6.7b). The index of average percent error of three readings was 4.65%. There were significant relationships between fish age and otolith length (Otolith length = 2767.1 + 680.04Age, $n = 360$, $F = 381.55$, $p<0.001$), width (Otolith width = 2158.2 + 658.56Age, $n = 360$, $F = 332.67$, $p<0.001$) and weight (LogOW = 2.7566 + 5.2386Age, $n = 360$, $F = 701.62$, $p<0.001$) in both male and female based on the regression analysis (Fig. 6.8). The result of fish age determination by using length frequency analysis and reading annual ring on the otoliths was not significantly different (ANOVA, F = 2.08, $p>0.05$).

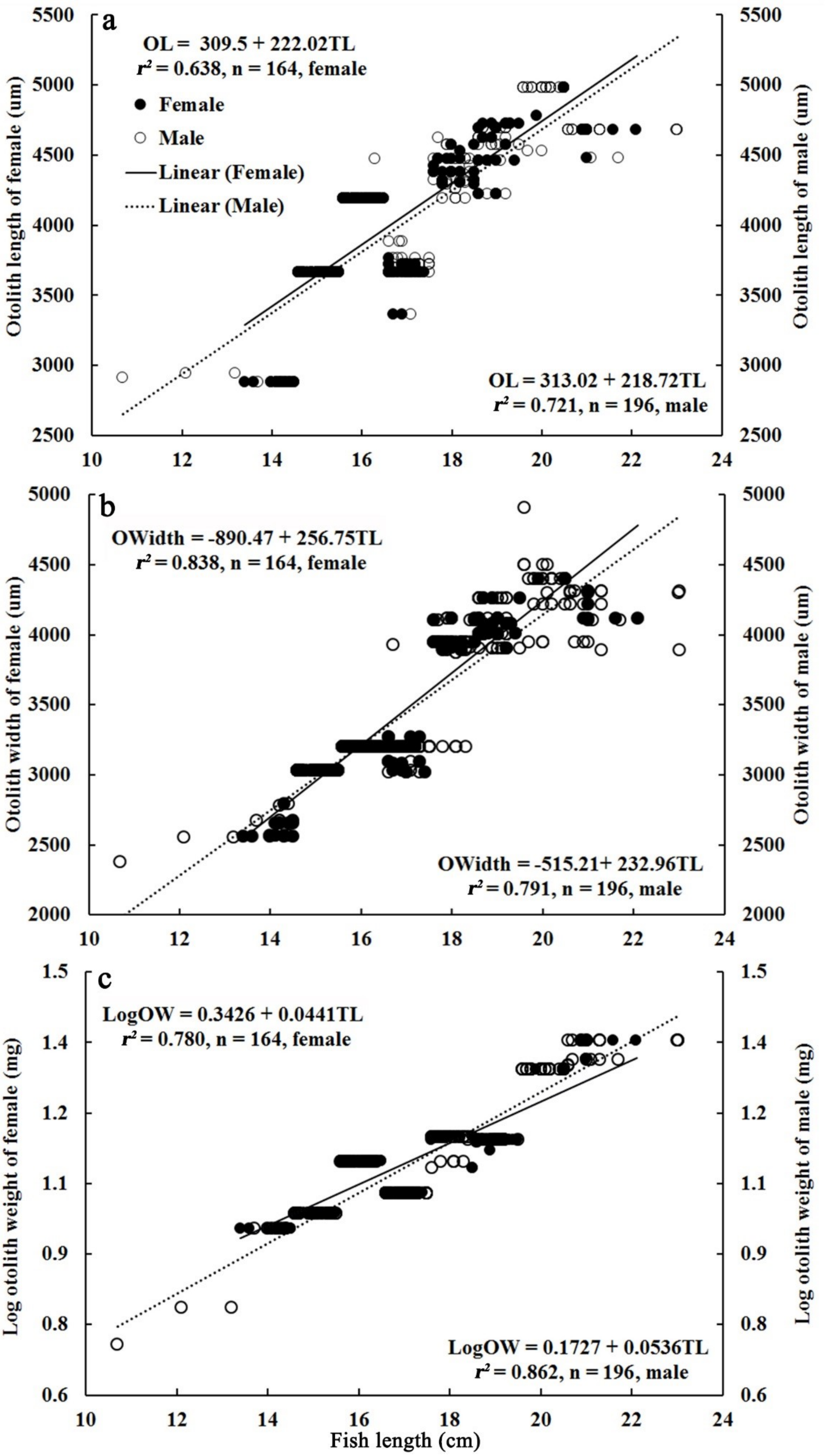

Figure 6.6 Relationships between total length of male and female fish from (a) otolith length (OL), (b) otolith width (OWidth), and (c) otolith weight (LogOW)

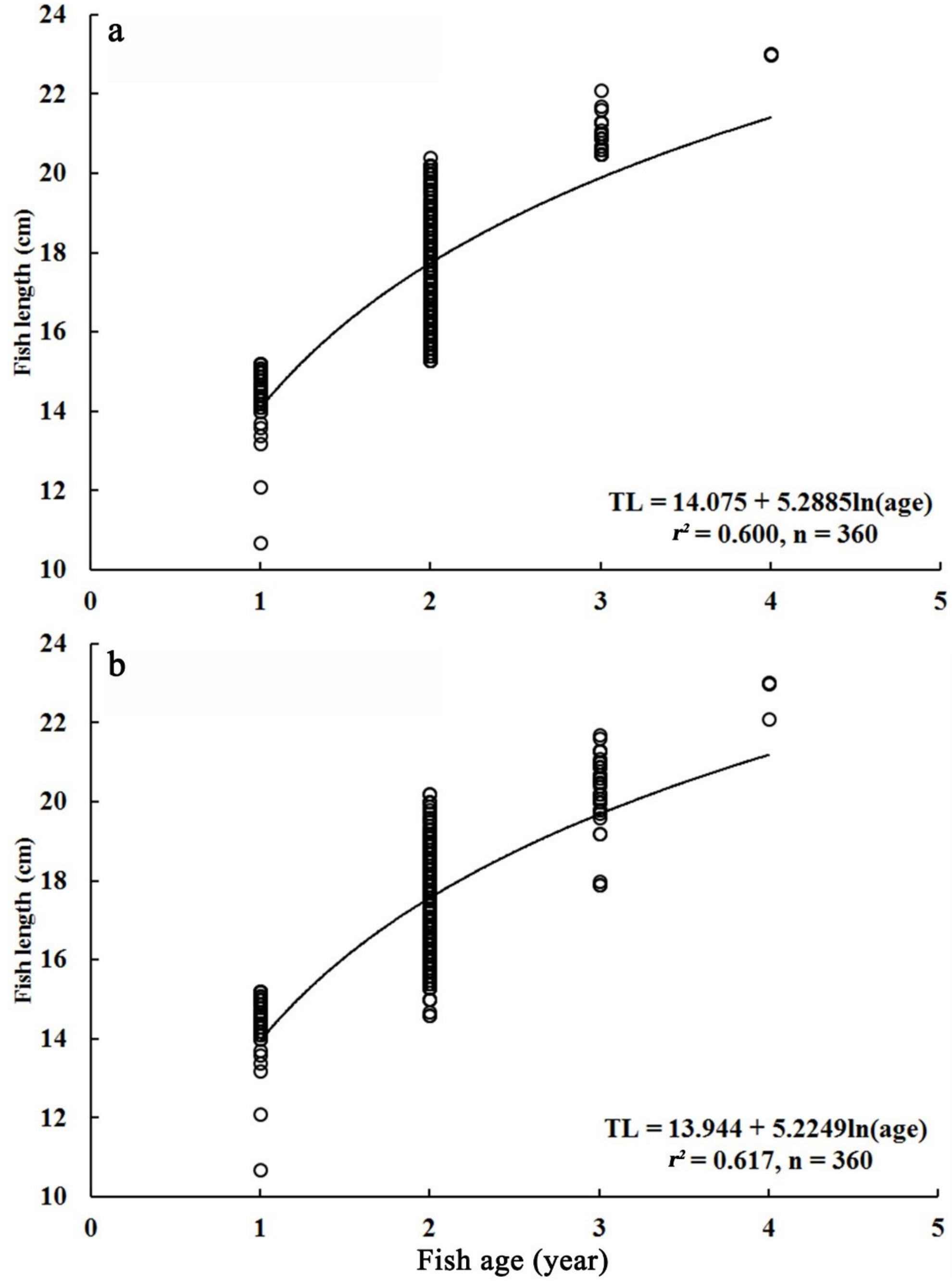

Figure 6.7 Relationships between fish total length and fish age obtained from (a) the length-frequency analysis and (b) otolith annual ring readings

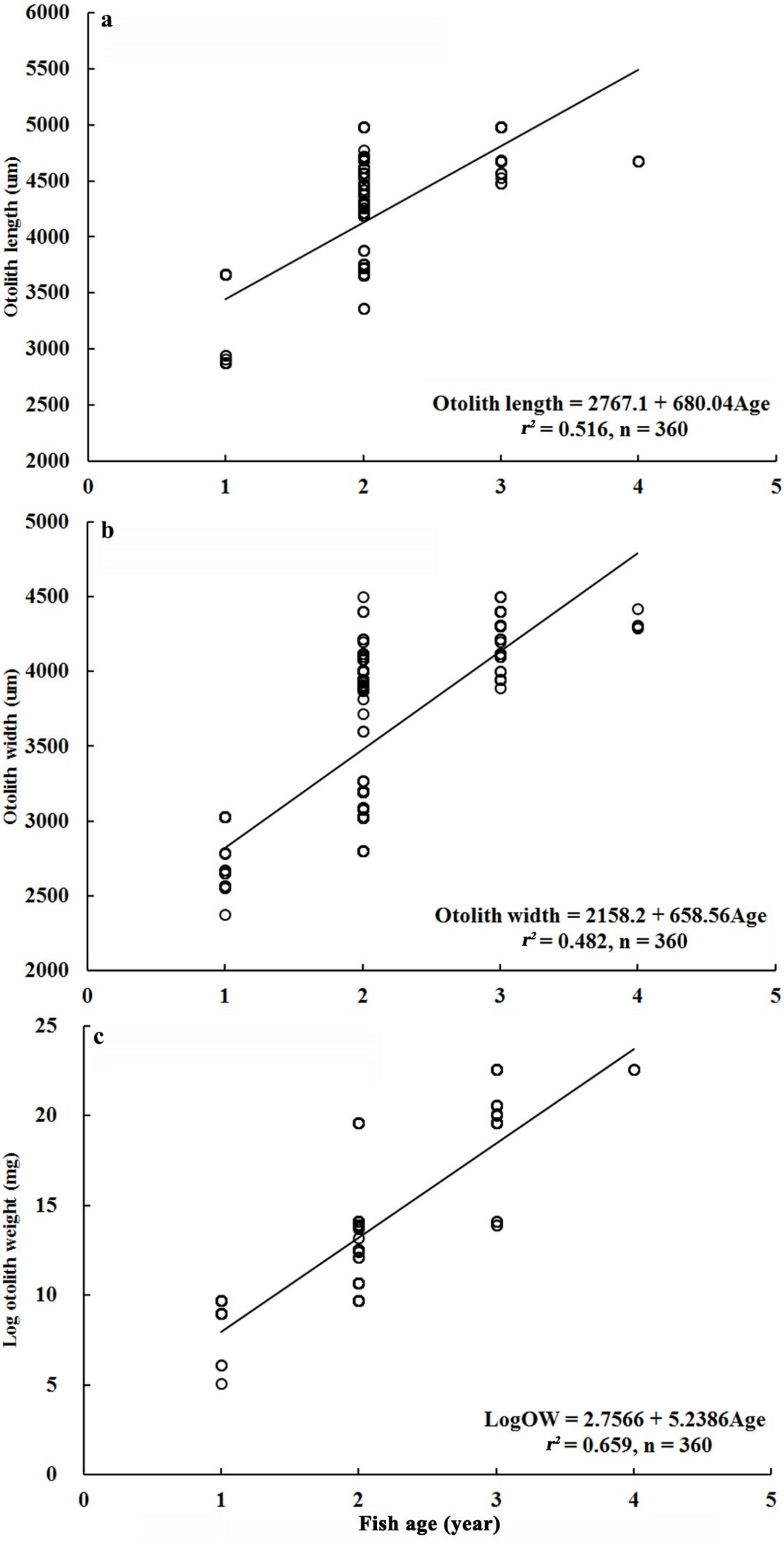

Figure 6.8 Relationships between fish age and (a) otolith length, (b) otolith width, and (c) otolith weight (LogOW)

6.5. Discussion

6.5.1. Sex ratio

In the present study, the sex ratio of *P. serperaster* was not significantly different from the 1:1 female to male ratio. Similarly, Tran et al. (2007) reported that the male to female ratio of another goby (*P. elongatus*) living in the Mekong Delta was also near 1:1. Moreover, the number of male and female of other gobiid species *Boleophthalmus boddarti* occupying the same habitat is also close to 1:1 (Dinh 2014). The environmental temperature and salinity in the study of Tran et al. (2007) and Dinh (2014) are similar to this study, which may contribute to the relatively similar male to female ratio in *P. serperaster*, *P. elongatus* and *B. boddarti* populations. The goby *Periophthalmodon schlosseri* in Malaysian waters also shares the same sex ratio (Mazlan and Rohaya 2008), and the 1:1 male to female ratio is found in four-spotted goby *Deltentosteus quadrimaculatus* collected from İzmir Bay, central Aegean Sea (Metin et al. 2011). It seems that the temperature and salinity could not regulate sex ratio of gobiid species, corroborating observations on most other fish species (Nikolsky 1963).

6.5.2. Population structure

Pauly (1987) suggested that the analysis of the structure of a fish population requires at least 1500 fish specimens collected over six months, and the length frequency distribution should display distinct peaks over time. The present study adopted this sampling criterion by collecting 3002 fish specimens during 12 months, and the sequentially arranged monthly abundance histograms displayed two distinct peaks in population abundance. Thus, it is reasonable to believe that the results from such a large population size would provide a reliable dataset for the analysis of population parameters. The results from the length-frequency analysis in the present study showed the migration of two main cohort juveniles from the sea to the estuary and then to the Kinh Ba River for foraging, which is concomitant to the two spawning migration events from July to October during wet seasons (personal observation). Similarly, Tran (2008) also reported that *P. elongatus* juveniles move from the marine to brackish habitats for feeding, indicating that estuaries are the main nursery ground for gobies and other fish larvae.

Direct comparisons on von Bertalanffy growth parameters may not make precise biological sense as the fish growth curves are not linear and the growth rate varies with fish length and age. Therefore, the growth comparison should be made from a multivariate perspective so that both fish asymptotic length (L_∞) and growth parameters (K) are considered, and growth performance index (Φ') is used for growth comparison between fish species (Pauly and Munro 1984; Etim et al. 2002; Tran et al. 2007). Besides, when comparing growth parameters between different tilapia populations, Moreau et al. (1986) used another growth index $\omega = KL_\infty$ for growth comparison, but found that the growth performance index (Φ') is the best growth index as it exhibits the least degree of variation. The growth parameter Φ' is species-specific, i.e., its values are usually similar within the related taxa and have narrow normal distributions (Tran et al. 2007). The Φ' value of *P. serperaster* was higher than its neighbour species *Pseudapocryptes elongatus* and other gobiid species, except for *P. schlosseri* (Table 6.2). The differences in L_∞ and K indices between these species could lead to the variation of Φ', and it seems that a longer fish may lead to a greater value of the growth index.

Table 6.2 The von Bertalanffy growth parameters and growth-performance index for various gobiid species

Species	Φ'	L_∞ (cm)	K (yr^{-1})	Source
Periophthalmodon schlosseri	3.01	29	1.44	Mazlan and Rohaya (2008)
Periophthalmus papilio	2.28	19.39	0.51	Etim et al. (1996)
Periophthalmus barbarus	2.41	21.6	0.55	Etim et al. (2002)
Pseudapocryptes elongatus	2.64	26	0.65	Tran et al. (2007)
Parapocryptes serperaster	2.67	25.2	0.74	This study

The fishing mortality (F) of *P. serperaster* was slightly lower than that of the natural mortality (M), suggesting that its population has been underexploited. However, the mortalities including total mortality (Z) of this goby are slightly higher ($Z = 3.07$ yr^{-1}, $M = 1.57$ yr^{-1} and $F = 1.51$ yr^{-1}) than the *Pseudapocryptes elongatus* goby in the same area ($Z = 2.91$ yr^{-1}, $M = 1.44$ yr^{-1} and $F = 1.47$ yr^{-1}) as the commercial fishing activity for *P. serperaster* has been recently increased. The two annual recruitment peaks of *P. serperaster* population indicate that this goby can spawn more than once per year, matching the two recruitment peaks of the goby *Pseudapocryptes elongatus* (Tran et al. 2007). However, the length at first capture ($L_c = 14.60$ cm) in *P. serperaster* was greater than that in *P. elongatus* (12.85 cm), implying that *P. serperaster* is less exploited than *P. elongatus*. *P. serperaster* stock has not been subjected to overfishing as the exploitation rate of this goby ($E = 0.49$) is less than the maximum exploitation rate ($E_{max} = 0.83$). Furthermore, fish were fully exploited below the length at first maturation in both sexes (15.63 cm for male and 15.9 for female, unpublished PhD thesis data).

Pauly and Soriano (1986) used four-quadrant models to describe fish yield related to fish size. Quadrant A represents under fishing where the ratio of fish length at first capture (L_c) to the asymptotic length (L_∞) is 0.5–1, and the exploitation rate (E) is 0–0.5. Quadrant B represents eumetric fishing where L_c/L_∞ = 0–0.5 and E = 0–0.5. Quadrant C represents developed fishery where L_c/L_∞ = 0.5–1, and E = 0.5–1. Quadrant D represents overfishing where L_c/L_∞ = 0–0.5, and E = 0–0.5. In comparison, the fish yield isopleths of this goby belong to quadrant A as the ratios between the length at first capture (L_c) and the asymptotic length (L_∞) was 0.57 and the exploitation rate (E) was 0.49. Therefore, the population of this goby has not been overexploited and the current use of fishing gears is suitable to sustain the population growth of this goby species. Although the fish stock of *Pseudapocryptes elongatus* has not reached the point of overexploitation, more small fish were caught in the study of Tran et al. (2007) as the yield isopleths L_c/L_∞ (0.45) and E (0.51) belong to the quadrant D.

The longevity of *P. serperaster* in this study was quite high (4.05 yr) and the age at first maturity was one-year old. Comparing to other gobies, the maximum lifespan of *P. serperaster* is slightly shorter than that of *P. elongatus* (4.55 yr) (Tran et al. 2007), but is longer than *P. schlosseri* (2.14 yr) (Mazlan and Rohaya 2008) and *Gobius vittatus* (2.94 yr) (Kovačić 2007). The variation of longevity among gobies could be due to geographic latitude, predation and fishing activities.

6.5.3. Otolith dimensional morphometry and age determination

In the present study, the left otolith was used to determine fish age. The positive relationships between fish total length (TL), otolith length (OL), width (OWidth) and weight (Log_{10} of otolith weight, LogOW) suggest that the otolith can be used to determine the age of tropical fish such as *P. serperaster*. Similarly, Matic-Skoko et al. (2011) and Pilling et al. (2003) also found that the above three variables for otolith dimensional measurements in both *Phycis phycis* (Gadidae) and *Lethrinus mahsena* showed positively close relationships with fish length. Moreover, the utilisation of otolith dimensional measurements has been successfully used on other fish species such as *Melanogrammus aeglefinus* (Cardinale and Arrhenius 2004), *Gadus morhua* and *Pleuronectes platessa* (Cardinale et al. 2000). Therefore, the growth rings on the otolith can be used as a quick, easy and reliable method for age determination on tropical fishes.

The ages of *P. serperaster* derived from the length-frequency distribution and otolith annual ring readings were similar, indicating that length frequency analysis can be used as an alternative for fish age determination on this goby, though length distribution was not recommended for age determination on older and slow growing fishes (Campana 2001). Similarly, length-frequency distribution is also successfully used to determine the age of *Pseudapocryptes elongatus* (Tran 2008) possibly due to the short lifespan of fish and fast growth. In the present study, the method of using annual rings on the otolith for age determination was applied successfully for *P. serperaster*, a tropical goby fish. Likewise, this method was applicable for the age determination on *P. elongates*, another tropical fish living in the same habitat as *P. serperaster* (Tran 2008). However, when a large number of specimens are available, the method of length frequency should be first considered for age determination as it does not require tedious preparation and treatment of otoliths for small and short life goby species.

In the use of otolith for age determination, the index of average percent errors (*IAPE*) was 4.65% in this study, which is less than the threshold value (5%) recommended by Beamish and Fournier (1981), suggesting that the result of three readings on the growth rings on the otolith is reliable. This index (*IAPE*) was used as the principal index for comparing the results of otolith annual ring determination within a reader or between readers. For example, Matic-Skoko et al. (2011) apply this index for comparing the reliability between two readers and two reading times for age determination on *Phycis phycis* (Gadidae); Newman et al. (2000) used the *IAPE* to test the precision of age estimates for *Lutjanus erythropterus*, *L. malabaricus* and *L. sebae*; and this index is also used by Tran (2008) to examine the age precision on the goby *P. elongatus*.

In addition, the results of aging on this goby with the length-frequency method and reading annual rings on the otoliths were similar, suggesting that the length-frequency can also be used for age determination on tropical fish species. This study shows that despite a similar annual temperature condition in the growing area of this *P. serperaster*, growth rings can still be used for fish age determination. The development of a reliable tool to measure the age and length relationships is useful for fishery management and future aquaculture planning of this goby species.

In conclusion, the sex ratio of *P. serperaster* was close to 1:1 and its stock was high in population recruitment and was not over exploited in the Mekong Delta. Otolith measurements can be used for age determination for both male and female gobies. The use of length-frequency distribution of a large sample size validated the result of age determination using otolith. The age and growth relationships can be used to determine the proper size of fish at catch and manage the sustainable fishery in trophic areas.

6.6. Acknowledgments

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CHAPTER 7: GENERAL DISCUSSION

7.1. General discussion

Parapocryptes serperaster is a bottom burrowing species in the Gobiidae and widely distributed in the Indo-West Pacific regions (Murdy 1989; Talwar and Jhingran 1991; Kottelat et al. 1993; Khaironizam and Norma-Rashid 2000), including the Mekong Delta (Rainboth 1996; Tran et al. 2013). Its external morphometric and taxonomic identification keys are provided by Murdy (1989) and Khaironizam and Norma-Rashid (2000). Although *P. serperaster* is an important commercial fish (Ip et al. 1990) and adapts to a wide range of salinity from marine to freshwater (Rainboth 1996), its habitat, growth, reproductive biology and population structure had not been well studied. This thesis provided useful information on its habitat use, growth and life history toward to sustainable fishing management and future aquaculture. Upon the completion of this thesis, the major findings are summarised as follows:

1. This goby mainly uses the burrow as a place for protection from predators;
2. This goby displays a pattern of isometric growth and omnivorous feeding habit, mainly feeding on detritus, diatoms, bluegreen algae and copepods;
3. This fish is a multiple spawner and reaches sexual maturity when one-year old for both males and females;
4. The goby stock is not subject to overfishing in the study region;
5. The otolith was successfully used for age determination in this goby species.

The outcomes of this thesis have provided better understanding on the habitat use, food and feeding, reproduction and population demography of this goby species, which contribute to fundamental knowledge of fishery management and aquaculture of this goby and other small gobiid fishes in the monsoonal region.

7.1.1. Habitat use and features

Burrowing activity is an important adaptation for gobies living in a shallow and muddy habitat. Like other bottom burrowing gobiid species, *P. serperaster* also build burrows that comprise several slightly circular openings, a few branching tunnels, multi-bulbous chambers and various short cul-de-sac side branches, based on physical burrow structure analysis. The burrows structure of *P. serperaster* had fewer openings and branches than burrows made by *Odontamblyopus lacepedii* (Gonzales et al. 2008) and *Taenioides cirratus* (Itani and Uchino 2003) living in mudflats, but slightly more than the con-specific *Pseudapocryptes elongatus* inhabiting in the same mudflats in the Mekong Delta (Tran 2008). The U-shaped *P. serperaster* burrow was similar to the burrow of *Boleophthalmus boddarti* (Clayton and Wright 1989), but the W-shaped was different from the *B. boddarti* burrow. The U- and W-shaped *P. serperaster* burrows were different from those of other gobiid species such as *Pseudapocryptes elongatus* (Y-shaped) (Tran 2008), *Taenioides cirratus* (Itani and Uchino 2003) and *Odontamblyopus lacepedii* (Gonzales et al. 2008).

The larger *P. serperaster* can make more sophisticated burrows as the burrow dimension was positively correlated with fish size, and the burrow structure and dimensions varied with fish size, but not seasons. *P. serperaster* constructed shelters through body movement at the time coincident to high tide based on laboratory observations, which is also found in anguilliform fish (Herrel et al. 2011). However, as the casts showed that configuration of burrows was so complicated, this gobiid species may use a combination of mouth excavation and body twisting to dig a burrow. Mouth excavation is found in other gobies such as *Periophthalmodon septemradiatus* (Bhatt et al. 2009), *Valenciennea longipinnis* (Takegaki and Nakazono 1999a), *Boleophthalmus boddarti* (Clayton and Wright 1989), and yellowhead jawfish *Opistognathus aurifrons* (Colin 1973).

Burrows could provide a low tide retreat and protection from predators, but were not used for spawning and feeding for this goby species, whereas the monogamous goby *Valenciennea longipinnis* (Takegaki and Nakazono 1999b; Takegaki 2001), the goby *Zosterisessor ophiocephalus* (Mazzoldi et al. 2000), *Periophthalmodon schlosseri* (Ishimatsu et al. 2009) and mudskipper *Periophthalmus modestus* (Ishimatsu et al. 2007) use burrows for reproduction.

7.1.2. Growth pattern and body condition

The body length of *P. serperaster* did not significantly vary with gender, suggesting that male or female gobies can reach a marketable size at the same age, which is also found in other gobies *Pseudapocryptes elongatus* (Tran 2008), *Periophthalmus barbarus* (Chukwu and Deekae 2011) and *Parachaeturichthys ocellatus* (Panicker et al. 2013). The present study showed that this goby reached a maximum total length of 24 cm compared to 17.3 cm and 23 cm in previous studies (Murdy 1989; Rainboth 1996), indicating that the length of this fish can vary with different locations. The length-weight relationships were similar between female and male *P. serperaster*, and the positive relationships between length and weight are also found in the goby *P. elongatus* (Tran 2008), the Atlantic mudskipper *Periophthalmus barbarus* (Chukwu and Deekae 2011) and the goby *Parachaeturichthys ocellatus* (Panicker et al. 2013).

Gonadal development can lead to a high value of the length-weight regression slope (*b*) in mature fish as adult male and female *P. serperaster* showed a significantly higher *b* value than juveniles. Kalaycı et al. (2007) found that gonadal development leads to a different *b* value in both male and female *Gobius niger* in Turkey, but not in the mudskippers *Periophthalmus barbarus* (King and Udo 1998; Chukwu and Deekae 2011) and *Parachaeturichthys ocellatus* (Panicker et al. 2013). Similarly, the large size of female gonads during the spawning season resulted in a slightly high value of *b* in females compared to males. *P. serperaster* showed isometric growth since its slope value was close to the standard threshold value (i.e., 3) as defined by Froese and Binohlan (2000). Like *Ilisha melastoma* in Pakistan (Mahmood et al. 2012) and *P. elongatus* in the Mekong Delta (Tran 2008), the growth pattern of *P. serperaster* was not significantly different between seasons. Male and female gobies showed a similar growth pattern in both wet and dry seasons, indicating that *P. serperaster* can successfully adapt to climate change in this area.

P. serperaster lives in a favourable environment as its body condition (*K*) value was higher than other fish in the same habitat like *Pseudapocryptes elongatus* (Tran 2008). Similarly, *Ilisha melastoma* lives in a favourable environment in Pakistan as its condition factor is close to the wellbeing threshold (Mahmood et al. 2012). The body condition factor of *P. serperaster* decreased as fish grew, and the *K* of male *P. serperaster* was greater than of females in the wet season. It suggests that the condition factor can be affected by fish developmental stage, and it was high in females towards the end of gonadal maturation, which was also found in *Ilisha melastoma* (Mahmood et al. 2012), but not *Periophthalmus barbarus* (King and Udo 1998; Chukwu and Deekae 2011). The change in *K* value of *P. serperaster* was not significantly different between wet and dry seasons, suggesting that the nutritional condition in the area is favourable for the growth of this species.

7.1.3. Food and feeding habit

P. serperaster was omnivorous based on the relative gut length (*RGL*) analysis, which was also supported by the result of fish stomach analysis. The *RGL* is usually used to classify the trophic guild in most fish species (Geevarghese 1983; Elliott and Bellwood 2003; Karachle and Stergiou 2010). In the present study, the *RGL* was successfully applied to *P. serperaster*, suggesting that this index is useful to determine feeding intensity.

The gut fullness index reached a high value before and after the spawning period, indicating that the feeding intensity of *P. serperaster* varied over time and this goby species actively fed during the pre- and post-spawning periods to obtain energy needed for gonad development and compensate energy loss because of spawning (Dinh et al. 2015). The feeding intensity of *P. serperaster* varied with season possibly due to seasonal variation in food availability, but not fish size, suggesting that food competition is not severe between small and large fish.

P. serperaster primarily ingested detritus, coinciding with a high proportion of this item in the environment. A small percentage of the algae in the phylum of Bacillariophyta, Cyanophyta and copepods was found in the stomach of *P. serperaster*, suggesting that these prey items were random food, which was supported by their low occurrence in the environment. This finding is similar to a related goby *P. elongtus* that feeds on detritus and the algae in the phylum of Bacillariophyta (Tran 2008), and the mudskipper *Boleophthalmus pectinirostris* that also feed diatoms (Yang et al. 2003). In contrast, Khaironizam and Norma-Rashid (2000) revealed that *P. serperaster* mainly ingested Bacillariophyta and that the food availability in the environment was the main factor involved in food selection, which is found in the round goby *Neogobius melanostomus*, a non-random or nonopportunist feeder adapted to a different environment (Thompson and Simon 2014). The diet of the common goby *Pomatoschistus microps* varies with prey availability in the environment since the fish stomach comprises a group of shrimp-like crustaceans (Mysidacea) in the upper Tagus estuary (Salgado et al. 2004) but not in fish found in the Mondego estuary due to low prey abundance in the environment (Leitão et al. 2006). Similarly, high rainfall in the wet season leads to the change of nutrient input and prey availability in the environment (Nedeco 1993), resulting in the variation of diet composition of *P. serperaster* between dry and dry seasons. A seasonal change in feeding intensity is also found in the Bayad fish *Bagrus bajad* in Egypt (Bakhoum and Fatas 2003) and the naked goby *Gobiosoma bosc* (D'Aguillo et al. 2014) in western Atlantic estuaries.

The index of relative importance (*IRI*) is used as a tool to determine fish feeding habit and used as a biological indicator to classify the type of food items (Gumus et al. 2002), but it is difficult to apply for omnivorous, detritivorous, zooplanktivorous and phytoplanktivorous fish due to small prey size. To overcome the difficulty of small prey size, the percentage of food weight or volume was replaced by food biovolume (e.g., area of prey) to estimate the *IRI* of each prey. Based on the estimate of food biovolume, *P. serperaster* mainly fed on detritus and some diatoms (*Navicula* spp., *Nitzschia* spp. and *Gyrosigma* sp.), but this fish rarely consumed Cyanophyta and copepods (Vu and Duong 2013), and *Acartia* sp., *Calanus* sp. and *Paracalanus* sp. (copepods) rarely appeared in the environment. The biovolume method is consistent with the result of other methods for food item determination and can be used to determine the seasonal and intraspecific variations in small fish that feed on small-sized prey. Such variation is also found in round goby *N. melanostomus* (Thompson and Simon 2014) and the mudskipper *B. pectinirostris* (Yang et al. 2003), suggesting that the biovolumetric method is useful to study the dietary composition of small fishes such as gobiids.

P. serperaster is a generalist feeder as various food types (14 prey types) were found in its stomach, enabling *P. serperaster* to adapt to a variety of environmental conditions, which is also found in other gobiids such as *P. modestus* and *P. magnuspinnatus* (Baeck et al. 2013), *Achondrostoma arcasii, Pseudochondrostoma duriense*, *Salmo trutta* and *Gasterosteus gymnurus* (Sánchez-Hernández et al. 2011), *P. minutus* and *P. microps* (Salgado et al. 2004; Leitão et al. 2006). By contrast, the goby *Economidichthys pygmaeus* has a narrow diet breadth and is a specialist feeder on copepods and chironomids (Gkenas et al. 2012). In this study, although 19 types of food items belonging to six main taxonomic categories were found in the environment, more species of microalgae might have been found if more samples had been collected. *Navicula* spp. were mostly consumed whereas *Spirulina* sp. was rarely ingested by fish in the wet season. On the other hand, *Nitzschia* spp. were mainly consumed whereas *Anabaena* spp. were rarely ingested in the dry season. *Navicula* spp. were mostly ingested, whereas Cyanophyta species and copepods were rarely fed by all sized fish in this study. The diet overlap of prey items between the fish stomach and water column suggests that *P. serperaster* may feed in the water column, which occurs in the co-occurring *P. elongatus* on the same site (Tran 2008).

7.1.4. Reproductive biology

P. serperaster is a multiple spawner due to the coexistence of four oocyte types (oogonia, primary oocytes, secondary oocytes, and vitellogenic and hydrated mature oocytes) in the stage V ovary. Surprisingly, there was no completely spent ovary in this multiple spawning species as no true stage VI ovaries were found in this study. Besides, the male goby can release sperm several times during the reproductive season as the stage IV testes comprised spermatocytes at different developmental stages including primary spermatocyte, secondary spermatocyte, spermatid and spermatozoa. The high runoff after precipitation may increase food availability for larval fish nursery due to high nutrients in the incoming water into the coastal area (Nedeco 1993; Nguyen et al. 2000; Tran 2008). In the same study region, the spawning season of a related goby species *P. elongatus* is from June to November with two spawning peaks in July and in October (Tran 2008), overlapping the spawning period of *P. serperaster* seems to be a semi-estuarine resident and an oviparous fish since a few *P. serperaster* juveniles were collected along the coastline using a larval fish net during the study period (Dinh et al. 2014).

The spawning season of *P. serperaster* from July to October coincides with the main period of precipitation in the Mekong Delta. During the spawning period, the male *Gobius vittatus* tend eggs and embryos, resulting in more females than males in fish catches (Kovačić 2007b), which is also found in the goby *Periophthalmus barbarus* in Imo estuary, Nigeria (Etim et al. 2002). Similarly, the matured males might tend to eggs in the mud substrate as there was no male *P. serperaster* with stage V testis caught during the breeding season in the present study. The egg tending activity by the male during the spawning season also occurs in other gobiid fishes such as *Periophthalmus magnuspinnatus* (Baeck et al. 2008), *Periophthalmus modestus* (Ishimatsu et al. 2007; Ishimatsu and Graham 2011), and *Periophthalmus minutus* (Takeda et al. 2012). The monthly change of gonadosomatic index (GSI) of *P. serperaster* was similar between males and females, and its GSI values were high before the spawning period due to the relative increase of the ovary mass to body weight. Based on the monthly GSI observations, Tran et al. (2007) reported that the spawning season of *Pseudapocryptes elongatus* ranges from June to October, and Macinnis and Corkum (2000) reported that the goby *Neogobius melanostomus* in Canada spawn from May to July. In the present study, the GSI was low in April, which coincides with the early stage of gonad development and reached the highest value in September, matching the histological development of the stage V ovary. With the development of ovary maturation and the increase of yolk content and hydratation of oocytes, the value of GSI reached a maximum during the spawning season. The GSI declined from September onward after the egg was released due to degeneration of follicular layers.

Wootton (1990) reported that the size and age at first maturity in fish primarily depend on species and environmental factors. The size of fish at first sexual maturation is regulated by biotic factors such as parental care and predation (Abrams and Rowe 1996), e.g., the marbled goby *Pomatoschistus marmoratus* show that parental care leads to a larger size of males at first maturation than females (Mazzoldi et al. 2002). Besides, abiotic factors such latitude also affect the size at first sexual maturation in fish. For instance, *P. marmoratus* reach the first sexual maturation at 2.4 cm in Suez Canal (Fouda et al. 1993) but 2.7 cm in Mauguio Lagoonare (Bouchereau et al. 1993). In the present study, the size at first maturation in the male *P. serperaster* was similar to the female, suggesting no sexual dimorphism in growth for both sexes of this gobiid species.

Most gobies are repetitive spawners, and the fecundity and egg size depend on fish size (Miller 1984; Kovačić 2007b). The present study, likewise, revealed that *P. serperaster* is a serial spawner, and its fecundity is positively related to fish size. The total fecundity of *P. serperaster* is lower than some other gobiid species such as *Valenciennea strigata* (Reavis 1997), *Amblygobius phalaena* (Takegaki 2000), and *Periophthalmus barbarus* in mangrove swamps of Imo estuary, Nigeria (Udo 2002). However, the fecundity of *P. serperaster* is in the similar range to *Periophthalmus papilio* (e.g., *P. barbarus*) in mangrove swamps of Lagos Lagoon, Nigeria (Lawson 2011), *Periophthalmus barbarus* in new Calabar River, Nigeria (Chukwu et al. 2010) and *Pseudapocryptes elongatus* in the Mekong Delta (Tran 2008), but higher than other gobiid species such as *Boleophthalmus dentatus* in the northern Arabian sea (Hoda and Akhtar 1985), *Boleophthalmus dussumeri* in Karachi coast (Hoda 1986), *Neogobius melanostomus* (Macinnis and Corkum 2000), *Pomatoschistus marmoratus* in Venetial lagoon (Mazzoldi et al. 2002) and *Crystallogobius linearis* (Caputo et al. 2003). It seems that the fecundity of gobiid is species-specific and is also related to the size of fish.

7.1.5. Population and age structure

Temperature variation can strongly regulate the sex ratio of fishes. For example, in Lake Thun, Switzerland, a monitoring program showed that the sex ratio of a population of grayling *Thymallus thymallus* changed from approximately 65% males before 1993 to approximately 85% males in 2011, along with a gradual temperature elevation over the last few decades (Wedekind et al. 2013). In this thesis, however, the sex ratio of *P. serperaster* was not significantly different between dry and wet seasons, suggesting that the abundance of male and female populations of this goby was not regulated by the seasonal variation in temperature. The 1:1 male to female ratio is also found on other gobiid species living in the same habitat with *P. serperaster* such as *Pseudapocryptes elongatus* (Tran 2008) and *Boleophthalmus boddarti* (Dinh 2014), suggesting that the low variation of temperature in the study region between dry and wet seasons (26.5–30 ºC) may explain the similar sex ratio of gobies between seasons in study area.

The initial size of *P. serperaster* when it entered fishery catch was 14.6 cm *TL*, and the longevity was 4.05 yr. This fish reached first maturity at one-year old, and its fishing, natural and total mortalities were 1.57 yr^{-1}, 1.51 yr^{-1} and 3.07 yr^{-1}, respectively. Comparing to other gobies, the maximum lifespan of *P. serperaster* is slightly shorter than *P. elongatus* (4.55 yr) (Tran et al. 2007), but longer than *Gobius vittatus* (2.94 yr) (Kovačić 2007a) and *P. schlosseri* (2.14 yr) (Mazlan and Rohaya 2008). The variation of longevity among gobies could be due to geographic latitude, predation and fishing activities. Its stock was not over exploited as the exploitation rate ($E = 0.49$) was less than the maximum exploitation rate ($E_{max} = 0.83$).

The proper readable otoliths of 164 females and 196 males were used for age identification as the positive relationships exist between the fish total length, otolith length, width and weight. Similarly, Matic-Skoko et al. (2011) and Pilling et al. (2003) also found that the above three variables for otolith dimensional measurements in both *Phycis phycis* (Gadidae) and *Lethrinus mahsena* were positively and closely related to fish length. The utilisation of otolith dimensional measurements has been successfully applied to other small fish species such as *Melanogrammus aeglefinus* (Cardinale and Arrhenius 2004)*, Gadus morhua* and *Pleuronectes platessa* (Cardinale et al. 2000). The ages of *P. serperaster* derived from the length-frequency distribution and otolith readings were similar, indicating that length frequency analysis can also be used as an alternative for fish age determination on this goby, though the length distribution was not recommended for age determination on older and slow-growing fishes (Campana 2001). The otolith morphometry is a reliable way to age this fish and possibly is also applicable for other tropical gobies.

7.2. Conclusion

This thesis presents findings on the habitat use, growth pattern and body condition variation, food and feeding habits, reproductive biology, and population and age structure of *P. serperaster*. The outcomes of the research not only contribute to better understanding of the biology and fishery management this gobiid species but it also provides crucial information useful to the study of other similar gobiids in the monsoonal region. In summary, the following five conclusions are drawn:

(1) The sex ratio of *P. serperaster* was not significantly different between the dry and wet seasons, but the length and weight of this goby differed between genders and seasons. This gobiid species can reach a maximum length at 24 cm. The burrow structure of *P. serperaster* was sophisticated and strongly varied with fish size, but shared a similar pattern in dry and wet seasons. This gobiid species actively excavated its burrow by body movement and used the burrow as a place to live and protect from predation.

(2) *P. serperaster* showed isometric growth at all seasons. This goby lived in a condition suitable for growth as the *K* value was close to the threshold of wellbeing category. This goby has adapted to the variation of precipitation between the dry and the wet seasons as its condition factor varied with genders and fish sizes, but not with seasons.

(3) *P. serperaster* is omnivorous, feeding mainly on detritus and algae, and its feeding habits varied with food availability in the environment. The food composition varied with fish size and season. This fish was a generalist feeder with a

wider diet breadth in young than in old individuals. Its feeding intensity peaked at the periods before and after spawning.

(4) *P. serperaster* is a serial spawner shedding eggs over four months during the wet season. Although male and female *P. serperaster* reached sexual maturity at one-year old, the female was smaller than the male in body size. As a wet season spawner, climate change and variation of rainfall pattern could significantly impact its spawning success. The effect of climate change on this goby species may spin off on other benthic fishes in this region, which may further influence the ecosystem function via the impact on their predators through food web interaction.

(5) The stock recruitment rate was high and the fish population was not overly exploited in the study region. The male and female gobies can be aged using otolith measurements that were validated by the analysis of length-frequency distribution with a large size of sample. The proper size of fish at catch should be determined by the study of population demography to develop a sustainable fishery in the tropical area.

7.3. Further study

Although this thesis provided new knowledge of habitat, growth pattern and condition factor variation, food and feeding habit, reproductive biology and population structure, which may be applied for other small-bodied fish species in the monsoonal areas, some questions remain unresolved. Therefore, future research should focus on tackling the following issues:

(1) Although the utilisation of burrows of this gobiid species has been studied, the use of burrows for other activities such as spawning and feeding was not found in this study. A longer term observation in the field and in the laboratory is suggested to further understand the usage and function of burrows in this goby.

(2) This goby is a potential species for aquaculture as isometric growth was observed in all seasons. The knowledge gained in this thesis should be applied to the study on the feasibility and practicality for farming this goby under captivity.

(3) The food composition of this goby was investigated, but the nutritional requirement was not addressed. It is recommended to use stable isotope analysis to

reveal the contribution of different food items to nutrient absorbance and utilisation by this fish. The evaluation of prey digestibility and nutritional requirement is needed before the development of aquaculture of this goby.

(4) This goby is a wet season spawner with high fecundity, but the specific conditions and requirement for spawning are not clear. Further research should focus on artificial spawning, such as hormone injection and environmental manipulation, of this fish under captivity.

(5) Although this goby population is not subject to overfishing during the study period, current fishing methods such as fishing gears and fishing season should be evaluated for sustainable fishery management. The population survey of this gobiid fish should be continued for next five years to provide further information to stabilise the exploitation rate for this goby and other species.

7.4. References

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APPENDIX

Haematoxylin solution

Distilled water: 100 mL, glycerine: 100 mL, ammonium sulphate: 3 g, acetic acid: 10 ml

Eosin Y solution

Eosin Y: 1 g, ethanol 70%: 1 L, acetic acid: 5 mL

Albumin solution

Egg-white: 50 mL, glycerine: 50 mL, phenol: 5 mL

Baume Canada

Baume Canada: 25 mL, xylene: 25 mL

Bouin solution

Picric acid: 1,126 g, distilled water: 75 mL, formalin 40%: 25 mL, acetic acid: 5 mL

Gilson solution

Ethanol 60%: 100 mL, nitric acid 80%: 15 mL, acetic acid: 18 mL, mercuric chloride: 20 g, distilled water: 880 mL

Ovary staining procedure

Stage	**Chemicals**	**Time (stage I/II–III/IV–V)**	**Note**
Fixation	Bouin solution	At least 24 h	
Washing	Ethanol 50%	24 h	
Dehydration	Ethanol 70% I	15 min	
	Ethanol 70% II	15 min/1 h/2 h	
	Ethanol 90% I	15 min/1 h/2 h	
	Ethanol 90% II	15 min/1 h/2 h	
	Absolute ethanol I	15 min/1 h/2 h	
	Absolute ethanol II	15 min/1 h/2 h	
	n-Butanol I	15 min/1 h/2 h	

Stage	**Chemicals**	**Time (stage I/II–III/IV–V)**	**Note**
	n-Butanol II	15 min/1 h/2 h	
Ethanol removal	Xylene I	15 min/1 h/2 h	
	Xylene II	15 min/1 h/2 h	
Xylene removal	1 paraffin wax : 1 xylene	40 min/3 h/5 h	In the oven with 60 ºC
	Paraffin wax	1 h/5 h/10 h	
Moulding	Paraffin wax	At least 24 h	
Cutting			6-µm thick
Put the micro slide of sample onto the lame	Albumin solution		
	Hot water (80 ºC)		
Removal paraffin from the micro slide	Xylene I	1 min	
	Xylene II	1 min	
	Absolute ethanol	30 sec	
	Ethanol 90%	30 sec	
	Ethanol 70%	30 sec	
	Ethanol 50%	30 sec	
Haematoxylin staining	Distilled water	30 sec	
	Haematoxylin	10 sec	
	Running tap water	10 min	
	Distilled water	2 min	
Eosin Y staining	Ethanol 50%	1 min	
	Ethanol 70%	1 min	
	Eosin Y	10 min	
	Ethanol 80%	10 sec	
	Ethanol 90%	10 sec	
	Absolute ethanol	10 sec	
	n-Butanol I	1 min	
	n-Butanol II	2 min	
Clear micro slides	Xylene I	2 min	
	Xylene II	Until the micro slide was clear	
Lamella mounting	Baume Canada		

Testis staining procedure

Stage	**Chemicals**	**Time (stage I–II/III–IV)**	**Note**
Fixation	Bouin solution	At least 24 h	
Washing	Ethanol 50%	24 h	
Dehydration	Ethanol 70% I	3 min/ 15 min	

Stage	**Chemicals**	**Time (stage I–II/III–IV)**	**Note**
	Ethanol 70% II	3 min/15 min	
	Ethanol 90% I	3 min/15 min	
	Ethanol 90% II	3 min/15 min	
	Absolute ethanol I	3 min/15 min	
	Absolute ethanol II	3 min/15 min	
	n-Butanol I	3 min/15 min	
	n-Butanol II	3 min/15 min	
Ethanol removal	Xylene I	3 min/15 min	
	Xylene II	3 min/15 min	
Xylene removal	1 paraffin wax : 1 xylene	15 min/40 min	In the oven with 60 ºC
	Paraffin wax	40 min/1 h	
Moulding	Paraffin wax	At least 24 h	
Cutting			6-µm thick
Put the micro slide of sample onto the lame	Albumin solution		
	Hot water (80 ºC)		
Removal paraffin from the micro slide	Xylene I	1 min	
	Xylene II	1 min	
	Absolute ethanol	10 sec/30 sec	
	Ethanol 90%	10 sec/30 sec	
	Ethanol 70%	10 sec/30 sec	
	Ethanol 50%	10 sec/30 sec	
Haematoxylin staining	Distilled water	10 sec/30 sec	
	Haematoxylin	10 sec	
	Running tap water	10 min	
	Distilled water	2 min	
Eosin Y staining	Ethanol 50%	1 min	
	Ethanol 70%	1 min	
	Eosin Y	10 min	
	Ethanol 80%	10 sec	
	Ethanol 90%	10 sec	
	Absolute ethanol	10 sec	
	n-Butanol I	30 sec/1 min	
	n-Butanol II	30 sec/2 min	
Clear micro slides	Xylene I	2 min	
	Xylene II	Until the micro slide was clear	
Lamella mounting	Baume Canada		